# Left Ventricular Diastolic Function in Adolescents with Overweight and Obesity

## The Moderating Influence of Physical Activity, Cardiorespiratory Fitness, and Metabolic Health

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# Abstract

Lifelong preservation of left ventricular diastolic function (LVDF) is an important goal for reducing the burden of diastolic heart failure and related adverse health outcomes. LVDF is strongly linked with obesity. The global obesity pandemic has already increased the burden of diastolic heart failure in adults, but the prevalence of overweight and obesity is rising fastest in children, potentially setting them on a course to early heart disease. Public health measures designed to tackle the problem of adolescent obesity have focused on lifestyle interventions, such as exercise promotion and dietary improvements, with uncertain impact on LVDF. This thesis aimed to investigate how obesity, physical activity (PA) and innate cardiorespiratory fitness (CRF) influence LVDF in adolescents. From these data, it was also aimed to identify a PA target that might be sufficient to prevent adolescent impaired LVDF.

To demonstrate the need for a focus on LVDF in this age group, Study 1 was a meta-analysis of the relationship of obesity with LVDF in adolescents. The results indicated that those with obesity were likely to have impaired LVDF and that such impairments were best demonstrated by septal tissue-Doppler imaging (TDI) methods of echocardiography (z-score difference=0.91; 95% CI=0.46, 1.37). This study also identified preliminary evidence for links of insulin resistance (IR) and CRF with LVDF, but there were no studies to address the relationship between PA and LVDF. Therefore, Study 2 leveraged data from 99 adolescents with either overweight/obesity or normal-weight and high PA (≥75$^{th}$ percentile) or low PA (≤25$^{th}$ percentile) to address this question. Vigorous physical activity (VPA) was the only PA intensity associated with LVDF, with the strongest links to septal TDI. Mediation analyses revealed that higher VPA had a direct benefit on LVDF as well as an indirect effect through decreased adiposity. This study also confirmed the link of IR and CRF with LVDF, independently from adiposity. As CRF was linked with LVDF and is a good measure of general cardiovascular health, Study 3 aimed to understand the intensity and duration of PA that are best associated with CRF in a large population of adolescents (n=339). Only VPA was associated with CRF, with relationships plateauing at around 20

minutes. This short duration of daily VPA contrasts with the World Health Organization's moderate-to-vigorous PA guidelines, which can be satisfied by only undertaking moderate PA, with no apparent independent benefit on LVDF or CRF, suggesting that specific VPA guidelines for adolescent cardiovascular health are needed.

This thesis provides novel insights into the effects of obesity and PA on LVDF in adolescents. A targeted approach, not simply focused on weight loss, has been identified, in theory, to prevent or reverse this adverse early phenotype and provides a roadmap for the duration of routine VPA that might be tested in a randomised, controlled clinical trial as a means to normalise LVDF.

# Publications and presentations relevant to thesis

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**Publications**

**Burden SJ,** Dawes H, Jones A. Time to move away from moderate-to-vigorous physical activity for cardiovascular health? *Pediatrics* (Opinion piece – in preparation for submission). Chapter 6

**Burden SJ**, Weedon BD, Gunawan P, Turner A, Whaymand L, Meaney A, Dawes H and Jones A. Physical Activity and Diastolic Heart Function in Adolescents. (In preparation for submission). Chapter 5

**Burden SJ**, Weedon BD, Gunawan P, Meaney A, Dawes H and Jones A. Septal Diastolic Function in Adolescents. (In preparation for submission). Chapter 5

**Burden SJ**, Weedon BD, Turner A, Whaymand L, Meaney A, Dawes H and Jones A. Intensity and Duration of Physical Activity and Cardiorespiratory Fitness. *Pediatrics*. 2022;150(1):e2021056003. Chapter 6

**Burden SJ**, Weedon BD, Whaymand L, Rademaker J, Dawes H, Jones A. The effect of overweight/obesity on diastolic function in children and adolescents: A meta-analysis. *Clinical Obesity*. 2021;11(5):e12476. Chapter 3

**Publications associated with this thesis**

Hauser J,* **Burden SJ**,* Karunakaran A, Muthurangu V, Taylor A, Jones A. Whole-body MRI assessment of the contributions of adipose and non-adipose tissues to cardiovascular remodeling in adolescents. *J Am Heart Assoc*. 2023;12(15):e030221. *co-first author

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**Presentations**

**Burden SJ**, Weedon BD, Gunawan P, Turner A, Whaymand L, Meaney A, Dawes H and Jones A. The case for vigorous physical activity in adolescents – evidence from the OxSOCRATES Study. STEM for Britain, 6th March 2023. Chapters 5 & 6

**Burden SJ**, Weedon BD, Gunawan P, Turner A, Whaymand L, Meaney A, Dawes H and Jones A. Left Ventricular Diastolic Function in Adolescents with Overweight and Obesity – The moderating Influence of Physical Activity, Cardiorespiratory Fitness, and Metabolic Health. Department of Women & Children's Health Monthly Meeting, King's College London, 1st December 2022. Chapters 3, 5 & 6

**Burden SJ,** Weedon BD, Gunawan P, Dawes H, Jones A. Better diastolic function in adolescents is associated with vigorous physical activity, independently of lesser intensities and adiposity. European Childhood Obesity Group (ECOG) 31st Annual Conference, 4th November 2022. Chapter 5

**News coverage**

American Academy of Pediatrics: *Teens Should Exercise Vigorously 20 Minutes a Day for Heart Health* https://www.aap.org/en/news-room/news-releases/pediatrics2/2022/study-teens-should-exercise-vigorously-20-minutes-a-day-for-heart-health/ Chapter 6

CBS News interview: *Study finds teens don't need as much daily exercise as you'd think to stay healthy* https://www.cbsnews.com/miami/news/study-teens-daily-exercise-stay-healthy/ Chapter 6

Washington Post: *20 minutes of vigorous exercise daily works wonders for teens* https://www.washingtonpost.com/health/2022/06/28/teen-fitness-exercise-daily/ Chapter 6

**Podcast**

American Academy of Pediatrics: *Vision and Concussions, Physical Activity and Cardiorespiratory Fitness – Episode 123* (Podcast) https://www.aap.org/en/pages/podcast/vision-and-concussions-physical-activity-and-cardiorespiratory-fitness/ Chapter 6 (note: skip to 22 minutes)

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# Abbreviations

| | |
|---|---|
| 20mSRT | 20-meter shuttle run test |
| A-wave | late mitral inflow peak velocity |
| a’ | late diastolic myocardial peak velocity |
| BCa | bias-corrected accelerated |
| BFEN | bandpass-filtered followed by Euclidean norm |
| BIA | bioelectrical impedance analysis |
| BMI | body mass index |
| BP | blood pressure |
| $Ca^{2+}$ | calcium |
| CI | confidence interval |
| CMR | cardiovascular magnetic resonance |
| CMRFs | cardiometabolic risk factors |
| $CO_2$ | carbon dioxide |
| COVID-19 | coronavirus disease 2019 |
| CPET | cardiopulmonary exercise test |
| CRF | cardiorespiratory fitness |
| CT | computed tomography |
| CVD | cardiovascular disease |
| CWD | continuous-wave Doppler |
| DEXA | dual-energy X-ray absorptiometry |
| -dP/dt | maximal rate of pressure decline during left ventricular relaxation |
| DT | E-wave deceleration time |
| D-wave | peak diastolic pulmonary vein velocity |
| EN | Euclidean norm |
| ENMO | Euclidean norm minus one |
| ENMOz | Euclidean norm minus one with negative values rounded to zero |
| E-wave | early mitral inflow peak velocity |
| E/A | E-wave/A-wave ratio |
| E/e’ | E-wave/e’ ratio |
| e’ | early diastolic myocardial peak velocity |
| e’/a’ | early-to-late myocardial peak velocity ratio |
| FFA | free fatty acid |
| FFM | fat free mass |
| FMI | fat mass index |
| HFEN | high-pass filtered Euclidean norm |
| HFEN+ | high-pass filtered Euclidean norm plus ENMO |
| HFpEF | heart failure with preserved ejection fraction (diastolic heart failure) |
| HFrEF | heart failure with reduced ejection fraction (systolic heart failure) |
| HIIT | high-intensity interval training |
| HOMA-IR | homeostatic model assessment of insulin resistance |
| HR | heart rate |

| | |
|---|---|
| IQR | interquartile range |
| IR | insulin resistance |
| IS | insulin sensitivity |
| IVRT | isovolumic relaxation time |
| LA | left atrium |
| lat | lateral |
| LPA | light physical activity |
| LV | left ventricle |
| LVDD | left ventricular diastolic dysfunction |
| LVDF | left ventricular diastolic function |
| MetS | metabolic syndrome |
| METs | metabolic equivalents |
| MICT | moderate-intensity continuous training |
| MPA | moderate physical activity |
| MRI | magnetic resonance imaging |
| MVPA | moderate-to-vigorous physical activity |
| $Na^+$ | sodium |
| NHS | National Health Service |
| $O_2$ | oxygen |
| OBU | Oxford Brookes University |
| OCMR | Oxford Centre for Magnetic Resonance |
| OW/Ob | overweight and obesity |
| OxSOCRATES | Oxfordshire Sedentariness, Obesity & Cardiometabolic Risk in Adolescents – a Trial of Exercise in schools |
| PA | physical activity |
| PE | physical education |
| PWD | pulsed-wave Doppler |
| rLPA | residualised light physical activity |
| rMPA | residualised moderate physical activity |
| rPA | residualised physical activity |
| rST | residualised sedentary time |
| RCT | randomised controlled trial |
| RPE | rating of perceived exertion |
| RER | respiratory exchange ratio |
| RV | right ventricle |
| SAT | subcutaneous adipose tissue |
| SERCA2a | sarcoplasmic reticulum ATPase |
| SD | standard deviation |
| SEM | structural equation modelling |
| sep | septal |
| ST | sedentary time |
| STE | speckle-tracking echocardiography |
| S-wave | peak systolic pulmonary vein velocity |
| TDI | tissue Doppler imaging |

| | |
|---|---|
| UK | United Kingdom |
| USA | United States of America |
| VAT | visceral adipose tissue |
| $VO_2$peak/max | peak/maximal oxygen consumption |
| Vp | mitral-to-apical flow propagation velocity |
| VPA | vigorous physical activity |
| WHO | World Health Organization |

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# Chapter 1: Introduction

Cardiovascular disease (CVD) remains the largest contributor to global mortality despite recent declines in its impact due to reduced smoking and better healthcare.[1] That beneficial trend has now slowed considerably or even reversed in some countries, including in the United Kingdom (UK) (Figure 1.1).[2-4] Although definitions of the threshold of premature CVD mortality varies greatly (<45–75 years),[2-6] evidence of a rise in premature CVD mortality is consistent across definitions.[2-4] It has been suggested that overweight and obesity (OW/Ob) is responsible for this.[7]

OW/Ob is a known risk factor in the pathogenesis of CVD, which accounts for two-thirds of deaths in affected individuals.[8] Although a greater proportion of adults globally are OW/Ob, its prevalence is rising fastest in children.[7] In England, this adverse trend has worsened dramatically as a result of the coronavirus disease 2019 (COVID-19) pandemic (Figure 1.2),[9] with severe childhood obesity becoming twice as common in just over a decade. A dramatic increase in early Type-2 diabetes and CVD in England can now be expected as these children age,[10-14] if nothing is done to manage this problem.

Large modelling studies indicate that CVD prevention should be the primary method to achieve the United Nations goal of a global 25% reduction in premature CVD mortality.[15] This is especially important for the prevention of heart failure with preserved ejection fraction (HFpEF – diastolic heart failure). Although HFpEF is currently prevalent in ~50% of heart failure cases,[16] the prevalence is steadily increasing.[17] This is probably explained by the increasing prevalence of OW/Ob, as 84% of HFpEF patients are overweight or obese.[18] Impairments in left ventricular diastolic function (LVDF) (ability of the left ventricle [LV] to relax and fill with blood) underpin HFpEF,[19, 20] and it is well known that adult obesity is a potent risk factor.[18, 21, 22] As childhood obesity is known to track into adulthood[23-26] and is an independent risk factor for adult impairments in LVDF,[27] we can expect a marked increase

in future HFpEF burden given recent OW/Ob trends. Therefore, identification of effective primordial prevention techniques in children and adolescents with OW/Ob are urgently required.

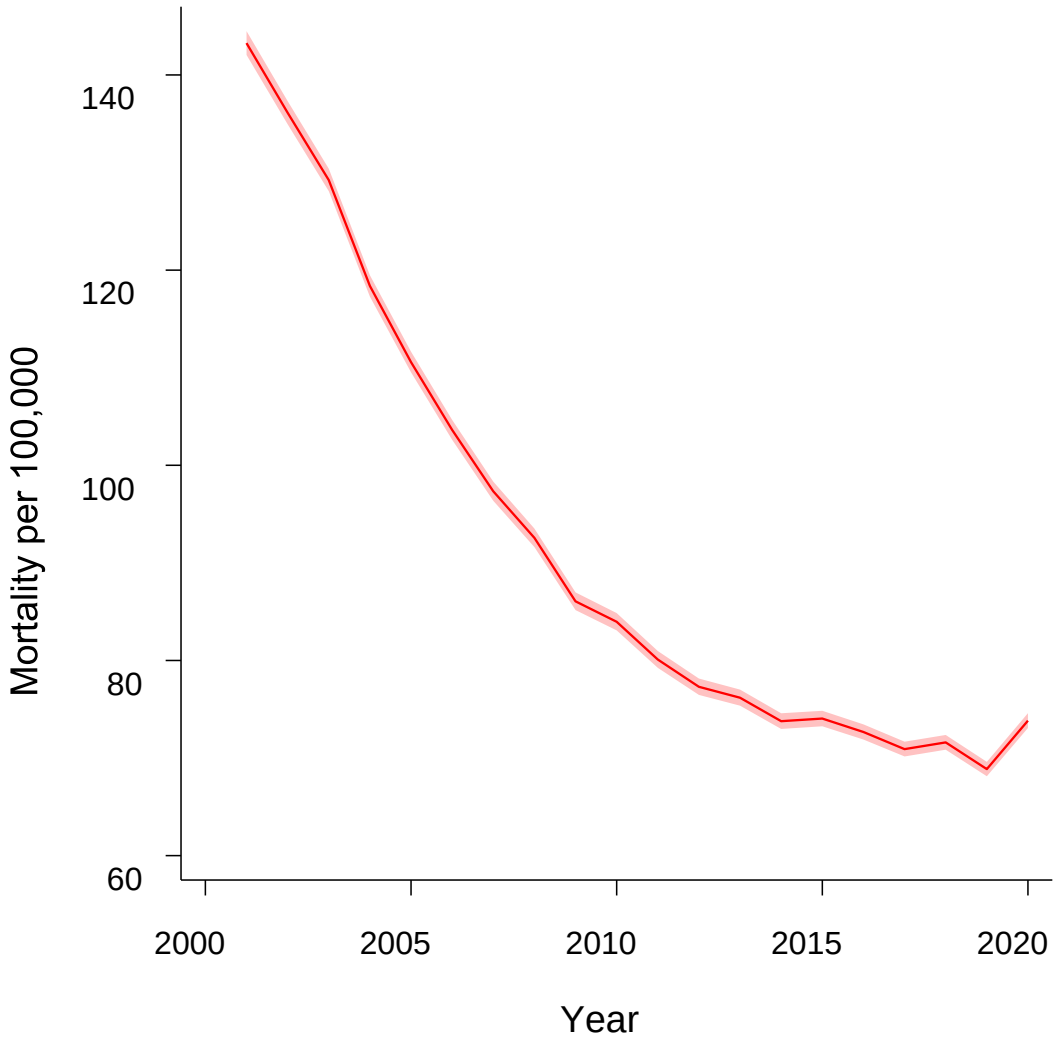


**Figure 1.1: Premature cardiovascular disease (under 75) mortality rate in England per year.** The data for this figure were obtained from Public Health England (https://fingertips.phe.org.uk/profile/public-health-outcomes-framework/data).

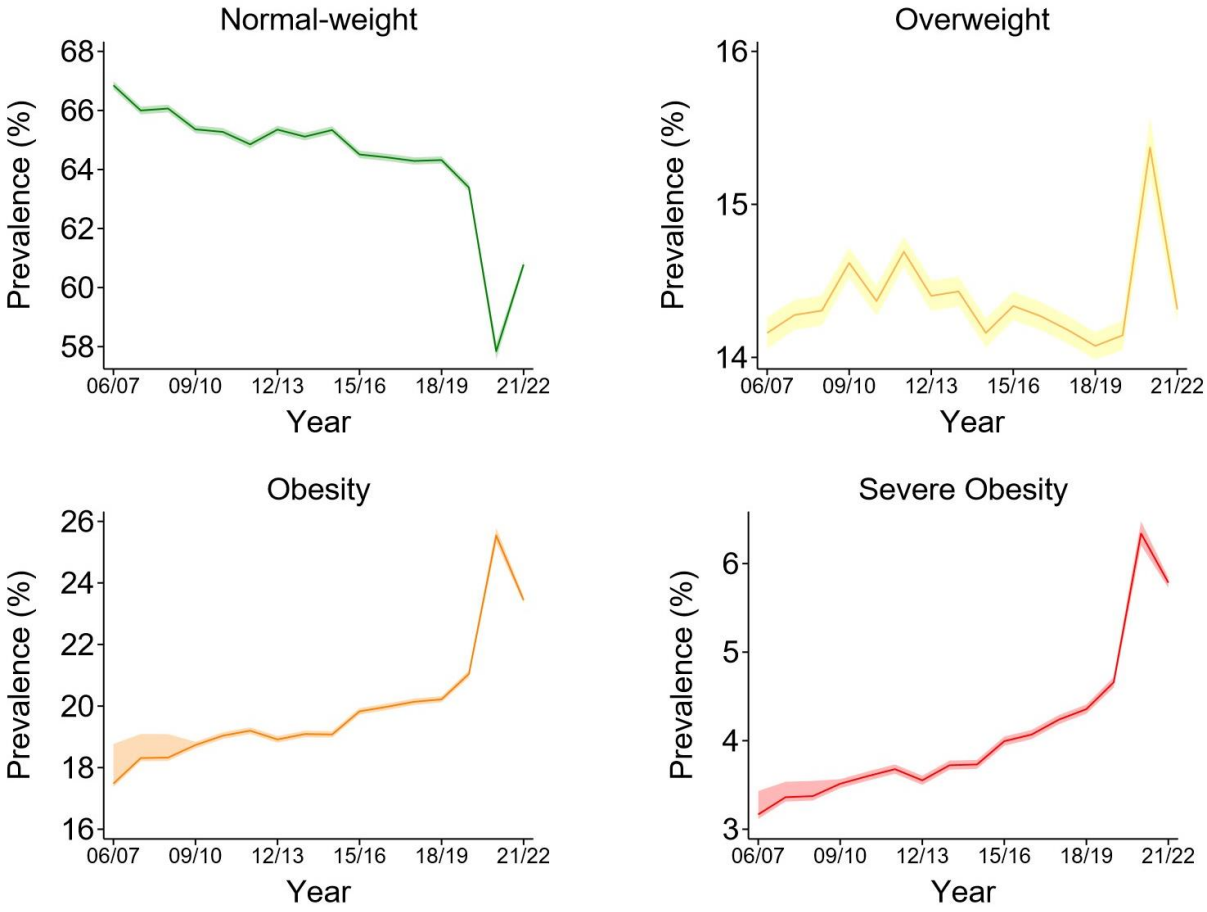


**Figure 1.2: Trends in the prevalence of normal-weight, overweight, and obesity in England between the 2006/07 and 2021/22 school years in Year 6 children.**

Data were collected as part of the National Child Measurement Programme and obtained through Public Health England (https://digital.nhs.uk/data-and-information/publications/statistical/national-child-measurement-programme/2021-22-school-year).

## 1.1 Overweight and obesity – increased adiposity

The World Health Organization (WHO) defines OW/Ob as "*abnormal or excessive fat accumulation that presents a risk to health*".[28] Increased calorie intake above that required for energy expenditure promotes adiposity. Dietary carbohydrates are the principal source of such calories. Both liver and skeletal muscle cells can store glucose in the form of glycogen, but only the liver can break it down again to provide glucose for systemic circulation. After eating, digested carbohydrates (mainly glucose) circulate systemically and are rapidly metabolised by the liver. Once glycogen stores are replete, excess carbohydrate is converted to fatty acids and are stored primarily in adipose tissue as triglycerides. There are different adipose tissue compartments for this storage, which include subcutaneous (SAT – adipose tissue beneath the skin) and visceral (VAT – adipose tissue surrounding internal organs), in non-adipose tissues such as muscle and liver, and in ectopic fat silos such as epicardial fat or perivascular fat. The increased lipid accumulation in fat cells leads to adipocyte hypertrophy by an increase in cell volume, but with no change in the total number of adipocytes.[29] However, adipose tissue lipid storage is limited. With increasing levels of adiposity, surplus lipids accumulate within non-adipose tissue, known as lipid spillover/lipotoxicity.[30] This is thought to be a result of leptin resistance with increasing levels of adiposity, leading to unoxidised fatty acids and lipid derivatives accumulating within internal organs, reducing function and ultimately resulting in organ dysfunction.[30] The consequences of this divergent fat storage for health are now being recognised. Cardiac steatosis, for example, is a hallmark of OW/Ob and Type-2 diabetes and is linked with LV dysfunction.[31-33]

It is now understood that metabolic diseases are not limited to people with obesity, but are also evident in those with normal-weight, whilst some people with OW/Ob do not present as metabolically unhealthy.[34-36] In those with metabolic disease, irrespective of obesity status, there is excess storage of lipids within VAT,[37] which is seen as "pathological adipose tissue". This is important because whilst greater VAT and SAT volumes are both associated with worse LVDF in independent analyses,[33, 38, 39]

the association with SAT is not demonstrable when VAT is accounted for in the same model and does not predict future HFpEF and CVD morbidity and mortality, suggesting VAT is most important.[39-41] Although VAT is more likely to be found in those with OW/Ob, some people store more fat as VAT, suggesting a highly individualised approach to fat storage. Why this is the case is not fully understood, but genetics, prenatal programming, and lifestyle behaviours are important and interlinked.[42-49] Certain combinations of genes can predispose some people to obesity (polygenic obesity) with family, twin, and adoption studies calculating the heritability of obesity to be between 40-70%.[48, 49] Prenatal programming can also alter the offspring genetic architecture, whereby maternal overnutrition in pregnancy can result in an upregulation of lipogenic and adipogenic genes in VAT, coupled with an increase in SAT in early postnatal life, as well as a predisposition to maintain this increased body fat because of developmental changes in the appetite regulatory network.[44] It is also known that lifestyle factors can also contribute to obesity. For example, children and adolescents with OW/Ob who are more vigorously active and/or have higher cardiorespiratory fitness have less VAT than less vigorously active/fit counterparts.[46]

Although VAT is the fat silo that presents the greatest risk to health, diagnosis of OW/Ob is currently based on body mass index (BMI), a measure of adiposity that cannot distinguish between SAT and VAT and is a poor predictor of metabolic disease,[50] as discussed in Section 2.2.

### 1.1.1 Traditional definition of excess adiposity

In adults (≥18 years-old), a BMI (weight/height$^2$ [kilograms [kg]/meters [m]$^2$]) of ≥25 kg/m$^2$ and ≥30 kg/m$^2$ defines overweight and obesity, respectively.[51] Due to the confounding effect of age and sex on BMI in children and adolescents, WHO defines child and adolescent OW/Ob using age- and sex-adjusted BMI z-scores, based on a reconstruction of the 1977 National Center for Health Statistics and WHO reference median.[52] For children and adolescent aged 5-19 years-old, overweight is defined as a BMI z-score of >1 and of >2 for obesity.[52] Other health organisations such as the International Obesity Task Force use similar definitions based on different sets of reference data.[53, 54] These have

been used to estimate the global prevalence of OW/Ob in children and adolescents, but there has been no international consensus over which definition or reference dataset to use.

### 1.1.2 Prevalence of increased adiposity

#### 1.1.2.1 *Globally*

From 1980 to 2013, OW/Ob increased by 47.1% in children and by 27.5% in adults.[7, 55] In 2013, 23.8% of boys and 22.6% of girls were classified as OW/Ob.[7] Those reaching the threshold for obesity have increased from 0.7% to 5.6% in girls and from 0.9% to 7.8% in boys, as well as an age-standardised, global increase in BMI of 0.32 kg/m$^2$ (girls) and 0.40 kg/m$^2$ (boys) per decade between 1975 and 2016.[55]

Although there are genes associated with obesity,[56, 57] the obesity epidemic cannot be explained by rapid changes in human genetics, as population wide changes in human genetics occur over many thousands of years.[58] Instead, lifestyle alterations such as over-eating and a lack of physical activity (PA) leading to excess calorie intake above what is required for energy expenditure, probably explain these trends. However, some people with "obese genes" could be more susceptible to obesity when exposed to lifestyle changes.[47, 59-61]

#### 1.1.2.2 *England*

Sedentary lifestyle and OW/Ob are more common in higher-income countries such as England.[7, 55] The recent adverse trends in overweight, obesity, and severe obesity shown in Figure 1.2 result from annual measures of height and weight in over 1 million children in the 10-11 year-old (Year-6) cohort by Public Health England.[9] The adverse impact of the COVID-19 pandemic is obvious. In the 2020/21 school year, ~41% of all Year-6 children had OW/Ob with 25.5% classified as obese.[9] The increase in prevalence of OW/Ob has been driven by an increase in obesity prevalence, whereas there has been no significant change in overweight prevalence before the COVID-19 pandemic.[9] Although obesity is seen as being more detrimental to health than overweight due to the higher proportion of adipose

tissue, overweight still possesses a greater risk to health when compared to individuals with normal-weight, highlighted by the dose-response relationship between the degree of adiposity and health outcomes,[62] and hence why it is important to highlight that the proportion of children with overweight *or* obesity is currently >40%.[9] Although the National Childhood Measurement Programme provides valuable insights into the OW/Ob prevalence in England, this only reflects 10-11 year-old children and therefore does not entirely reflect the population in this thesis. To the best of my knowledge, there is no nationwide dataset for adolescents. However, these findings are reflected by recent trends in other high-income countries, such as the United States of America (USA),[63] highlighting the impact of altered lifestyle behaviours on obesity.[64]

#### 1.1.2.3 *Long-term trends*

Large prospective longitudinal studies show a correlation between childhood and adulthood obesity.[23-26] Children with obesity are over five-times more likely (relative risk 5.21) to be obese in adulthood, while roughly 80% of adolescents with obesity continue to be obese in early adulthood and about 70% are still obese after 30 years-of-age.[23] It is estimated that over half (57.3%, 95% confidence interval [CI]: 55.2, 60.0) of the children living in the USA in 2017 will be obese at 35 years of age.[65] Thus, we can expect to see an increase in the prevalence of adult obesity as this cohort matures.[23-26] This will increase the burden on healthcare providers with major parallel economic costs.

### 1.1.3 Economic burden of increased adiposity

The direct cost of OW/Ob to the National Health Service (NHS) in the UK was £5.1 billion in 2006/07, increasing from £3.2 billion in 1992/93.[66] In 2014/15, Public Health England estimated that OW/Ob cost the NHS £6.1 billion with an indirect cost of £27 billion to wider society. By 2050, these figures are projected to reach £9.7 billion and £49.9 billion, respectively.[67] In the USA, children with obesity are estimated to account for an extra $14 billion per year in excess direct medical costs over their lifetimes.[68] Most of the healthcare burden from OW/Ob comes indirectly as a result of the diseases that OW/Ob promotes, such as CVD. The diagnostic, monitoring, and management options for CVD is

particularly costly. As CVD is the most dominant non-communicable disease worldwide,[8] understanding and identifying ways to prevent CVD through OW/Ob is of public health importance.

## 1.2 Obesity and cardiovascular disease

In 2015, over 52% of all disability-adjusted life years and over 65% of all deaths due to CVD were seen in adults with OW/Ob.[8] CVD commonly results in heart failure, which can be categorised into HFpEF or heart failure with reduced ejection fraction (HFrEF – systolic heart failure). However, HFpEF is the dominant type of heart failure in OW/Ob, with 84% of HFpEF patients having OW/Ob.[18] In a large longitudinal study of 51,451 adults, those with overweight (BMI 25 to <30 kg/m$^2$), obesity class I (BMI 30 to <35 kg/m$^2$), or obesity class II–III (BMI ≥35 kg/m$^2$) were at greater risk for HFpEF (hazard ratios: 1.38 [1.18–1.61], 1.56 [1.30–1.87], 2.72 [2.24–3.32], respectively) than HFrEF, with only the obesity class II-III group classified as having increased risk (hazard ratios: 1.03 [0.87–1.22], 1.20 [0.98–1.46], 1.49 [1.18–1.89], respectively).[69]

CVD and HFpEF traditionally present in middle- or old-age, but the pathophysiology underpinning such disease begins in childhood.[40, 70] Atherosclerosis and coronary artery disease form a major part of CVD, which begin when macrophages and foam cells deposit cholesterol esters in vessel walls, increasing the thickness of the arterial intima. Although present in almost all young adults, OW/Ob and its associated risk factors, such as inflammation and insulin resistance (IR), can further accelerate this process.[40] Furthermore, in autopsies of children and young adults, the degree of OW/Ob and its associated downstream cardiometabolic risk factors, such as hyperglycaemia, dyslipidaemia, and high blood pressure (BP), have been linked with the degree of atherosclerosis,[71] highlighting that CVD is not solely an adult disease.

Excess adipose tissue in early life also results in cardiac remodelling and changes in cardiac function.[72-75] This can be a result of direct effects on the myocardium and vasculature, and indirect effects through comorbidities associated with OW/Ob.[76] For example, OW/Ob activates the renin-angiotensin-aldosterone system and other sympathetic nervous systems that lead to an increase in BP, which is a

strong determinant of cardiac function.[77] Post-mortem studies in adults identified that individuals with obesity have an increased heart mass, LV dilation, right ventricle (RV) dilation, and LV and RV hypertrophy.[78-80] This cardiac remodelling has since been termed *obesity-related cardiomyopathy*, which is described as an increase in myocardial thickness, myocardial mass, ventricular dilation, and reductions in cardiac function; a result of chronic volume overload and metabolic abnormalities.[81, 82] Early signs of this, especially alterations in cardiac structure, are documented in children and adolescents with obesity,[72-75] but there is less of a consensus regarding LVDF. This is important as impairments in LVDF in young adulthood are linked with increased future morbidity and mortality,[83] highlighting the need to better understand its link in childhood.

## 1.3 Left ventricular diastolic function (LVDF)

### 1.3.1 Definitions and terminology

The LV cardiac cycle is split into two key steps: systole, the contraction of the LV, causing ejection of blood; and diastole, the relaxation of the LV with consequent filling. Diastole completes in four stages: 1) isovolumic relaxation; 2) rapid filling; 3) diastasis; and 4) left atrium (LA) contraction (Figure 1.3). LVDF describes the process of how the LV relaxes and fills with blood during these stages. Numerous physiological parameters influence LVDF, which is determined by a combination of active (energy-utilising) and passive forces.[20, 84] A description of some of the common measures of LVDF and what they represent, depicted in Figure 1.3, is provided in Table 1.1.

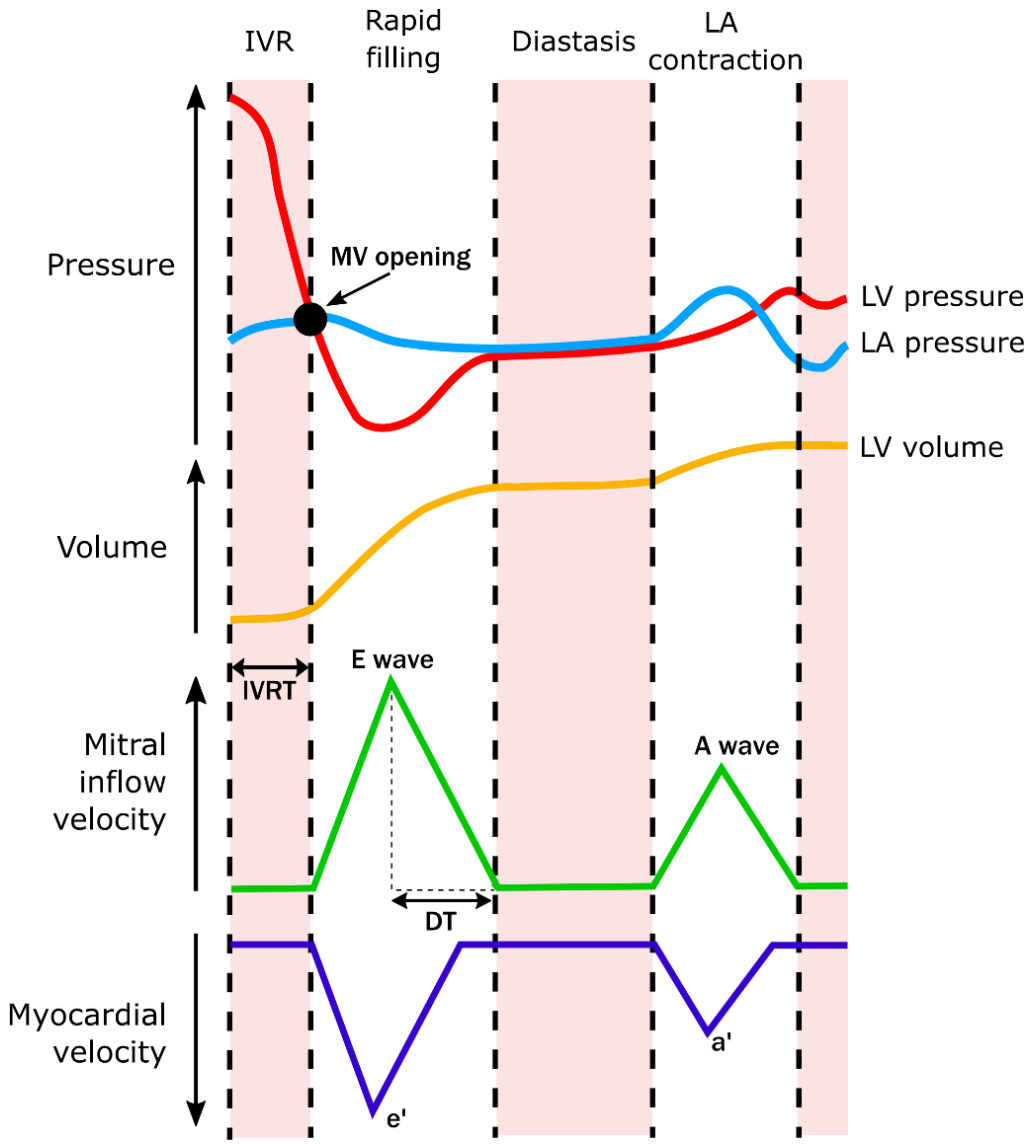


**Figure 1.3 Stages of diastole and echocardiography measures of diastolic function.**

Stage 1 - isovolumic relaxation (IVR), which occurs after aortic valve closure and before mitral valve (MV) opening. Left ventricle (LV) pressure falls rapidly until it reaches left atrial pressure, prompting MV opening; Stage 2 - rapid filling, where the MV is open and blood is suctioned towards the apex of the LV from the left atrium (LA), which occurs as the myocardium lengthens during falling LV pressure; Stage 3 - diastasis, after initial filling where LA and LV pressures equalize and flow ceases; Stage 4 - LA contraction, which generates an additional pressure gradient that drives more blood into the LV. A-wave indicates late mitral inflow peak velocity; a', late diastolic tissue peak velocity; DT, E-wave deceleration time; E-wave, early mitral inflow peak velocity; e', early diastolic tissue peak velocity; IVRT, isovolumic relaxation time.

**Table 1.1:** Typical echocardiography measures of LVDF

| LVDF Measure | Definition | Echocardiography Modality | Description |
|---|---|---|---|
| E-wave | Early mitral inflow peak velocity | PWD | Peak velocity of blood flowing between the LA and LV during early/rapid filling. Considered to reflect the relative driving force across the mitral valve (LA-LV pressure difference) during early diastole. Influenced by LA pressure, LV pressure at the end of isovolumic relaxation, and rate of LV relaxation. With impaired relaxation (grade 1 LVDD), peak E-wave velocities are reduced. |
| A-wave | Late mitral inflow peak velocity | PWD | Peak velocity of blood flow between the LA and LV during late/active filling. Considered to reflect the relative driving force across the mitral valve (LA-LV pressure difference) during late diastole. Influenced by LV compliance and LA systolic function. With impaired relaxation, peak A-wave velocities are increased. |
| E/A ratio | E-wave/A-wave ratio | PWD | Used to identify filling patterns of the LV. Acts as a marker of global LVDF. With impaired relaxation, E/A ratios are reduced. |
| DT | E-wave deceleration time | PWD | The interval between the peak E-wave velocity and the intersection of the deceleration of flow with the baseline. Used to identify filling patterns of the LV. DT is influenced by LV relaxation, LV pressure at the end of isovolumic relaxation, and LV stiffness. With impaired relaxation, DT is prolonged. |
| IVRT | Isovolumic relaxation time | CWD, PWD and TDI | Time between aortic valve closure and mitral valve opening. Considered to reflect early LV relaxation. Can be influenced by LAP and heart rate. With impaired relaxation, IVRT is prolonged. |
| Vp | Mitral-to-apical flow propagation velocity | Colour flow Doppler | Velocity of blood flow from base to apex of the LV. In a healthy individual, the early filling wave propagates rapidly towards the apex of the LV. Influenced by a number of factors including driving pressure, inertial forces, viscous friction, chamber geometry, systolic function, and contractile dyssynchrony. With impaired relaxation, Vp can be prolonged. |
| e’ | Early diastolic tissue peak velocity | TDI | Peak velocity of longitudinal myocardial motion during early diastole. Considered a measure of LV relaxation. Influenced primarily by LV relaxation but also by preload, LV systolic function, and LV pressure at the end of isovolumic relaxation. With impaired relaxation, peak e’ velocity is reduced. |
| a’ | Late diastolic tissue peak velocity | TDI | Peak velocity of longitudinal myocardial motion during late diastole. Influenced by LA systolic function and LV end-diastolic pressure. With impaired relaxation, a’ is increased. |
| e’/a’ ratio | e’/a’ ratio | TDI | Used to identify patterns of myocardial motion. With impaired relaxation, e’/a’ is reduced. |
| E/e’ ratio | E-wave/e’ ratio | PWD & TDI | e’ velocity is used to correct for the effect of LV relaxation on peak E-wave velocity and can be used to estimate LV filling pressures. With impaired relaxation, E/e’ is increased. |

| | | | |
|---|---|---|---|
| S wave | Peak systolic pulmonary vein velocity | PWD | Peak velocity of blood flow between the pulmonary veins and LA during LA diastole. Used to help understand LV filling pressures. Influenced by changes in LAP, LA contractility, LV contractility, right ventricle contractility. |
| D wave | Peak diastolic pulmonary vein velocity | PWD | Peak velocity of blood flow between the pulmonary veins and LA during LV diastole. Used to help understand LV filling pressures. Influenced by early LV diastolic filling and compliance. |
| S/D ratio | S wave/D wave ratio | PWD | Used to identify LA filling patterns and help understand LV filling pressures. A decrease in LA compliance and increase in LAP is associated with a decrease in S wave velocity and an increase in D wave velocity. This measure is not often used in children due to the reversal of S and D waves that are commonly seen in adulthood. |

Information for this table was adapted from Nagueh *et al* 2009[85] and Nagueh *et al* 2016.[19] CWD indicates continuous wave Doppler; LA, left atrium; LAP, left atrial pressure; LV, left ventricle; LVDD, left ventricular diastolic dysfunction; LVDF, left ventricular diastolic function; PWD, pulsed wave Doppler; TDI, tissue Doppler imaging.

### 1.3.2 Active relaxation

Active relaxation describes the process by which calcium ions ($Ca^{2+}$) are actively removed from the cytosol and taken up into the sarcoplasmic reticulum. The removal of $Ca^{2+}$ enables the detachment of actin-myosin cross-bridges and results in the decline of cellular tension and relaxation of the sarcomere units. This is an energy-dependent process that is regulated by enzymes such as sarcoplasmic reticulum ATPase (SERCA2a), the sodium ($Na^{+}$)/$Ca^{2+}$ exchanger, and phospholamban.[20, 86] *In vitro* experiments comparing tissue from normal hearts and those from patients with heart failure suggest that reduced expression/activity of SERCA2a and resultant high cytosolic $Ca^{2+}$ concentrations could be a contributing factor to LV diastolic dysfunction (LVDD) (an abnormal elevation in diastolic filling pressures to obtain adequate stroke volume at rest or during exercise).[19, 84, 87] However, in a rat model of LVDD resulting from chronic press-overload and hypertrophic LV remodelling, passive stiffening of the LV with fibrosis was found to be a contributor to LVDD, independent of active relaxation. Indeed, there was evidence of increased SERCA2a activity with improved $Ca^{2+}$ handling, compared to normal rats,[86] suggesting a role for enhanced active relaxation in compensating for other pathological processes in some forms of LVDD. It may be that these processes contribute at different stages of advancement towards irreversible diastolic heart failure or differentially according to the cause of the LVDD.

Active myocardial relaxation is more sensitive to disruptions in energy availability than myocardial contraction.[88, 89] In adults without identifiable cardiovascular risk factors, obesity is linked with a reduced phosphocreatine/adenosine-triphosphate ratio, a marker of energy availability in the myocardium, and is associated with LVDD.[33, 90] Although individuals with obesity have reduced phosphocreatine/adenosine-triphosphate ratios, the obese heart maintains delivery of adenosine-triphosphate to the myocardium through increased creatine-kinase shuttle activity, but fails to maintain this delivery with stress.[90] This can be improved with appropriate weight loss and is

accompanied by an improvement in LVDF.[90] This has not been studied in children with OW/Ob, which is warranted as it may be important in the early pathogenesis of LVDD.

In addition to active processes, there are also passive processes that aid the expansion of the LV.

### 1.3.3 Passive relaxation

Passive myocardial relaxation has been extensively researched and is primarily determined by myocardial stiffness, wall thickness, and chamber geometry.[91] In a population of middle-aged adults, LV hypertrophy was associated with a ~26% greater increase in transmural stiffness compared to controls, assessed by pressure-volume relationships determined with cardiac catheterisation, three-dimensional echocardiography, and manipulation of LV preload.[92] The increased stiffness associated with LV concentric hypertrophic remodelling in obesity is linked with reduced LVDF, independently of myocardial energetics and steatosis and unconfounded by diabetes and hypertension.[33] Geometric LV remodelling that might be expected to increase passive stiffness of the LV is demonstrable in children and adolescents with OW/Ob.[72-75]

Multiple pathophysiological mechanisms have been linked with passive relaxation and LVDD, but changes in titin stiffness and structural changes to collagen (fibrosis) are key elements.[91]

Titin, a molecular spring, helps to expand the LV after systole.[20, 93] It has two isoforms that are expressed in the heart; N2BA is longer and more compliant and N2B is shorter and stiffer.[20, 93] Titin stiffness is regulated by posttranslational modifications such as phosphorylation.[93] Hypophosphorylation of the N2B isoform can lead to increased stiffness of cardiomyocytes,[94, 95] contributing to LVDD aetiology.[96]

The extracellular matrix protein, collagen, also plays a significant role in LV relaxation. During systole, compression of collagen filaments stores energy, which is released in diastole to aid LV expansion.[97] However, increased collagen deposition, mainly collagen I, increased collagen cross-link formation, and glycation lead to a fibrotic state, stiffening the LV and impairing LVDF.[98]

Together, both active and passive relaxation contribute to LV relaxation, generating a negative pressure gradient ("suction"), passively filling the LV. Impairments in these processes can ultimately lead to the development of LVDD.

## 1.4 Left ventricular diastolic dysfunction (LVDD)

LVDD is defined as an abnormal elevation in diastolic filling pressures to obtain adequate stroke volume at rest or during exercise.[19, 84] This is a result of impaired relaxation, increased myocardial stiffness, and reduced diastolic suction.[19, 20, 84] The presence and extent of these pathological features is used to describe the grade of LVDD, which begins with impaired relaxation and decreased ventricular 'suction' in early filling (grade 1), advancing to increased LV stiffness and elevated LA pressure (grade 2), then high LA pressure and a non-complaint LV (grade 3), before becoming irreversible (grade 4). In later grades, LA size increases markedly and symptoms of HFpEF appear.[19]

Although not necessarily representative of younger individuals, adults with LVDD aged 51-82 years-old have 3.53 times greater relative risk of major adverse cardiovascular events and/or mortality, compared to age-matched controls.[99] Younger adults in the CARDIA study, aged 23-25 years at the time of echocardiography, with abnormal LVDF (defined in this study by an early-to-late mitral inflow peak velocity ratio [E/A ratio] < 1.3 and one marker of abnormal cardiac morphology) were two-times more likely to have a clinical CVD event over 20 years of follow-up, adjusting for multiple confounders.[83] Although it is unlikely that adolescents with OW/Ob will develop overt LVDD, they may have impaired LVDF, which could be a result of many modifiable and non-modifiable risk factors.

Lifestyle behaviours are the main drivers of modifiable risk, including the development of obesity and low levels of PA. Individuals with high CVD risk typically have multiple modifiable risk factors and it is likely that multiple such factors are involved in the development of LVDD.[81]

## 1.5 Adiposity and LVDF

Studies in adults have shown the link between obesity (based on BMI), LVDF,[21, 22] and HFpEF.[18] In the 30-year follow-up of children from the Cardiovascular Risk in Young Finns Study, increased adiposity in childhood was associated with worse LVDF in adulthood, independent of other childhood and adult risk factors.[27] However, there is no clear consensus in children and adolescents, with previous reviews failing to conclude whether OW/Ob is linked with reduced LVDF.[72-74] Although adolescents with obesity will likely present with worse cardiovascular health than child equivalents, most likely because of a greater number of years lived with obesity, it is of importance to also study children as cardiovascular remodelling, whether physiological or pathophysiological, has been identified in the first two years of life.[70] Studying the earliest pathology would also help identify the measures of LVDF that first indicate reduced function. As there have been more recent articles published, an up-to-date review on this subject is needed.

In adults, the degree of adiposity is linked with LVDF.[33, 38, 39] The increased volume overload (preload) seen with increasing levels of obesity, without a concomitant decrease in peripheral resistance (afterload),[100] is likely to be a dominant factor in LVDD. With greater preload, myocardial fibres are stretched to accommodate the greater blood volume, which leads to increased contractile force (myocardial work) being used to eject it, as described by the Frank-Starling law.[101] When the heart is subjected to abnormal loading conditions (increased pressure or volume overload), it undergoes a remodelling process to compensate for the increased workload by alterations in the arrangement of sarcomere units.[102] Volume overload triggers sarcomere series replication, whereby new sarcomere units are added in a linear fashion, resulting in the lengthening of cardiomyocytes to accommodate more blood and normalise wall stress.[102] Additionally, in response to a greater afterload (increased arterial blood pressure), which is commonly seen in obesity,[103] new sarcomeres are added in parallel to increase cardiomyocyte cross-sectional area. By adding sarcomeres in parallel, the cardiomyocyte fibres become thicker, which generates greater force and contractility to overcome the increased

afterload.[102] However, this comes at a price, as increased myocardial thickness results in greater LV stiffness.[92] Indeed, animal studies have shown that to ensure normal LV systolic function and to prevent stretch-induced myocardial damage in response to pressure overload, there was a resultant increase in LV stiffness, resulting in a left-upward shift in the end-diastolic pressure–volume relationship, indicating reduced LVDF.[104]

A number of factors may promote cardiac hypertrophy in OW/Ob, but excessive stretch of cardiomyocytes has been demonstrated to be a potent activator of the expression of genes that cause hypertrophy, independent of neuronal or humoral factors.[105] Humoral stimuli can also result in pathological LV hypertrophy.[106] For example, lipotoxicity associated with excess adiposity leads to IR and hyperinsulinaemia, triggering hepatic angiotensin II synthesis, as well as the activation of insulin-like growth factor-1 receptors, stimulating LV hypertrophy.[106-109] Obesity is also associated with systemic inflammation, and inflammatory markers have been shown to directly induce LV hypertrophy and myocardial fibrosis.[106, 110] There is a linear relationship between adiposity and LV hypertrophy, independent of hypertension status[111] that is linked with increased passive stiffness[92] and reduced LVDF.[33]

Increased adiposity is also linked with reduced myocardial energetics and myocardial steatosis.[33] An increase in free fatty acids (FFAs) in the circulation of people with obesity lead to greater cardiomyocyte FFA uptake and a subsequent shift to FFA metabolism,[112] which reduces adenosine-triphosphate yield and mitochondrial efficiency.[33, 81] These disruptions in cardiomyocyte energy availability in obesity are linked with impaired LVDF.[33] Additionally, over-spill of lipids into the myocardium results in the storage of lipids as triacylglycerol,[33] but this storage is limited, resulting in the creation of toxic reactive lipid species.[112] These toxic lipid species can lead to mitochondrial and cellular dysfunction, cardiac fibrosis, cardiomyocyte apoptosis, and ultimately LVDD.[33, 81, 112]

### 1.5.1 Visceral vs. subcutaneous adipose tissue in LVDF

LVDF is linked to specific patterns of adiposity, with some types of adipose tissue having a more dominant effect on outcome. Although greater VAT and SAT volumes are both associated with worse LVDF in independent analyses,[33, 38, 39] the association with SAT is not demonstrable when VAT is accounted for in the same model and does not predict future HFpEF, suggesting VAT may be more important in the pathogenesis of LVDD.[39, 41] This could be due to VAT having a greater influence on cardiometabolic risk factors (CMRFs) than SAT,[113-115] as well as being linked with myocardial steatosis, energetics, and LV hypertrophy, all of which are independently linked with worse adult LVDF.[33]

Another possible mechanism is that adipocytes secrete numerous adipokines that regulate an array of CMRFs, including inflammation, fibrosis, insulin sensitivity (IS), and BP.[116, 117] These adipokines can become dysregulated in obesity[117, 118] and can contribute to the development of atherosclerosis, IR, dyslipidaemia, and hypertension, amongst others conditions, in both adults and children.[12, 117, 119] Localised exposure of the myocardium to dysregulated adipokines and the resulting pathophysiology of poor cardiometabolic health likely contribute to the development of LVDD.

## 1.6 Cardiometabolic risk factors and LVDF

Poor metabolic health is most commonly diagnosed using the metabolic syndrome (MetS), which was first described to understand the pathological effects of IR and hyperinsulinaemia.[120] Although multiple definitions exist, MetS focusses on five key criteria: abdominal obesity, hypertriglyceridaemia, reduced high-density lipoprotein cholesterol, hypertension, and elevated fasting hyperglycaemia. Patients are diagnosed with MetS when any three of these criteria are met. However, there has not been a standardised definition of MetS in children and adolescents due to difficulties defining the individual components.[121] This has led to many groups using different definitions of MetS as well as other similar summary measures to identify poor metabolic health.[121]

Although not completely protected from cardiometabolic complications in comparison to metabolically-healthy normal-weight individuals, metabolically healthy adults with obesity are less likely (hazard ratio 1.1) to suffer from a myocardial infarction compared to metabolically unhealthy normal-weight (hazard ratio 1.9) and metabolically unhealthy adults with obesity (hazard ratio 2.0).[34] These findings are supported by meta-analyses[35] and large longitudinal studies[36] that show poor metabolic health results in higher risk of cardiovascular complications, irrespective of weight class, although the risk increases with greater BMI.

In support of the aforementioned CVD outcome studies, higher levels of CMRFs are associated with reduced LVDF in adults.[122, 123] CMRFs impair LVDF by impairments in active relaxation through mitochondrial and cellular dysfunction and impair passive relaxation through cardiomyocyte apoptosis, cardiac fibrosis, and alterations in titin properties.[33, 81, 95, 112, 124-127] Evidence suggests that this is caused by factors such as increased cardiomyocyte FFA metabolism and myocardial steatosis (section 1.5).[33, 81, 128-130]

As discussed in Section 1.5, increased arterial blood pressure (afterload) results in parallel sarcomere replication, increasing the thickness of the LV, generating greater force and contractility to overcome the increased afterload.[102] However, this comes at the consequence of increased LV stiffness, limiting LVDF.[92, 104] A recent systematic review and meta-analysis identified that paediatric hypertension is associated with worse LVDF.[131] Other meta-analyses found that children and adolescents with OW/Ob have higher systolic (4.5-7.5 mmHg) and diastolic (2.6-4.1 mmHg) resting BP and higher systolic (11.6 mmHg) and diastolic (6.1 mmHg) ambulatory BP compared to normal-weight counterparts,[132] suggesting that elevated BP in adolescent OW/Ob could be an important mechanism in any impaired LVDF. However, some studies using 24-hour ambulatory BP monitoring have found that there are no differences in LVDF in adolescents with hypertension and obesity compared to obese normotensive counterparts,[133, 134] suggesting that other obesity related mechanisms could also be responsible.[33] Although more research is clearly required to understand any independent effect of high BP in

adolescent OW/Ob, current evidence suggests that if sustained into adulthood, hypertension can lead to myocardial fibrosis and inflammation, further worsening LVDF and potentially resulting in LVDD and HFpEF.[135]

Peripheral and cardiomyocyte IR is thought to play an important role in FFA metabolism and myocardial steatosis.[136] Under normal conditions, insulin is an important hormone that regulates the uptake of glucose into cardiomyocytes by the glucose transporter type 4.[137] Glucose uptake is typically impaired in obesity, and excess adiposity, especially visceral adiposity, is linked with IR.[138, 139] At the cardiomyocyte level, IR can cause myocardial dysfunction by: impairing glucose transportation into the cell; impairing intracellular signalling kinase pathways; impairing $Ca^{2+}$ handling; reducing metabolic flexibility by shifting substrate metabolism towards FFA metabolism; impairing mitochondrial function leading to reduced adenosine-triphosphate yield; and causing endothelial dysfunction by impaired generation of nitric oxide or endothelial nitric oxide synthase, resulting in hypoxia and inhibition of angiogenesis, all of which can ultimately lead to fibrosis and myocardial cell death,[127-130, 136, 137] likely contributing to the aetiology of LVDD.

The impact of CMRFs on LVDF is also independent of the degree of adiposity assessed by BMI.[122, 123] One possible explanation for this could be that BMI explains less of the variance of VAT (56.3%) than it does of SAT (88.9%) in children,[140] which could be important given that VAT has a greater influence on CMRFs.[113-115] This is supported by a dose response relationship between the amount of VAT and the odds for having MetS, irrespective of BMI weight class.[37] For example, normal-weight adults in the highest quartile of VAT are 9.4-times (95% CI: 4.2, 20.7) more likely to have MetS compared to those in the lowest quartile.[37] Another possible explanation is that factors other than adiposity, such as genetics and PA, can also influence VAT and CMRFs.[42-45, 141-143] For example, IR is linked with LVDF in adolescents, independently of OW/Ob status.[144] Therefore, investigation into whether there is an independent relationship between CMRFs and LVDF in children and adolescents is warranted, as impaired LVDF may not be restricted to those with OW/Ob.

Numerous childhood cross-sectional studies have examined whether CMRFs are linked with LVDF, but there is a lack of longitudinal studies, Mendelian randomisation studies, and randomised clinical trials (RCTs) to assess possible causality. The number of cross-sectional studies in young people could be investigated by pooling results, confirming whether CMRFs are related to impaired LVDF.

Although CMRFs may be adversely linked with child and adolescent LVDF, this could be counterbalanced by regular PA and/or high levels of cardiorespiratory fitness (CRF). It is well known that regular PA and high levels of CRF are protective of CMRFs[145-154] and so investigation as to whether these are linked with LVDF is also warranted.

## 1.7 Physical activity, cardiorespiratory fitness and LVDF

Although commonly used interchangeably, PA, exercise, and CRF are separate entities (Table 1.2).[155-158]

The modern-day environment does little to promote PA and encourages sedentary behaviour in children and adolescents. In a global study of 1.6 million children aged 11-17 years, 81% did not meet the 2010 WHO guidelines of at least 60-minutes of moderate-to-vigorous PA (MVPA) per day. In the UK, it was estimated that 74.7% of boys and 80.5% of girls did not meet these guidelines.[159] This is supported by data from the International Children's Accelerometry Database, which show that 70% of UK 10-18 years-olds do not meet the guidelines.[160]

The health benefits of PA on metabolic health,[149-154] CRF[161-164] and cardiovascular morbidity and mortality[165, 166] are well known. These include favourable metabolic risk scores (MetS) and improvements in CRF through functional adaptations such as increased myocardial contractility, increased blood volume, angiogenesis, and greater cardiac output.[161-164]

**Table 1.2:** Fitness related definitions

| Term | Definition |
|---|---|
| **Cardiorespiratory fitness** | The capacity of the cardiovascular and respiratory systems to supply oxygen to the mitochondria within skeletal muscles cells during physical activity.[157, 158] Maximal oxygen consumption ($VO_2$max) is the current gold-standard to assess cardiorespiratory fitness. $VO_2$max is determined by stroke volume, heart rate, and arterial and venous oxygen content and is, therefore, influenced by cardiac, respiratory, cardiovascular, and cellular function.[157, 158] |
| **Exercise** | A type of physical activity consisting of planned, structured, and repetitive bodily movement done to improve and/or maintain one or more components of physical fitness.[158] |
| **Physical activity** | Any bodily movement produced by the contraction of skeletal muscles that results in a substantial increase in caloric requirements over resting energy expenditure.[156] Different levels of energy expenditure are used to classify physical activity into vigorous, moderate, and light intensities and information on the frequency and duration of these intensities can be measured.[167-173] Energy expenditure >1.5 metabolic equivalents (METs) is typically used to define PA, with different intensities defined as: light (>1.5 & ≤3.0 METs); moderate (>3.0 & ≤6 METs); and vigorous (>6.0 METs).[173] |
| **Sedentary behaviour** | Any waking behaviour characterized by an energy expenditure ≤1.5 metabolic equivalents, while in a sitting, reclining or lying posture.[155] |

Having high CRF, independent of other risk factors such as obesity, can protect against cardiometabolic complications and CVD outcomes,[145-148] while exercise training can reduce LV myocardial stiffness.[174] Higher subjective PA levels in childhood are associated with better LVDF in adulthood, independent of child and adult adiposity and BP.[27] However, there has been little research in healthy adults or adolescents on the link between objectively measured PA and LVDF. A small number of studies have examined whether exercise and/or diet programmes can improve LVDF in adolescents with OW/Ob, which warrant further investigation by pooling the results.

Increasing PA may protect against adverse CVD outcomes by counter-balancing the caloric surplus that promotes obesity and/or by improving metabolic health. Insufficient PA in adolescents may, therefore, increase numbers with OW/Ob, predisposing a larger population to CMRFs and potentially an increased risk of LVDD and premature CVD. The already high level of adolescents not meeting the PA guidelines has worsened dramatically because of the COVID-19 pandemic. A recent, global meta-analysis of studies comparing PA levels pre- and post-COVID-19 restrictions has reported that the duration of PA in children and adolescents has declined by an additional 20% (95% CI: 4-34%) for total PA and by 28% (95% CI: 13-41%) for MVPA.[64] Given this and the accelerated increase in the prevalence of childhood OW/Ob in recent years (Figure 1.2), understanding the link between OW/Ob, CMRFs, PA, and CRF with LVDF in adolescents is more important than ever to prevent future LVDD and HFpEF.

## 1.8 Thesis hypotheses and objectives

While evidence that OW/Ob promotes development of LVDD and HFpEF in adults has been demonstrated repeatedly, little evidence exists for children and adolescents. Public health measures designed to tackle the problem of adolescent OW/Ob have focused on lifestyle alterations such as exercise promotion and dietary improvements, with uncertain impact.[175, 176] In particular, the effects of these preventative measures on LVDF have not been demonstrated. Further investigation to address this and to establish possible targets to prevent impaired LVDF in adolescent OW/Ob is important for preventing future LVDD. Therefore, the following hypotheses and objectives for this thesis are as follows:

It is predicted that OW/Ob is related to LVDF in adolescents, while CMRFs, PA, and CRF are independently linked. More specifically, it is predicted that:

1. OW/Ob is associated with worse LVDF in adolescents;
2. More PA might have a direct effect on LVDF or it may indirectly improve LVDF by decreasing adiposity;

3. Poor cardiometabolic health and low CRF are linked with worse LVDF in adolescents, independently from OW/Ob.

Therefore, in order to investigate these hypotheses, this thesis aims to:

1. Statistically synthesise existing data in children and adolescents to determine;
    a. The extent to which OW/Ob is associated with various measures of LVDF;
    b. Which measures of LVDF best detect the adverse effects of OW/Ob;
    c. The associations of CMRFs with LVDF measures;
    d. The association of PA and CRF with LVDF measures;

    and

    e. The extent to which exercise and lifestyle modification have been shown to improve LVDF.
2. Determine the existence and extent of relationship between adiposity measures and LVDF in a population of adolescents with OW/Ob as compared to those with normal-weight;
3. Establish the existence and extent of relationship of different PA intensities, CRF and cardiometabolic health with LVDF in adolescents, independent of OW/Ob;
4. Identify possible ways to prevent the development of impaired LVDF through PA;
5. Discuss and propose future work to further explore the pathogenesis and prevention of impaired LVDF in children and adolescents from the findings of this thesis.

# Chapter 2: Methodological background

This chapter provides an in-depth discussion of the methods used to typically measure LVDF, adiposity, PA and CRF to provide sufficient background for this thesis. It starts by discussing the gold-standard method to assess LVDF and then moves on to echocardiography, which is the most widely used and accepted method for non-invasively measuring LVDF. This will include an overview of how Doppler ultrasound works and the typical measures of LVDF. After that, a discussion on methods to assess adiposity is provided, focussing on the methods typically used to define OW/Ob, discussing their limitations, and the alternative, more accurate methods to assess adiposity. Finally, a discussion on the methods used to typically assess PA and CRF is given.

## 2.1 Measuring LVDF

### 2.1.1 Invasive assessment of LVDF

The gold-standard to assess LVDF is by the measurement of the time-constant of LV relaxation, tau (τ), by the insertion of a high-fidelity catheter into the LV.[20, 177] Using this method, Weiss *et al*[177] first identified that LV pressure (P) can be fitted to the following function:

$$P = e^{-\frac{t}{\tau + B}}$$

where t is the time from the maximal rate of pressure decline during LV relaxation (-dP/dt) and B is a constant. This formula can be rearranged in terms of τ to give:[177, 178]

$$\tau = P \div -\frac{dP}{dt}$$

Both P and -dP/dt are measured using a high-fidelity catheter to determine τ (speed of LV relaxation). Longer durations of τ (slowed relaxation) are used to identify LVDD, which is diagnosed when τ is >48 ms.[179] Although direct measures of τ are the gold-standard to assess LVDF, this method is rarely used due to the invasive nature of cardiac catheterisation. Therefore, non-invasive measures of LVDF have been developed.

### 2.1.2 Non-invasive assessment of LVDF

#### 2.1.2.1 *Echocardiography*

Echocardiography is the most widely used and accepted method to assess LVDF.[19, 20] The Doppler shift phenomenon (an apparent change in the frequency of a sound wave upon reflection from a moving target) underpins the assessment of LVDF by echocardiography. Doppler transducers emit an ultrasound beam of a known frequency that is reflected after contact with tissues, such as blood or muscle, and subsequently received by the transducer. An increase in the frequency of the reflected beam indicates movement of tissue towards the transducer and *vice versa* for a decrease in frequency.[180] The Doppler shift degree is proportional to the velocity of the tissue, with a greater or lesser Doppler shift indicating increased or decreased velocity, respectively.[180] This information is processed and presented to the assessor as images or in wave-forms to determine direction, velocity, and magnitude of blood flow or myocardial movement.[180]

Different modalities of Doppler ultrasound are used to assess LVDF, including pulsed- and continuous-wave Doppler (PWD and CWD, respectively), tissue Doppler imaging (TDI), colour flow Doppler, and colour tissue Doppler. PWD and CWD measure the high frequency, low amplitude signals from blood flow, whereas TDI measures the low velocity, high amplitude signals of myocardial motion. A description of the LVDF measures obtained from these modalities is given in Table 1.1.

***Pulsed-wave Doppler***

In PWD, the assessor places the cursor (sample volume) at a specified depth on the 2D ultrasound image. Short bursts, at predetermined intervals, of ultrasound are emitted from the transducer to the sample volume, allowing for Doppler shifts to be recorded at specific locations (depth acuity).[180] Measurements obtained by PWD include: early mitral inflow peak velocity (E-wave); late mitral inflow peak velocity (A-wave); E-wave/A-wave (E/A ratio); E-wave deceleration time (DT); peak systolic pulmonary vein velocity (S-wave); peak diastolic pulmonary vein velocity (D-wave); and S-wave/D-wave (S/D ratio) (Table 1.1).

Colour flow imaging is a form of PWD that can be used to assess intracardiac flow. Each pixel within the image acts as its own sample volume and displays the Doppler shift as a coloured dot on the image, with different colours and intensities representing directionality and velocity of blood flow.[180] In the measurement of LVDF, colour flow is used to optimally align the sample volume with blood flow but can also be used to assess mitral-to-apical flow propagation velocity (Vp) (Table 1.1). Due to the intermittent sampling of PWD and susceptibility for aliasing with high-velocity signals, PWD is limited mainly to low-velocity signals (e.g. blood flow).[180]

***Continuous-wave Doppler***

Unlike PWD, CWD simultaneously emits and receives a continuous ultrasound beam along a path specified by the assessor. CWD can be used to assess isovolumic relaxation time (IVRT) (Table 1.1). Due to the continuous nature of CWD, CWD can accurately assess high velocity blood flow but lacks spatial localisation.[180]

***Tissue Doppler imaging***

TDI utilises the same fundamentals of traditional Doppler imaging but is adjusted by removing the high-pass filter and reducing the gain amplification, meaning that the low-frequency Doppler signals from myocardial motion are not eliminated and are more evidenced than the low-amplitude blood-

flow signals. As longitudinal myocardial fibres are most parallel to the ultrasound beam when in an apical view, TDI is used to measure longitudinal myocardial relaxation velocities.[181] LVDF measures that are obtained with TDI include: early diastolic tissue peak velocity (e'); late diastolic tissue peak velocity (a'); e'/a' ratio; and is included in the calculation of E/e' ratios (Table 1.1). TDI is useful for the assessment of LVDF as peak e' velocities are associated with τ in both animal[182-184] and human studies.[185-187]

Both spectral and colour can be used to measure myocardial longitudinal velocities. Spectral TDI measures peak Doppler shifts at a sample volume specified by the assessor, whilst colour TDI measures mean velocities within each pixel and displays the Doppler shift as a coloured dot. Colour TDI measures assess mean regional velocities and are therefore lower than peak spectral TDI velocities.[188] Therefore, colour TDI may be better suited in the assessment of myocardial relaxation homogeneity, whereas spectral TDI may be better suited for precise velocity quantification.

***Strain-rate imaging***

Assessment of LVDF is currently driven by Doppler-based measures. However, speckle-tracking echocardiography (STE) to measure strain and strain-rate is a newer modality that may add to the assessment of LVDF. STE assesses the deformation of the LV during diastole as an analogue for LV relaxation.[19, 20, 189] Myocardial strain refers to the deformation of the myocardium, whereas myocardial strain-rate refers to the rate at which the deformation occurs, both of which take place in three different planes; circumferential, radial, and longitudinal. After acquisition of cine loops, STE uses an offline algorithm that tracks speckle artefacts throughout the cardiac cycle that are naturally generated when using echocardiography.[189] Detection of the spatial movement of these speckles allows for the calculation of strain and strain-rate. As TDI only provides longitudinal myocardial velocity, STE provides a more comprehensive analysis of LV relaxation, providing deformation in each of the three planes, as well as globally. As STE tracks speckles, it can differentiate between true relaxation and passive motion,[189] which is not possible for TDI even though the LV apex remains

relatively stable during the cardiac cycle. Furthermore, some indices of strain-rate may perform better than typical TDI measures.[20]

#### 2.1.2.2 *Limitations to echocardiography*

There are some important limitations that should be considered when assessing LVDF by echocardiography in children and adolescents. Due to the invasive nature of directly measuring τ, there are no studies in healthy children, adolescents, or adults to assess the sensitivity and specificity of echocardiography for measuring LV stiffness. In adults with known CVD, e' has been shown to be negatively correlated with τ (r≈0.5-0.6)[185, 190] and the E/e' ratio to be positively related with LV filling pressures (r≈0.6-0.9).[186, 191] In a study of 38 children/adolescents with either LV noncompaction, dilated, restrictive, or hypertrophic cardiomyopathy (conditions typically associated with LVDD), peak septal e' velocity was the only LVDF measure associated with elevated LV filling pressures (r=-0.38, p=0.02).[192] However, this study was limited by echocardiography being performed up to three months after cardiac catheterisation, during which time LVDF profiles could have changed. There was also no control group, although it is not reasonable to perform cardiac catheterisation in healthy children. In another study with similar participants, a peak septal e' velocity of ~11 cm/s seemed best able to discriminate between cardiomyopathy (n=116) and control (n=50) groups (see Figure 3B in Dragulescu *et al*).[193] Thus, due to the lack of sensitivity and specificity data, it is difficult to ascertain whether echocardiography measures of LVDF in children/adolescents are truly reflective of early LV stiffening, but some studies suggest that septal TDI is linked.

Although there are recommendations to measure and diagnose LVDD in adults,[19] there are no accepted recommendations for children and adolescents. This makes it difficult for clinicians and researchers to implement LVDF measures into practice or research. Furthermore, although there have been efforts to establish a paediatric nomogram for LVDF, these are limited to specific populations.[194, 195] This has led to many studies using their own reference populations and criteria to diagnose LVDD, limiting comparability between studies.[193, 195]

Physiologically, many LVDF measures are influenced by LV loading conditions, although some measures may be less influenced than others.[185, 191, 196] The volume overload associated with obesity may, therefore, reduce the ability of some LVDF measures to detect early impairments in LVDF.[185]

Incorrect alignment of the ultrasound beam can also influence results because Doppler ultrasound measures the vector of motion that is parallel with the ultrasound beam. However, this can be minimised by ensuring minimal angulation (<20°) between the ultrasound beam and blood flow/cardiac motion.[19, 85]

Some of the errors associated with echocardiography may be mitigated by other non-invasive techniques such as cardiac magnetic resonance (CMR).

#### 2.1.2.3 *Cardiac magnetic resonance*

Although CMR is the gold-standard to assess cardiac structure, it is used less frequently than echocardiography to assess LVDF. CMR measures of LVDF include mitral flow velocities, myocardial velocities, and strain and strain-rate, which correlate well with their corresponding echocardiography measures.[197, 198] CMR is an attractive supplement to echocardiography as the limitations with echocardiography, such as angulation of the ultrasound beam, do not impact CMR. However, CMR is less frequently used due to cost and lower versatility compared with echocardiography,[197] with only one study, to the best of my knowledge, reporting CMR LVDF in children and adolescents with obesity.[199]

To summarise, echocardiography is the most frequently used and accepted modality for assessment of LVDF. The gold-standard assessment of LVDF, τ, is very rarely used in children and adolescents due to the invasive nature of cardiac catheterisation. Therefore, almost all studies that have assessed LVDF in this population have used echocardiography. However, it remains unclear which measure(s) of echocardiography may be best suited to identify early impairments in LVDF due to the lack of

paediatric guidelines and normative data. This disparity extends to the measurement of adiposity, with a number of different methods used to quantify adiposity, as discussed below.

## 2.2 Measuring adiposity

### 2.2.1 Anthropometrics

The most commonly used anthropometric method to assess total body adiposity is BMI. As BMI is easily measured, it is the method of choice to initially screen and classify normal-weight, overweight, and obesity in adults.[51] However, although BMI has a good specificity for detecting obesity, its sensitivity is poor (36-50%).[50, 200]

In children and adolescents, normal BMI varies by age and sex, meaning that BMI z-scores (or percentiles) based on national/global normative data adjusted for age and sex are needed to define weight classes.[52-54, 201] However, much like BMI in adults, BMI z-score is a relatively poor measure of adiposity in children and adolescents.[202-205] Neither metric is able to differentiate between lean mass and adipose mass and divergent quantities of VAT and SAT may result in similar overall mass but very different metabolic consequences (Figure 2.1). For example, some individuals have more than usual amounts of muscle, which weighs more than fat, and low adiposity but still be classified as overweight or obese using BMI. BMI can also overestimate and underestimate adiposity, depending on ethnicity, in children and adolescents.[206-209] The lack of standardisation in the reporting of BMI z-scores has meant that there is large heterogeneity in their calculation, limiting comparison between studies, as highlighted later in Chapter 3. These limitations have led to the development of other easily obtained anthropometric measures to assess adiposity in children and adolescents, although BMI and BMI z-score are still the most widely reported measures.

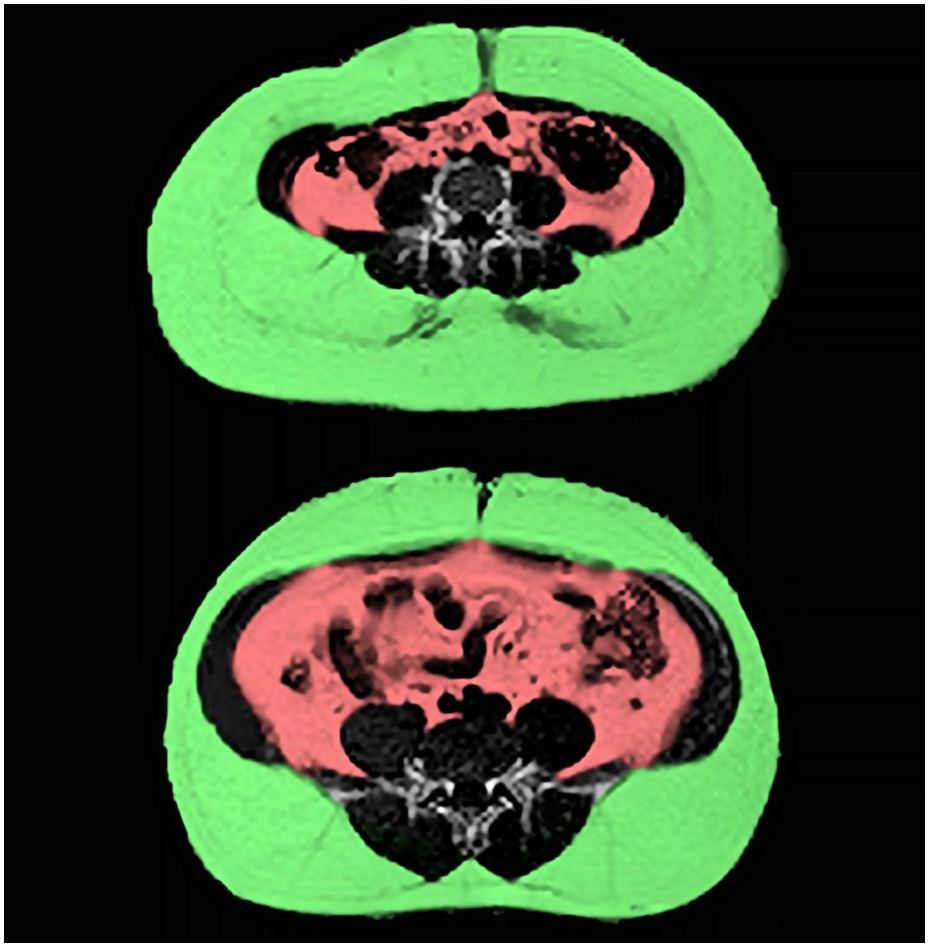

**Figure 2.1: Divergent visceral (red) and subcutaneous (green) adipose tissue in two subjects with the same body mass index.**

Reproduced with permission from Dr Alexander Jones.

Measures of waist circumference and the waist-hip ratio are an alternative method to assess adiposity. Abdominal fat, or "*android*" obesity, was first investigated by Jean Vague in 1947[210] and has since been shown to be more closely related with cardiometabolic health complications than "*gynoid*" obesity (lower-body adiposity).[50] The premise for waist circumference and the waist-hip ratio assumes that greater abdominal fat levels result in a greater waist circumference and smaller waist-hip ratios. This has since been confirmed with high sensitivity and specificity to identify trunk fat mass in both adults and children/adolescents.[211, 212] Furthermore, waist circumference and the waist-hip ratio are often more strongly associated with CVD outcomes than BMI,[213, 214] including LVDF measures in children and adolescents.[215]

Although waist circumference and waist-hip ratio measures are inexpensive and simply obtained, they have not been readily adopted into clinical practice. This could be due to the heterogeneity in the anatomical location at which measurements have been obtained, with eight different locations reported for waist circumference alone.[216] Lack of standardisation may push clinicians and researchers to lean towards more simplistic measurements of adiposity such as BMI.

Other simplistic anthropometric measurements can be obtained to assess adiposity. These include, but are not limited to, waist-height ratio, thigh and calf circumferences, waist-thigh ratio, neck circumference, sagittal abdominal diameter, and various skin-fold thicknesses.[50] However, the clinical utility of these measurements is unclear given that there is no clear advantage for their use over waist circumference and BMI.[50] An important limitation of anthropometric measures is that they do not adequately capture disease risk when compared to imaging techniques of fat distribution[217] and do not accurately predict whole-body composition.[218] However, as BMI z-scores are the most commonly reported metric in the literature and are used to define OW/Ob in adolescents, BMI z-score was used as the primary measure of adiposity in this thesis.

### 2.2.2 Bioelectric impedance analysis

Bioelectric impedance analysis (BIA) is a useful method to quantify adiposity when more detail than anthropometric measures is required and imaging methods are not available. BIA follows the principle that tissues absorb electricity differently based on their water and electrolyte content.[50] A low-amplitude, alternating electrical current is applied to the body to measure impedance between electrodes at the extremities of the body (typically hands and feet), which can be used to estimate measures of total body fat. This can be further refined by using a multisegmental BIA approach to estimate regional body fat (e.g. trunk, arms, and legs). In children and adolescents, BIA performs well when compared with more comprehensive analyses of body fat,[219-224] but underestimates total body fat percentage compared to reference standards.[218] BIA machines are also portable, relatively easy to use, and inexpensive when compared to imaging methods.

To determine total adiposity by BIA, a metric of total body fat percentage is commonly calculated by dividing measures of fat mass by total body weight. However, dividing by weight may be inadequate for deriving total adiposity.[225, 226] Wells provides a clear explanation of the limitations of assessing adiposity by percentage body fat stating: "*[percentage fat] ignores between subject variation in FFM. Individuals will differ in percentage fat either if they have identical FFM but divergent fat-mass, or if they have identical fat mass but divergent fat-free mass. Percentage fat, like BMI, therefore contains information about two divergent aspects of body composition and cannot distinguish effectively between them [and] risk[s] allocating individuals to obese or non-obese status partly on the basis of their relative lean deposition.*"[226] Instead, it has been proposed that fat mass should be divided by height squared,[225, 226] similar to BMI, providing a metric of the relative fat proportion of BMI, independent of body size. As BMI and BMI z-scores do not perform well at discriminating between those with and without excess adiposity,[202-205] the fat mass index (FMI) from BIA was also used in this thesis to assess adiposity.

BIA has some important limitations. Although providing more information than anthropometric measures, BIA cannot differentiate between SAT and VAT, which are dissimilarly associated with cardiometabolic health and CVD outcomes.[113-115] In children and adolescents, BIA can under or overestimate adiposity measures.[218, 227] A systematic review comparing BIA with reference standards found BIA had high levels of bias, wide limits-of-agreement, and inferior diagnostic test accuracy.[218] Attention must also be paid to the choice of equation/algorithm used to estimate adiposity from BIA. Many companies do not disclose their algorithms, meaning that inter-machine differences can limit comparison between studies and limit proper validation with reference methods. Furthermore, some of the algorithms were developed using adult populations and so may be less accurate in adolescents.[218, 227, 228] However, when used alongside BMI, BIA offers additional information to inform the findings with this thesis.

### 2.2.3 Imaging

Imaging methods such as dual-energy X-ray absorptiometry (DEXA), computed tomography (CT), and magnetic resonance imaging (MRI), have been described to accurately assess body composition,[50, 218] providing detailed information on specific fat depots, which are associated with CVD risk and outcomes.[229]

#### 2.2.3.1 *Magnetic resonance imaging*

In the research setting, MRI is seen as the "gold-standard" to assess body composition by obtaining single-slice or whole-body images by applying various magnetic fields and assessing the rate at which protons return to their equilibrium state.[50, 230] First investigated by Dixon,[231] modern MRI sequences are able to generate water- and fat-only images that can be used to assess specific fat-depots and whole-body composition.[232] Dixon MRI provides an accurate assessment of adiposity as it enables true volumetric three-dimensional imaging, which is not possible by DEXA.[232] Each MRI image volume element (voxel) is representative of the amount of fat within the voxel, relative to pure fat. Thus,

summing the voxel values from a whole-body scan provides a metric of total body fat. This can also be segmented into specific fat deposits, such as SAT and VAT, or organ-specific fat.

Although there have been some studies to quantify whole-body fat from Dixon MRI in adults,[232-234] there have been none in adolescents.[218] This is primarily because MRI has been limited to a small number of two-dimensional slices due to a lack of efficient tools to segment three-dimensional images.[232] However, this is becoming more accessible with the development of computer-based algorithms,[232-234] including an article I co-authored,[235] reducing the time and effort needed to manually segment anatomical regions. Furthermore, as MRI uses magnets, there is no radiation exposure, making it suitable for longitudinal studies or repeated measurements.

There are some limitations to MRI. Although single-slice metrics correlate well with whole-body MRI,[236, 237] single-slice imaging may not be as sensitive to differences in adiposity as multi-slice or whole-body imaging is, following weight loss.[238-240] The main limitation to adiposity assessed by MRI is that it is primarily used in the research and not the clinical setting due to cost, need for sophisticated machinery and trained personnel, and sophisticated data processing techniques.

#### 2.2.3.2 *Computed tomography*

CT is another imaging method that is used as a reference standard in the assessment of adiposity. Sliced images of the body are produced by multiple X-ray images to determine the volume or surface area of specific tissues. Measurements from a number view angles can be reconstructed to generate two- or three-dimensional maps of pixels to characterise tissue and fat deposits. The main limitation of CT is the radiation dose administered. Although this is small, this is more of a concern for children as they have more remaining years of life for which a CT-induced cancer could develop as well as being inherently more radiosensitive than adults.[241] To minimise this risk, as well reducing cost, CT scans typically assess adiposity by a single-slice image of the abdomen between the L4-L5 intervertebral space,[50] which correlate well with single-slice MRI metrics.[242] However, there is no consensus on the

exact methodology to obtain single-slice images by CT, reducing comparability between studies.[50] This method also does not provide a true assessment of whole-body fat given the nature of single-slice CT.

#### 2.2.3.3 *Dual-energy X-ray absorptiometry*

DEXA is an attractive option to analyse adiposity due to the relative lower cost compared to MRI and CT as well requiring much less radiation than CT scans.[50, 243] DEXA uses a dose of radiation equivalent to one day at sea level. It uses two energies, which are differentially attenuated by various tissues, in order to differentiate between fat, muscle, bone, and other tissues.[50] Because of its ease-of-use and relatively low cost compared to other imaging techniques, DEXA has been used extensively in the clinical and research setting.[50]

Although DEXA has been shown to correlate well with CT and MRI measures of adiposity in children and adults, studies suggest that DEXA may over- or underestimate adiposity in people with a high or low body fat percentage, respectively.[50, 240, 243] Direct volumetric, compartmental measurements are also not possible with DEXA, as only a two-dimensional image is produced. This means that DEXA derived volumes are estimates, indirectly obtained from anatomical models.[232] Studies have also indicated that DEXA cannot accurately assess longitudinal VAT changes in adults and children.[240, 244]

## 2.3 Measuring physical activity

PA is commonly measured and categorised into four key domains; type (mode of PA), duration (length of time spent in PA), frequency (how often PA is performed), and intensity (energy expenditure during PA). Guidelines for children and adolescents focus primarily on the daily duration of MVPA.[173]

PA intensities have traditionally been defined on the basis of basal and resting metabolic rate (BMR and RMR, respectively). BMR can be referred to as the energy required to maintain "*metabolic activities of cells and tissues and the energy needed to maintain blood circulation, respiration, and gastrointestinal and renal function*".[245] RMR is defined as an oxygen consumption ($VO_2$) of 3.5 mL/kg/min and is 10-20% higher than BMR due to energy expenditure for processes other than vital

functions.[246] Metabolic equivalents (METs) are widely used to express energy expenditure, with RMR (3.5 mL/kg/min) being used to define 1 MET.[246] Energy expenditure >1.5 METs is used to define PA in adults, with different intensities defined as: light (>1.5 & ≤3.0 METs); moderate (>3.0 & ≤6 METs); and vigorous (>6.0 METs).[173] Sedentary behaviour is defined as ≤1.5 METs.[173] Given that 1 MET was defined using an adult male, conventional METs cannot readily be applied to children of both sexes as RMR in children can range from 1.2-1.7 METs.[170-172] However, current values for child METs are also imperfect, as studies typically estimate RMR through the prediction equations by Schofield instead of directly measuring RMR.[167, 168]

Although imperfect, once definitions of intensity have been set, PA can be measured in a number of different ways, which can be categorised into objective and subjective measures.

### 2.3.1 Objectively measured PA

#### 2.3.1.1 *Energy expenditure*

Direct, objective measures of PA using calorimetry are considered the gold-standard method for assessing energy expenditure. Calorimeters measure the heat loss from a participant, and since kcal's are thermal units, these can easily be converted into energy expenditure units. The use of direct calorimeters are restricted to lab-use only as they are large, require good ventilation, and if exercise is to be involved, need to be big enough to contain exercise equipment as well. As calorimeters are financially burdensome and require highly qualified staff to operate, indirect calorimetry is instead considered as the reference method for assessing energy expenditure.[247, 248]

Indirect calorimetry measures energy expenditure by assessing ventilatory volumes as well as the amount of oxygen ($O_2$) consumed and carbon dioxide ($CO_2$) exhaled. Indirect calorimetry commonly employs an open-circuit system whereby a participant inspires room air, or gases of known concentrations, and the expired $O_2$ and $CO_2$ are measured, allowing for the calculation of energy expenditure.[247, 248] This method is mainly limited to laboratory-use, but some portable systems have been developed.

Laboratory-based restrictions have led to the development of the doubly-labelled water (DLW) method.[247, 248] The principle of the DLW method is the difference in the elimination rates of two stable isotopes (oxygen-18 [$^{18}O$] and deuterium [$^{2}H$]) from the body. After ingesting water labelled with the isotopes ($^{2}H_2{}^{18}O$), $^{2}H$ and $^{18}O$ are eliminated from the body over a certain time period and the difference in the elimination rates between these isotopes is used to indicate $CO_2$ production.[247, 248] When this is combined with RMR and the thermic effect of food, equations can be used to calculate energy expenditure over a set period of time.[247, 248] The DLW method is not burdensome on the participant and allows for the continuation of normal PA, but it is expensive and does not provide any specific details on the four domains of PA.

#### 2.3.1.2 *Wearable devices*

Wearable devices have aimed to provide scalable signals of activity and rest that overcome some of the limitations in the above methods and include heart rate (HR) monitoring, pedometers, and accelerometers.

The assessment of HR is based on the assumption that PA leads to an increase in the oxygen demand of active muscle cells, increasing HR to supply oxygenated blood to active tissue. Normally, under controlled conditions, there is a linear relationship between HR and energy expenditure during MVPA, although relationships at lower intensities are weaker where other variables affect HR and energy expenditure differently.[247-252] When performing steady-state exercise, the error rate for HR has been reported to be as low as 3%, although this can vary during non-steady-state exercise and in different populations.[253] HR monitoring also has the advantage that it can capture non-ambulatory PA such as cycling and weight lifting, which are limited when using other methods such as accelerometery. Therefore, as HR monitors are relatively non-invasive and inexpensive, many researchers use this method to assess PA and energy expenditure.[247, 248 254] However, HR monitoring has some important limitations. HR is known to be influenced by sympathetic nerve activity, which can increase HR due to factors such as temperature, emotional state, and caffeine consumption. Other factors such as skeletal

muscle mass, CRF levels, sex, and age can also influence HR during exercise.[247, 248] HR is also not necessarily indicative of when PA is being performed, with factors such as an increase in HR prior to exercise (anticipatory response), HR lag once PA has started, and different HR recovery times following the cessation of PA.[247, 249, 253, 255, 256] Therefore, HR monitoring may not be the best method to measure the sporadic nature of PA in children.[257]

Another type of wearable device to assess energy expenditure and PA is the pedometer. Early forms of pedometers were designed to be worn on the hip to count the number of heel-strikes during ambulation, but more recent pedometers utilise algorithmic assessment of signals from microelectromechanical systems to identify steps.[258] It has been determined that 80% of pedometers had excellent test-retest reliability and an accuracy of >95%, which increased with increased walking speed.[258] Furthermore, to meet the WHO PA guidelines, it has been suggested that children and adolescents should perform >9000 steps per day.[259] However, although this method of PA is easily understood by the general population and is relatively low cost, there are some important limitations to pedometry. The principle limitation is that pedometers cannot measure non-ambulatory activities such as cycling or weight lifting.[256] They are also inaccurate when determining the intensity of PA, with a step being recorded irrespective of whether walking, running, or stair climbing, for example, were involved.[256, 258] However, step rate has been proposed as a method to determine PA intensity, with values of 100 steps/min and 130 steps/min corresponding to MPA and VPA, respectively, in adults.[260] Pedometer derived steps also frequently rely on proprietary algorithms, which may not be applicable to all populations, especially children.[261]

Accelerometers are also commonly used and provide more information about PA than pedometers. Accelerometers are small, lightweight, and can be worn in a number of locations (wrist, ankle, hip, leg, and centre of mass) to provide a measure of acceleration during movement.[247, 248] Accelerations are typically measured in three planes (triaxial: vertical, mediolateral, and anterior-posterior) that are commonly transformed into a single vector magnitude, or Euclidean Norm (EN).[262] Data can be

summed into epochs and different thresholds and methodologies are applied to classify sedentary behaviour and intensities of PA.[168, 169, 262-266] Classification of the thresholds between intensities is based on energy expenditure assessed by indirect calorimetry during laboratory-based exercises in a small group of participants whilst simultaneously wearing an accelerometer.[168, 169, 262, 267] Once PA intensities have been defined, information on the frequency and duration spent at each intensity can be derived. Unlike, HR monitors, accelerometers are able to capture the sporadic nature of PA in children, which is important at higher intensities of PA as this typically occurs in very short bouts.[257] However, similar to pedometers, accelerometers are unable to accurately measure PA during non-ambulatory activity, and amplitudes may vary between individuals, which may, therefore, underestimate or introduce variability into PA metrics. Another limitation is the heterogeneity of methods used to collect and analyse accelerometry data, making it very difficult to compare studies,[262, 263, 266] but attempts have been made to standardise this.[268] Insufficient wear time also limits the accurate determination of habitual PA,[269] but wearing the accelerometer on the wrist has been shown to increase wear compliance in children.[270]

Accelerometers have proven popular in research due to their low-cost, low-burden on participants, versatility, and their ability to provide accurate PA data in children and adolescents.[247, 248, 267] Thus, large, population-based studies have begun to use accelerometers to assess PA.[271] Furthermore, accelerometers have been integrated into multi-sensor systems to tackle some of the limitations of wearable devices,[258] including the CamNtech Actiheart activity monitor, which provides measures of both HR and triaxial accelerometery to better understand energy expenditure.[272]

Whether it is accelerometery or multi-sensor systems, both have the potential to provide a more detailed and accurate assessment of PA than subjective, retrospective recall of PA.[273]

### 2.3.2 Subjectively measured PA

Subjectively assessed PA involves self-reporting PA by either questionnaires and/or diaries/log-books.[247, 248] These have been used in epidemiological and cohort studies to assess the average PA

levels of large populations,[159, 274] including our finding of physical inactivity being associated with COVID-19 outcomes.[275] The most common questionnaires that are used in children and adolescents are the PA questionnaire for children (PAQ-C) and adolescents (PAQ-A).[276, 277] Many studies have used subjective PA due to very low cost and ease of use when screening large populations and information of the type of activity and contextual setting. However, the main limitations of this approach are low accuracy and reliability.[248, 278] Additionally, diaries/log-books require significant time each day to fill-out, potentially resulting in less participant engagement. Given that parents may fill out these assessments on behalf of their children, their assessments may be biased, with some individuals under- or over-reporting certain activities. Furthermore, individuals may have different interpretations of PA intensities, which could result in PA misclassification. Therefore, objective PA, assessed using wrist-worn accelerometers, was used in OxSOCRATES and this thesis.

## 2.4 Cardiorespiratory fitness

CRF is defined as the capacity of the cardiovascular and respiratory systems to supply oxygen to the mitochondria of exercising muscles during physical activity.[157, 158] $VO_2$max is the current gold-standard to assess CRF, which is determined by stroke volume, HR, and arterial and venous oxygen content and is, therefore, influenced by cardiac, cardiorespiratory, cardiovascular, and cellular function.[157, 158] A variety of tests and protocols have been developed to measure or estimate $VO_2$max, requiring either maximal or submaximal effort. As submaximal tests do not directly measure $VO_2$max, estimation equations validated against maximal tests are used.[157] Maximal tests are used in preference to submaximal tests due to their greater accuracy.[157] However, submaximal tests are commonly used when a maximal test cannot be performed for reasons such as safety, cost, or setting.

### 2.4.1 Cardiopulmonary exercise tests

$VO_2$max assessed by indirect calorimetry during a maximal exercise test is the current gold-standard for assessing CRF.[279] Using the Fick principle, $VO_2$ is defined as:

$$\dot{V}O_2 = \dot{Q}(C_a - C_v)$$

where $C_a$ indicates arterial oxygen content; $C_v$, mixed venous oxygen content; $\dot{Q}$, cardiac output.

During a maximal exercise test, the capacity of the cardiovascular and respiratory systems to supply oxygen to exercising muscle is assessed by stressing these systems to their maximum and measuring the difference between maximally inspired and expired fractions of oxygen ($VO_2$max). Maximal exercise tests that directly measure $VO_2$ are commonly referred to as a CPET.

During a CPET, indirect calorimetry assesses fractions of $O_2$ and $CO_2$ and pulmonary ventilation whilst participants breathe through an open circuit to calculate $VO_2$ at regular intervals. At maximal effort, energy to continue exercising is delivered mainly by anaerobic respiration, resulting in lactic acidosis, muscle fatigue, and ultimately the termination of exercise.[280] At this point, it is assumed that the participant's true physiological limit has been reached, identified by a plateau in $VO_2$. However, few children exhibit this classic plateau and so, $VO_2$peak, the point at which maximal effort is terminated, is used instead as it has been shown to be equivalent to $VO_2$max.[281-283]

Step or ramp protocols are typically employed on either treadmill- or cycle-ergometers to measure $VO_2$peak. There are many different protocols for children and adolescents, and many groups use their own customised protocols. The Bruce protocol is commonly used when performing a treadmill CPET in children and adolescents,[284] while the Godfrey protocol is commonly used during a cycle CPET.[285] Treadmill-determined estimates tend to be higher than those determined by cycle ergometers due to the larger muscle mass that is being exercised, greater venous return, higher stroke volumes, and thus greater cardiac output.[286, 287] The method of CPET must therefore be considered when comparing studies.

Although maximal CPETs are currently the gold-standard for assessing CRF, there are some important limitations. Due to the need for sophisticated equipment and trained staff, CPETs are rarely performed outside of exercise laboratories or hospitals. Another limitation is the comparability between

laboratories due to the different software and equipment used to filter and analyse the data obtained. It is also well known that people who regularly exercise by cycling will perform better on a cycle CPET compared to a running CPET, and *vice versa* for individuals who train by running.[287] Finally, unless laboratories have enough equipment and staff to perform multiple CPETs at once, CPETs are restricted to testing one participant at a time. Therefore, other office or field based tests can be used to tackle some of these limitations.

### 2.4.2 Field and office based tests

The 20-meter shuttle run test (20mSRT), also known as the beep/bleep test, multistage fitness test, or progressive aerobic cardiovascular endurance run test, is the most commonly used field-based test for children and adolescents worldwide.[288-290] It has been validated for use in children and adolescents with moderate/high validity against CPETs[291-293] and has the attraction of allowing the testing of large cohorts. The total number of laps completed or the stage and level reached are recorded. Although $VO_2$peak can be estimated from these, it is prone to prediction errors.[294, 295]

Distance or timed run tests are also used to assess CRF in a large populations at once. A meta-analysis of distance- and time-based walk/run tests concluded that when a CPET is not feasible, the 1.5 mile and 12 minute walk/run tests are most strongly correlated with $VO_2$peak[296] and are comparable to the 20mSRT.[292, 293]

Office-based tests, such as the 6-minute walk test, can also be used to estimate CRF in children/adolescents and are summarised in the recent statement from the American Heart Association.[157] Other performance-based tests that were not summarised, such as the 1-minute sit-to-stand test,[297] amongst others, can also be used when there are limited resources.

A key problem for all exercise testing in adolescents is determining whether participants have truly performed a maximal test, which strongly depends on the motivation of the subject and can be particularly difficult to assess in adolescents.[298] Staff carrying out such tests can mitigate against this

problem by maximising their encouragement of all participants.[298] Criteria have been proposed to identify maximal tests, as discussed in Chapter 5.

CRF was assessed by two methods in this thesis; the 20mSRT and a cycle-ergometer CPET.

# Chapter 3: Left ventricular diastolic function in children and adolescents with overweight or obesity: a systematic review and meta-analysis (Study 1)

This chapter sets out to review the existing literature to establish the extent to which childhood and adolescent OW/Ob is associated with early impairment of LVDF. In addition, this chapter reviews whether CMRFs, PA and/or CRF are associated with LVDF as well as the benefit of early intervention.

I have published this work in *Clinical Obesity* as the first author.[299]

## 3.1 Introduction

An array of interrelated indices obtained by echocardiography or CMR exist to indirectly assess LVDF, as discussed in Chapter 2 (see also Table 1.1 and Figure 1.3). Although these are all subject to influence by pathological processes, including impaired relaxation, increased myocardial stiffness, and elevated LA pressure, there is evidence that some measures may be better at differentiating particular elements of LVDF than others. For example, some echocardiography measures are less influenced by

LV loading conditions.[185, 191, 196] It has been suggested that these differences may improve the ability to detect early LVDD,[185] particularly in conditions such as obesity where volume overload occurs.

Detection methods for LVDD in adults are well-established,[19] but it is unclear which measures best detect the earliest stages of LVDD and would, therefore, be most suitable in adolescents and particularly in those with OW/Ob. Reliable early detection is important as LVDD reversibility is still potentially achievable and lifestyle habits may be less fixed in the young. Furthermore, although there are many studies of LVDF in children and adolescents with OW/Ob, diverse methods and group definitions have made it difficult to adequately summarise findings by conventional meta-analysis, with study heterogeneity being identified as the main limiting factor in earlier attempts.[72-74]

### 3.1.1 Previous review articles

The study of LVDF in children with obesity was first addressed in 2001 by Harada and colleagues.[300] The authors identified that LVDF was impaired in children with obesity compared to controls. Since then, many studies have been published and have been aggregated into review articles.

The first review identified some studies that indicate a reduction in LVDF in adolescents with OW/Ob, but it was identified that more research was needed due to the few papers that had been published at that time.[74] In a later review, it was stated that LVDF seems to be impaired in children and adolescents with OW/Ob, but the authors failed to discuss the results of any studies reporting maintained LVDF and drew no overall meaningful conclusions.[72] In the most recent review, Koopman and Mertens[73] indicate that LVDF is likely impaired in children with obesity. They concluded that although PWD measures in children with obesity are highly variable, they may indicate reduced LVDF. It was also concluded that TDI and strain-rate measures seem best able to detect mild impairments in LVDF, although more studies were required. Given that this article did not conduct a thorough literature search and was published in 2014, a more up-to-date, systematic review of the available data is warranted. Furthermore, there has been no meta-analysis of the available data, which should be possible given that most studies share common measures of OW/Ob and LVDF. With the

publication of more recent articles, statistical synthesis of the data would confirm whether or not OW/Ob is associated with LVDF at a young age.

Additionally, an up-to-date analysis to understand the link between CMRFs and LVDF in children and adolescents with OW/Ob is also warranted to help understand the early aetiology of LVDD. It is also currently unclear whether PA and/or CRF offer any protection against LVDD.

### 3.1.2 Insulin resistance and cardiometabolic risk factors

Although most studies focus on measures of adiposity, a significant number have addressed other CMRFs that often accompany OW/Ob and may directly impair LVDF. Meta-analyses report IR as the single most important CMRF associated with cardiovascular events,[301] while large community studies show that increasing levels of IR lead to a progressive decline in LVDF, independent of confounders.[302] The authors state that several factors such as altered cardiac progenitor cell function, hyperglycaemia, renin-angiotensin-aldosterone system activation, microangiopathy, and autonomic neuropathy could explain the independent relationships.[302] Measures of IR may, therefore, be important in the identification of early LVDD and so aggregation of such data within a review article is also warranted.

### 3.1.3 Physical activity, cardiorespiratory fitness, and intervention

As discussed in Chapter 1, PA and CRF may confer protection against the adverse CVD effects of OW/Ob, while exercise and lifestyle programmes can improve LVDF, CMRFs and CRF in adults with obesity.[303] Although this has been studied less in children, it is likely that childhood interventions would have greater benefit, before irreversible cardiovascular remodelling could occur, and could explain why some adult intervention studies report no benefit.[304] Therefore, aggregation of the current data within a systematic review is warranted.

### 3.1.4 Aims

In this systematic review and meta-analysis, the aims were to determine in a population of children and adolescents: (1) the extent to which OW/Ob is associated with varying measures of LVDF; (2)

measures of LVDF which are most strongly associated with OW/Ob; (3) the associations of IR and other CMRFs with LVDF measures; (4) the association of PA and CRF with LVDF measures and; (5) the extent to which exercise and lifestyle modification have been shown to improve LVDF in childhood and adolescence.

## 3.2 Methods

The protocol for this review was registered with PROSPERO International Prospective Register of Systematic Reviews (identifier CRD42020177470). This review was completed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 guidelines.[305]

### 3.2.1 Criteria for considering studies for this review

#### 3.2.1.1 *Types of studies*

Cross-sectional studies, controlled intervention studies, and pre-post studies that examined group-differences and/or associations of childhood and adolescent OW/Ob with LVDF were included. Inclusion was limited to full-text articles reported in English and published in peer-reviewed journals. Studies published in grey literature sources and conference or meeting abstracts without a full text were excluded.

#### 3.2.1.2 *Participants*

Individuals aged <18 years were included in accordance with the international definition of childhood. Additionally, individuals aged 10-24 years were included and defined as adolescents in accordance with the widest accepted definition to ensure that articles that used this definition were not rejected.[306] It has been suggested that this definition of adolescence corresponds best with contemporary features of adolescent growth, such as neurocognitive maturation that continues past the age of 20 years, and social role transitions, such as partnering, parenting, and semidependency that are continuing to extend into the third decade of life.[306]

Almost every study used individual criteria to define their OW/Ob and control groups. Furthermore, the pathological group in some studies was obesity only whilst others included overweight in this group. In other studies, overweight was grouped with normal-weight as a control group. Therefore, study-specific group definitions are reported in the results, but were ignored in the meta-analysis, as this heterogeneity did not allow for meaningful group-based comparisons.

#### 3.2.1.3 *Outcome measures*

Primary outcomes were measures of LVDF. Where both septal and lateral TDI measures were reported without their commonly reported mean, this was calculated using the recommended Cochrane method (Appendix 1).[307] TDI measurements were sometimes reported without mention of the site of measurement. These were assessed separately and were identified as "cannot determine" measures. There were insufficient data for some measures of LVDF, such as pulmonary vein peak velocities and diastolic strain-rate, to be included in the meta-analysis. To address this, a systematic review was completed to ensure that all measures of LVDF were summarised.

### 3.2.2 Search methods for identification of studies

#### 3.2.2.1 *Electronic searches*

I devised search terms to include common terms and key words such as obesity, children, and diastolic function. Dr Alexander Jones checked these for completeness and correctness. Search hedges using these terms (Appendix 1) were applied in PubMed.gov (1958 to present), Cumulative Index to Nursing and Allied Health Literature (CINAHL; 1992 to present), Cochrane Central Register of Controlled Trials, and ClinicalTrials.gov (1997 to present), Embase (1974 to present), and Web of Science (1987 to present). The reference lists of included studies, as well as pertinent reviews,[72-74] were also searched, yielding four further studies.[308-311] The final search was completed on 11th July 2020.

### 3.2.3 Data collection and analysis

#### 3.2.3.1 *Study selection*

Four authors, including myself, independently reviewed results of the search to include/exclude studies for full-text screening. Inclusion and exclusion criteria for progression to the full-text screening are documented in Appendix 1. A preliminary screen of all potential papers for full-text review was completed to ensure that all reported LVDF.

Two independent full-text screens were completed to include/exclude studies for the review. Inclusion and exclusion criteria to be included is provided in Appendix 1. The IR meta-analysis was limited to using Homeostatic Model Assessment for IR (HOMA-IR) due to the lack of data using other methods.

For all analyses, when the same data were apparently reported in separate/duplicate publications, the article with the greatest number of subjects was selected and the other(s) excluded. However, if the article with fewer subjects reported additional LVDF measures, these measures were included as a separate study. Consensus on disagreements was achieved by discussion between reviewing authors or with the inclusion of a fifth author.

#### 3.2.3.2 *Data extraction and management*

I extracted data using a pre-defined form that was verified for completeness and correctness by two other authors. The following data were extracted: (1) study characteristics and methods; (2) subject/group demographics; (3) HOMA-IR results; (4) measures of LVDF and their results, and where applicable; (5) correlation statistics with adiposity CMRF, CRF, and/or PA measures.

#### 3.2.3.3 *Risk of bias assessment*

Four authors independently executed quality assessment of the included studies and any discrepancies were resolved by discussion. Modified versions of the Study Quality Assessment Tools by the National Heart, Lung, and Blood Institute (NHLBI) were used to assess study quality and risk of

bias (Appendix 1).[312] Scores of "good" (least risk of bias), "fair" (susceptible to some bias) and "poor" (significant risk of bias) were given to each study based on study design and implementation. Studies that were scored as "poor" overall but were otherwise methodologically sound (e.g. correctly reported LVDF measures and reported BMI, age, and sex) were included in the meta-analysis. A sensitivity analysis was completed to ensure that these studies did not influence the results.

#### 3.2.3.4 *Data synthesis*

Measures of LVDF were transformed into standard units of measurement where necessary. Mean ± standard deviation (SD) were calculated from alternative descriptions of central tendency and dispersion (e.g. median), using the recommended Cochrane tools (Appendix 1).[307, 313]

#### 3.2.3.5 *Statistical analysis*

Analysis was completed using STATA (version 16.1, StataCorp, College Station, Texas). Although group data are reported in study descriptions, the marked heterogeneity in the mean BMI of control and OW/Ob groups across studies limited the ability to do a conventional, group-based meta-analysis reliably. To overcome this, mean (SD) BMI values for all groups, regardless of how those groups were defined by authors, were used to assess continuous associations of BMI with LVDF measures, using weighted, random-effects linear regression. These models were adjusted for age and sex to account for their known effect on BMI. These models also took account of the fact that some group means (e.g. a normal and an obesity group) were drawn from the same study. Each study was treated as a unique level in the random-effects regression, allowing the pairwise differences within studies to be captured by the model without reliance on specific group definitions. This enabled estimation of the linear relationships of BMI with multiple measures of LVDF and their relative strength, giving insight into which measurements may be most useful for early detection of impaired LVDF.

HOMA-IR values were similarly used to assess continuous associations with LVDF measures, using weighted, random-effects linear regression. These models were also adjusted for age and sex.

To account for individual study size and measure variance, each group estimate in the random-effects regression models was weighted using the inverse-variance method (1/standard error$^2$).[307] The standard error of each measure was calculated using the SD and N reported for each group.

Histogram plots were used to assess normality of variables. Any non-normally distributed variables were transformed into normal distributions using the Tukey Ladder of Powers using the transformation with the smallest chi-squared value. Measures were further transformed to their z-scores, to allow correlation coefficients (r) to be calculated. Fisher z-test was used to compare the strength of these correlations with the strongest association as a reference (Appendix 1). Robust z-scores, which do not depend on parametrically distributed data, were also calculated (Appendix 1) and the analyses were repeated to check that non-parametric distributions were not responsible for the findings. The brand of echocardiography machine was further included as a variable in repeat analyses to determine whether differences in technologies influenced the strength of relationship to LVDF measures. A sensitivity analysis was completed by repeating the analysis but excluding any studies reported as "poor". A further sensitivity analysis was completed by excluding any study that included participants older than the American Academy of Pediatrics definition of adolescence (11-21 years).[314]

$R^2$ was reported for each model, and effect sizes, standard error, 95% CIs, z-statistic, and p-value were reported for each independent variable in the models. $P<0.05$ was considered statistically significant.

## 3.3 Results

### 3.3.1 Study characteristics

Searches identified 7,311 studies. 111 full-text articles were assessed after title/abstract screening (Figure 3.1). A total of 71 studies (Appendix 1, Table 1.1; sample sizes n=20-799; representing 9,975 participants) were eligible, with 53 studies in the systematic review, 55 studies in the BMI meta-analysis (Figure 3.1 and Appendix 1, Table 1.1; sample sizes n=20-650; representing 6,782 participants), and 31 studies in the HOMA-IR meta-analysis (Figure 3.1 and Appendix 1, Table 1.1; sample sizes n=20-650; representing 3,878 participants).

All studies assessed LVDF by echocardiography. One study that assessed LVDF by CMR was identified but was excluded from the systematic review due to different ages of participants between groups.

The number of studies and number of participants for each LVDF measure are reported in Appendix 1, Table 1.2. Six studies were scored as good, 48 as fair, and 17 as poor for quality and risk of bias (Appendix 1, Table 1.1). Mean age, percentage of males, and mean BMI ranged from 8.9 to 18.4 years-of-age, 0 to 100%, and 15.8 to 60.0 kg/m$^2$, respectively. There was marked heterogeneity in group-definitions, with >20 definitions identified for groups with OW/Ob groups and >20 for control groups (Appendix 1, Table 1.1). Furthermore, there was marked overlap of BMI between control and OW/Ob groups and marked dispersion of BMI within groups across studies, presenting a major challenge to the reliable use of conventional group-based meta-analysis (Figure 3.2). However, a preliminary group-based meta-analysis was done by ignoring the group definitions, which produced reassuringly similar results. For example, the OW/Ob groups reported worse septal e' velocities (mean difference = -1.16 cm/s, $p<0.001$), septal a' velocities (mean difference = 0.77 cm/s, $p<0.001$) and septal e'/a' ratios (mean difference = -0.31, $p<0.001$) compared to controls.

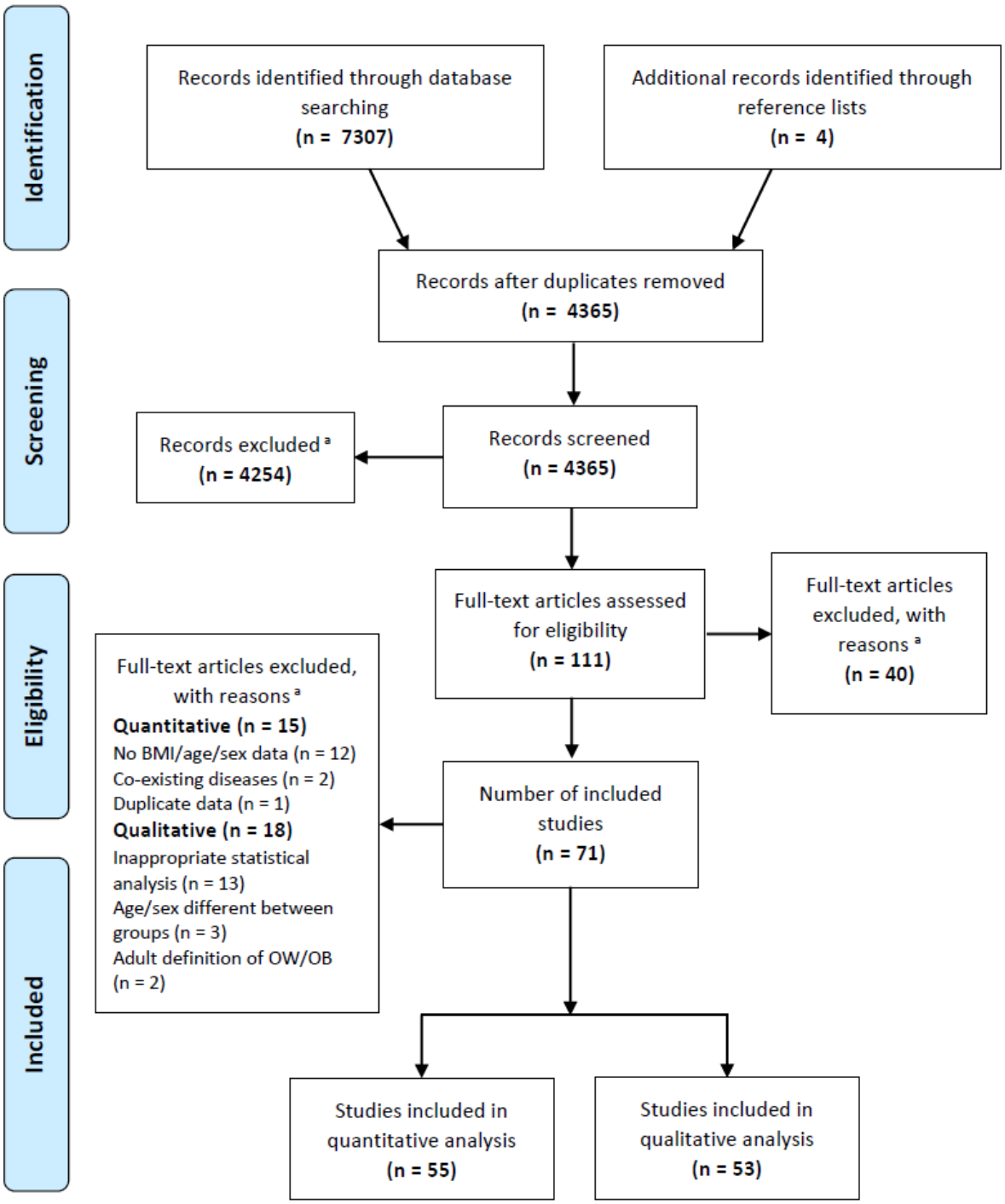


**Figure 3.1 Flow diagram of study identification, screening, eligibility and inclusion/exclusion.**

Echo indicates echocardiography; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; n, number of studies; OB, obese; OW, overweight. [a]Exclusion criteria and reasons can be found in Appendix 1.

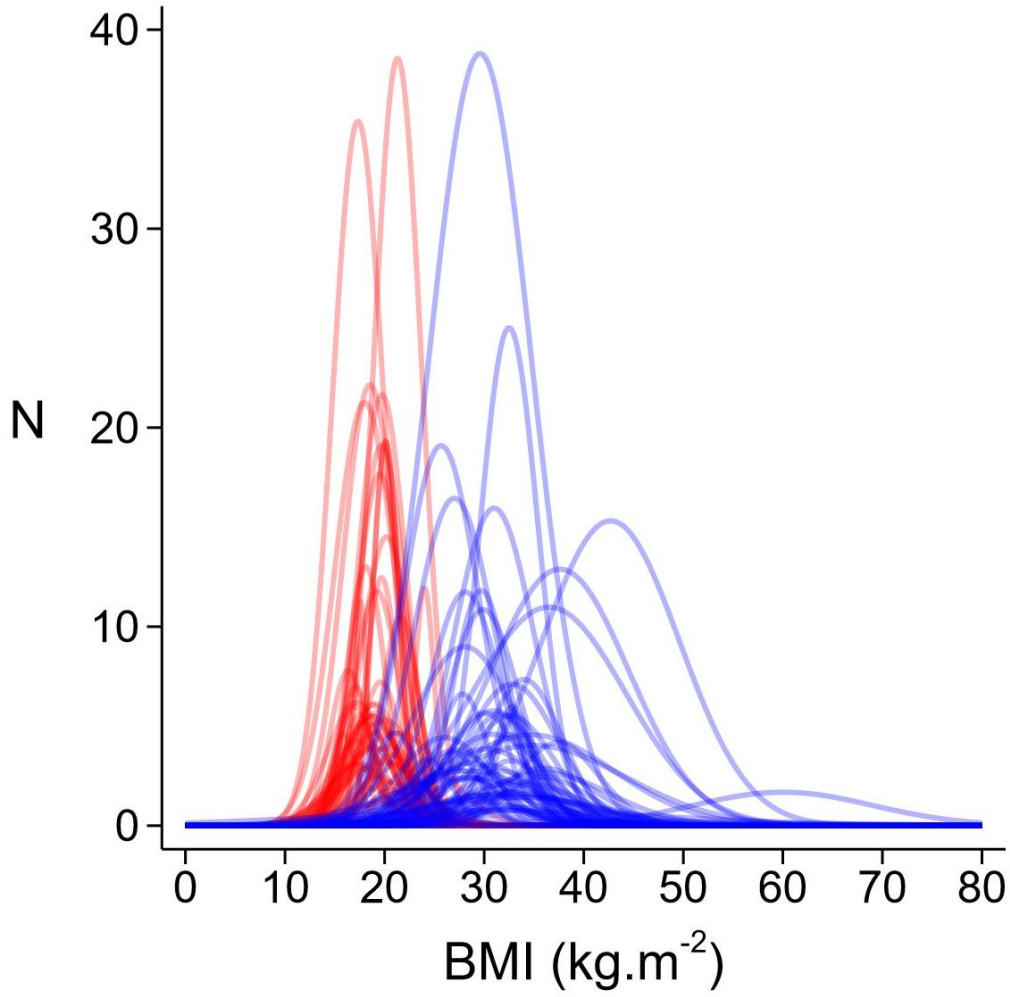


**Figure 3.2 Distribution of body mass index (BMI) in control (red) and overweight/obese (blue) groups included in the meta-analysis.**

Groups were defined as per the definitions in individual studies. A normal distribution curve was generated using the reported sample size (*N*), mean BMI, and BMI standard deviation. Significant overlap of BMI distributions between groups and marked variability of distributions within groups highlight that it was not possible to perform traditional group-based meta-analysis reliably.

### 3.3.2 Objective 1 – The association of OW/Ob with measures of LVDF

Objective 1 was to determine the association of OW/Ob with LVDF. This was done by meta-analysis and by systematic review. A small subset of papers addressed this question directly as a study outcome and the findings of these are summarised in Appendix 1, Table 1.3.

#### 3.3.2.1 *Meta-analysis*

The associations of BMI with measures of LVDF, after adjustment for age and sex, are given in Table 3.1 and Appendix 1, Table 1.4. There was evidence of reduced myocardial motion indicated primarily by strong associations of septal e'/a' ratios and septal a' peak velocities with BMI. BMI was associated with all other measures of LVDF, apart from DT. Independent associations of LVDF with age are reported in Appendix 1, Table 1.4. There were no independent effects of sex distribution in the studies.

Findings were not altered meaningfully by attempts to normalise non-normal distributions, although there was a marginal improvement for associations of BMI with a' (r=0.441) and with DT (r=0.158). The latter did not become statistically significant after such normalisation. Repetition of the analyses using robust z-scores or with adjustment for the type of echocardiography machine used in each study made no meaningful difference to the results (data not shown). Sensitivity analyses to exclude poor quality studies also made no meaningful difference to the results. Sensitivity analyses to exclude studies with adolescents aged >21 years made no overall meaningful difference to the results. However, averaged e'/a' ratios were no longer significantly associated with BMI (Appendix 1, Table 1.5).

#### 3.3.2.2 *Systematic review*

Forty-three studies eligible for systematic review reported matching LVDF measures to those included in the meta-analysis (Figure 3.3).[133, 134, 144, 215, 308-310, 315-350] A full list of these results is given in Appendix 1, Table 1.6. A number of studies also assessed adiposity by DEXA, reporting links with LVDF (Appendix 1).[326, 346, 351]

**Table 3.1:** Associations of BMI with each left ventricular diastolic function measure, ranked by strength of association (r)

| Measure (units per 10 point change in BMI) | Number of Studies | References | Correlation Coefficient (r) | b | 95% CI | Fisher's z-test |
|---|---|---|---|---|---|---|
| **e'/a' sep** ($1/kg/m^2$) | 13 | 215, 308, 311, 317, 323, 345, 349, 352-357 | -0.689 | -0.240 | **-0.299, -0.180** | 0.000 |
| **a' sep** ($cm.s^{-1}/kg/m^2$) | 16 | 215, 308, 311, 317, 323, 327, 339, 345, 349, 352-358 | 0.621 | 0.743 | **0.522, 0.965** | 0.239 |
| **e'/a' lat** ($1/kg/m^2$) | 12 | 215, 308, 315, 317, 345, 349, 352-355, 359, 360 | -0.593 | -0.366 | **-0.525, -0.208** | 0.318 |
| **a' lat** ($cm.s^{-1}/kg/m^2$) | 14 | 215, 308, 315, 317, 339, 345, 349, 352-355, 358-360 | 0.432 | 0.877 | **0.558, 1.195** | 0.883 |
| **E/e' sep** ($1/kg/m^2$) | 16 | 215, 308, 311, 323, 324, 327, 338, 339, 347, 353, 355, 357, 358, 361-363 | 0.431 | 0.814 | **0.593, 1.035** | 0.902 |
| **e' sep** ($cm.s^{-1}/kg/m^2$) | 19 | 215, 308, 311, 317, 318, 323, 327, 338, 339, 345, 347, 349, 352-358 | -0.413 | -0.747 | **-1.057, -0.437** | 1.012 |
| **E/e' average** ($1/kg/m^2$) | 16 | 215, 308, 324, 326, 328, 333, 338, 339, 346, 349, 353, 355, 358, 361, 363-365 | 0.387 | 0.666 | **0.552, 0.781** | 1.046 |
| **a' average** ($cm.s^{-1}/kg/m^2$) | 14 | 215, 308, 310, 317, 326, 339, 345, 349, 352-355, 358, 364 | 0.343 | 0.589 | **0.255, 0.924** | 1.176 |
| **e'/a' average** ($1/kg/m^2$) | 11 | 215, 308, 317, 345, 346, 349, 352-355, 366 | -0.306 | -0.155 | **-0.262, -0.048** | 1.208 |
| **e' average** ($cm.s^{-1}/kg/m^2$) | 20 | 215, 308, 310, 317, 318, 326, 328, 333, 338, 339, 345, 349, 352-355, 358, 364-366 | -0.294 | -0.912 | **-1.302, -0.522** | 1.463 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **e' lat** ($cm.s^{-1}/kg/m^2$) | 20 | 215, 308, 315-318, 331, 336, 338, 339, 345, 349, 352-355, 358-360, 367 | -0.247 | -1.161 | **-1.571, -0.752** | 1.649 |
| **E/e' lat** ($1/kg/m^2$) | 18 | 133, 215, 308, 310, 317, 324, 331, 336, 338, 339, 349, 353-355, 358, 360, 361, 363 | 0.237 | 0.462 | **0.336, 0.589** | 1.645 |
| **IVRT** ($ms/kg/m^2$) | 17 | 14,24,28,29,35,40,43,46,48,52,53, 57-59,61-63 | 0.222 | 2.861 | **0.961, 4.762** | 1.718 |
| **A-wave** ($cm.s^{-1}/kg/m^2$) | 34 | 12,14,15,18-48 | 0.216 | 2.636 | **1.660, 3.612** | 1.933 |
| **E-wave** ($cm.s^{-1}/kg/m^2$) | 33 | 12,14,15,18-47 | 0.178 | 1.774 | **0.355, 3.193** | 2.104 [a] |
| **E/A** ($1/kg/m^2$) | 42 | 12,14,15,18-30,32,34-38,40-60 | -0.147 | -0.056 | **-0.080, -0.032** | 2.275 [a] |
| **DT** ($ms/kg/m^2$) | 14 | 14,15,21,24,26,29,34-36,40,44,53,58,61 | -0.005 | -0.220 | -8.987, 8.546 | 2.408 [a] |

Associations that were statistically significant ($p<0.05$) are represented by bold 95% confidence intervals (CI). Fisher z-test, which accounts for sample size, was used to compare the strength of the correlation coefficients (r) with the strongest association, septal (sep) e'/a', as a reference. Larger values of Fisher's z indicate that correlation coefficients are more likely to be statistically different (less strongly associated) with respect to the reference association. Those that were significantly different ($P<0.05$) are marked with an [a]. Tissue Doppler imaging (TDI) measures are reported as an average of recordings from the septal and lateral wall (lat) of the left ventricle, and individually as sep and lat. A-wave indicates late mitral inflow peak velocity; a', late diastolic tissue peak velocity; b, unstandardized regression coefficient; BMI, body mass index; DT, E-wave deceleration time; E-wave, early mitral inflow peak velocity; e', early diastolic tissue peak velocity; E/A, E-wave/A-wave ratio; E/e', E-wave/e' ratio; e'/a', e'/a' ratio; IVRT, isovolumic relaxation time.

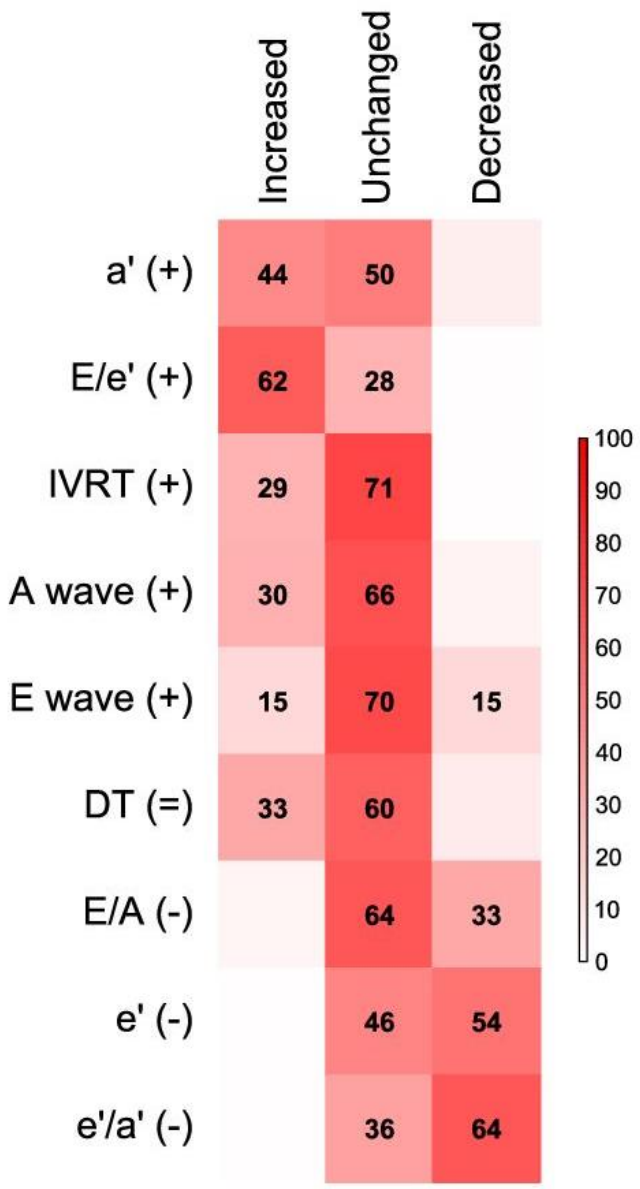


**Figure 3.3 Percentage of studies included in the qualitative analysis reporting increased (+), unchanged (=), or decreased (−) measures of left ventricular diastolic function in children/adolescents with OW/Ob compared to controls.**

Darker red squares indicate a greater percentage of studies. Measures are ranked by the strength of association (*r*) from strongest positive to strongest negative as identified in the meta-analysis. The directionality of greater percentages from top left to bottom right supports the meta-analysis results. A-wave indicates late mitral inflow peak velocity; *a*', late diastolic tissue peak velocity; DT, E-wave deceleration time; E-wave, early mitral inflow peak velocity; e', early diastolic tissue peak velocity; E/A, E-wave/A-wave ratio; E/e', E-wave/e' ratio; e'/a', e'/a' ratio; IVRT, isovolumic relaxation time.

### 3.3.3 Objective 2 – Comparison of measures of LVDF

Standardized r coefficients are reported in Table 3.1. BMI was most strongly associated with septal e'/a' ratios after adjustment for age and sex. Septal TDI measures were more strongly associated with BMI than lateral or averaged equivalents. Other TDI measures of LVDF, especially those including a' peak velocities, were more strongly associated with BMI than conventional measures of mitral inflow peak velocities (e.g. E-wave). Associations of BMI with E-wave peak velocity, E/A ratio, and DT were significantly weaker than that of septal e'/a' (Table 3.1), suggesting inferiority for early detection of reduced LVDF in childhood and adolescent OW/Ob.

### 3.3.4 Objective 3 – The association of HOMA-IR and other CMRFs with LVDF

#### 3.3.4.1 *Meta-analysis*

Evidence of reduced LVDF with increasing levels of HOMA-IR are reported in Table 3.2 and Appendix 1, Table 1.7. The strongest association was with the averaged E/e' ratio. Other TDI measures of LVDF and IVRT were more strongly associated with HOMA-IR than conventional measures of mitral inflow velocities. E-wave peak velocity, DT, septal e' peak velocity, and lateral a' peak velocity were not associated with HOMA-IR. Associations of HOMA-IR with measures of LVDF were not statistically different than that of averaged E/e' ratios (Table 3.2). There were insufficient data on septal E/e' ratios to be included in the meta-analysis. Independent associations of LVDF with age and sex are reported in Appendix 1, Table 1.7.

#### 3.3.4.2 *Systematic review*

Objective 3 was also to systematically review the association of other CMRFs with LVDF. Of the 51 studies included in the systematic review, all reported data on at least one CMRF but only 13 related these to LVDF.[144, 322, 323, 326-328, 339, 343, 344, 346, 351, 364, 368]

IR was the most common CMRF reported as associated with LVDF.[144, 322, 323, 326, 328, 344, 351] IR was assessed by HOMA-IR in all studies. One study found no relationship with HOMA-IR but reported

associations between fasting insulin levels and strain-rate in early diastole.[368] Three studies grouped participants with obesity by the presence (or not) of IR using thresholds of HOMA-IR.[144, 322, 323] Two studies reported reduced LVDF in participants with obesity and IR[144, 323] and one reported no difference.[322] Three studies grouped participants with obesity by indirect measures of IR; two using dysglycaemia[360, 361] and one by Type-2 diabetes.[346] LVDF was impaired further in participants with obesity and poor cardiometabolic health compared to normal cardiometabolic obese equivalents.[346, 361] Dysglycaemia was also associated with e' in one study.[364] Additionally, the inflammatory marker C-reactive protein was associated with LVDF in four of the studies reporting associations with IR.[144, 323, 328, 344]

Four studies reported associations of BP with LVDF.[322, 339, 346, 364] However, increased BP was associated with LVDF in only one study after adjusting for confounders.[346] Additionally, three studies grouped participants with obesity by the presence (or not) of hypertension.[133, 134, 369] Two studies grouped participants with obesity using 24-hour ambulatory BP monitoring and found no difference in LVDF in the hypertensive obesity group compared to the normotensive obesity group.[133, 134] One study grouped participants with obesity by office BP and found a significant difference in LVDF between groups.[369]

When participants with obesity were grouped by metabolic health status, defined by the MetS or other similar clusters of risk factors, two studies associated poor metabolic health with reduced LVDF[322, 370] and one study found no such association.[318] In another two studies, participants were grouped by the presence, or not, of MetS, irrespective of their obesity status. In these, the E/A ratio and DT were impaired in one study[321] and no differences in LVDF were found in the other.[371] Another study reported that an adverse metabolic risk score was associated with lower e' velocity and higher E/e' ratio, indicating reduced LVDF.[328] In children and adolescents with obesity, those with non-alcoholic fatty liver disease had reduced LVDF compared to those without in two-out-of-three studies.[343, 345, 351]

**Table 3.2:** Associations of HOMA-IR with each left ventricular diastolic function measure, ranked by strength of association (r)

| Measure (units per 1 point change in HOMA-IR) | Number of Studies | References | Correlation Coefficient (r) | b | 95% CI | Fisher's z-test |
|---|---|---|---|---|---|---|
| **E/e' average** | 7 | 326, 328, 338, 339, 343, 344, 364 | 0.600 | 0.509 | **0.296, 0.723** | 0.000 |
| **IVRT (ms)** | 13 | 134, 325, 338, 340, 342-344, 349, 359, 362, 370, 372, 373 | 0.463 | 3.56 | **1.310, 5.810** | 0.376 |
| **e'/a' sep** | 5 | 317, 323, 345, 349, 354 | -0.412 | -0.098 | **-0.163, -0.033** | 0.370 |
| **a' sep (cm/s)** | 6 | 317, 323, 339, 345, 349, 354 | 0.402 | 0.387 | **0.241, 0.534** | 0.426 |
| **e' average (cm/s)** | 12 | 317, 318, 326, 328, 338, 339, 343-345, 349, 354, 364 | -0.332 | -0.673 | **-1.125, -0.220** | 0.714 |
| **e'/a' lat** | 7 | 317, 345, 349, 354, 359, 360, 372 | -0.291 | -0.094 | **-0.132, -0.056** | 0.725 |
| **e' lat (cm/s)** | 12 | 316-318, 336, 338, 339, 345, 349, 354, 359, 360, 372 | -0.247 | -0.73 | **-1.146, -0.313** | 0.940 |
| **a' average (cm/s)** | 8 | 317, 326, 339, 343, 345, 349, 354, 364 | 0.247 | 0.295 | **0.187, 0.404** | 0.828 |
| **e'/a' average** | 5 | 317, 343, 345, 349, 354 | -0.174 | -0.056 | **-0.103, -0.009** | 0.839 |
| **E/A** | 24 | 133, 134, 316-319, 323, 325, 326, 328, 330, 332, 336, 338-340, 342-345, 349, 360, 362, 372, 373 | -0.159 | -0.035 | **-0.059, -0.011** | 1.308 |
| **A-wave (cm/s)** | 16 | 134, 316-319, 323, 326, 328, 336, 339, 340, 343-345, 349, 360 | 0.157 | 1.169 | **0.605, 1.733** | 1.238 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **E/e' lat** | 9 | 133, 317, 336, 338, 339, 349, 354, 360, 372 | 0.156 | 0.161 | **0.017, 0.306** | 1.084 |
| **a' lat (cm/s)** | 7 | 317, 339, 345, 349, 354, 359, 360 | 0.137 | 0.19 | -0.044, 0.423 | 1.060 |
| **DT (ms)** | 14 | 134, 319, 328, 336, 338-340, 343, 344, 359, 360, 362, 372, 373 | -0.103 | -2.654 | -7.007, 1.699 | 1.365 |
| **e' sep (cm/s)** | 8 | 317, 318, 323, 338, 339, 345, 349, 354 | -0.098 | -0.132 | -0.399, 0.136 | 1.203 |
| **E-wave (cm/s)** | 16 | 134, 316-319, 323, 326, 328, 336, 339, 340, 343-345, 349, 360 | 0.012 | 0.091 | -1.004, 1.185 | 1.644 |
| **E/e' sep[a]** | - | - | - | - | - | - |

Associations that were statistically significant (P<0.05) are represented by bold 95% confidence intervals (CI). Tissue Doppler imaging (TDI) measures are reported as an average of recordings from the septal and lateral wall (lat) of the left ventricle, and individually as sep and lat. Larger values of Fisher's z indicate that correlation coefficients are more likely to be statistically different (less strongly associated) with respect to the reference association. Those that were significantly different (P<0.05) are marked with an [a]. A-wave indicates late mitral inflow peak velocity; a', late diastolic tissue peak velocity; b, unstandardized regression coefficient; CI, confidence interval; DT, E-wave deceleration time; E-wave, early mitral inflow peak velocity; e', early diastolic tissue peak velocity; E/A, E-wave/A-wave ratio; E/e', E-wave/e' ratio; e'/a', e'/a' ratio; HOMA-IR, homeostatic model assessment of insulin resistance; IVRT, isovolumic relaxation time.

[a] There were an insufficient number of studies on septal E/e' to be included in the analysis.

### 3.3.5 Objective 4 – Relationship with cardiorespiratory and the reversibility of impaired LVDF

#### 3.3.5.1 *Relationship with cardiorespiratory fitness*

Four studies reported correlations between measures of CRF and LVDF in the obese,[310, 326, 328, 333] while no studies reported PA alongside LVDF measures.

Dias *et al*[326] found in a population of 20 adolescents that strain rate in early diastole was associated with $VO_2$peak adjusted for total body mass (r=0.58, p=0.008), although this relationship was marginally removed when $VO_2$peak was adjusted for FFM instead (r=0.43, p=0.059). In the other study by Franssen *et al*[328] (n=58), some measures of LVDF were associated with heart rate recovery, but the results of other metrics of CRF, including $VO_2$peak, were not reported.

Two studies used exercise stress echocardiography. Ingul *et al*[333] reported reduced e' velocity and increased E/e' ratio in the obese during peak exercise, which seemed to be exaggerated with exercise, although no statistics between rest and exercise LVDF were performed. Similarly, Schuster *et al*[310] found that the obese with a lesser degree of obesity had increased e' velocities throughout the exercise test, whereas the obese with a higher degree of obesity had decreased e' velocities compared to controls. They suggested that this could be due to a compensatory mechanism that maintains normal function in the less obese, but that fails as obesity progresses.

#### 3.3.5.2 *Reversibility of reduced LVDF*

Six exercise/lifestyle interventions were available for review.[332, 333, 341, 374-376]

The pilot study by Ingul *et al*[333] showed that a 3-month aerobic interval training protocol improved DT, IVRT, and e' velocity at rest, as well as e' velocity and E/e' ratio during exercise. Waist circumference and CRF parameters were improved post-intervention, but there were no changes in weight or BMI. The larger follow-up study by the same team failed to demonstrate improvements in these or other measures of LVDF, but only a small number of the recruited participants completed the study (high

intensity interval training [HIIT] intervention [52%, n=17] and moderate intensity continuous training [MICT] intervention [75%, n=24]).[332]

Hansen *et al*[376] found that a 3-month football training intervention did not improve LVDF, but the children in this study were young and were overweight, not obese, which may have limited the extent to which they could benefit from intervention.

Naylor *et al*[374] found that an 8-week resistance training intervention increased e' velocity and decreased A-wave velocity and the E/e' ratio, suggesting improvements in LVDF, but it also increased a' velocity, which contrasts with this. The intervention reduced total body fat percentage, increased lean body mass, and did not alter BMI.

Obert *et al*[341] performed a 9-month lifestyle intervention consisting of aerobic interval training, moderate PA (MPA), and a calorie-restricted diet. Post-interventional analysis revealed improvements in e' velocity, E/e' ratio, and longitudinal strain rate in early diastole, but with no changes in other measures. The intervention also improved multiple measures of adiposity and CMRFs. However, the individual benefit of increasing PA and/or dieting was unclear.

Karaagac *et al*[375] performed an intervention of diet and exercise over 6-months. They reported significant reductions of E-wave velocity and e' velocity, and an increase of the E/e' ratio after this intervention, suggesting a worsening of LVDD, but they concluded otherwise, making this study difficult to interpret. Furthermore, the individual benefit of increasing PA and/or dieting was unclear.

Two non-exercise interventions were available. Although not thoroughly described, Zeybek *et al*[349] provided a calorie restricted diet that improved some lateral TDI measurements. However, no changes in septal TDI measurements or PWD measurements were seen. Bariatric surgery improved measures of PWD and TDI.[353]

## 3.4 Discussion

This study presents the first meta-analysis that examined LVDF in children and adolescents with OW/Ob. Evidence suggests that elevated BMI in the young is associated with reduced LVDF and that the strongest associations are found when septal TDI measures are used. This could suggest that impaired LVDF in children and adolescents with OW/Ob begins in the septum. IR, as indicated by HOMA-IR, was likewise associated with reduced LVDF. Limited data also support reversibility of LVDF abnormalities with exercise/lifestyle intervention.

### 3.4.1 LVDF in childhood and adolescent OW/Ob

Elevated BMI in childhood and adolescence is adversely associated with all measures of LVDF, apart from DT, which coincides with the findings of the systematic review. Impaired longitudinal myocardial motion of the LV, identified by decreasing e' peak velocities and e'/a' ratios, and increasing a' peak velocities, was most strongly associated with increasing BMI.

LVDD begins with impaired relaxation and decreased ventricular 'suction' in early filling. In this study, there was evidence of reduced myocardial motion in early diastole (e') with increasing BMI. As e' is inversely related with the time constant of LV relaxation, τ, the results likely represent a gradual reduction in myocardial relaxation with increasing adiposity.[20] To overcome any abnormalities in early relaxation and maintain normal LV end diastolic volume, a more forceful 'atrial kick' is required, which can be identified by greater a' peak velocities. The results confirmed this with increasing a' peak velocities with increasing BMI. Although the results do not represent large differences in LVDF, such LV motion abnormalities probably represent the early stage of LVDD and may, therefore, be useful for identifying those most at risk of future cardiac events. In support of this, young adults in the CARDIA study, aged 23-25 years at the time of echocardiography, with abnormal LVDF (defined by an E/A ratio <1.3 and one marker of abnormal cardiac morphology) were 1.8 times more likely to have a clinical CVD event over 20 years of follow-up.[83]

### 3.4.2 Measures with strongest relationship to BMI

This study was able to compare the strength of the associations of BMI with different LVDF measures, yielding insight into which measurements are most useful for early detection of impaired LVDF in this context. Myocardial tissue peak velocities assessed by TDI were the LVDF measures that were most strongly associated with BMI. Of these, the strongest association was with the septal e'/a' ratio, which is in accord with a study of Type-1 Diabetic children.[377] These results also coincide with earlier studies of LVDF that identified the e'/a' ratio as the best marker of early longitudinal compliance abnormalities.[185]

Of the two components of e'/a', a' was more strongly associated with BMI than e'. The stronger relationship with a' may be explained by this measure being less influenced by volume overload, which is typically seen in obesity.[196] Therefore, it is reasonable to suggest that measures of both a' and e'/a' should be considered the best markers to identify early impairments of LVDF in children and adolescents with obesity, particularly in the septum.

It was found that TDI peak velocities assessed at the septal mitral annulus were more strongly associated with BMI than the lateral equivalent or their average. Stronger associations of BMI with septal TDI measures may reflect preferential remodelling of the septum prior to similar changes in the lateral myocardium.[75, 336] As myocardial hypertrophy leads to reduced compliance of the myocardium and worse LVDF,[378] earlier septal remodelling may explain our findings. It should also be noted that lateral TDI measures are technically more difficult to obtain reliably, particularly in children with obesity. We suggest that clinicians should focus on septal TDI measures when screening for impaired LVDF in children with obesity, while lateral and averaged measures can be used to supplement these, if necessary. However, there have been no paediatric guidelines for the diagnosis of LVDD, or studies reporting the minimal, clinically relevant detectable change for LVDF measures such as the septal e'/a' ratio. Thus, future work will be needed to determine what values of septal TDI, as well as other LVDF measures, are needed to detect clinically relevant early impairments in LVDF.

The E/A ratio has traditionally played a central role in paediatric clinical practice for the assessment of LVDF and this was confirmed with 53 of the 71 studies reporting E/A ratios. However, this measure is more influenced by ventricular loading conditions (fluid volume status) than TDI equivalents and, unlike TDI measures, summarises global ventricular compliance, rather than, for example, longitudinal septal compliance. This probably makes it less sensitive to the earliest pathological changes in LVDF, which may be localised, compensated for by other elements of diastolic function elsewhere in the ventricle, and preferentially affecting particular myocardial fibre groups/directions in the heart. In this study, the E/A ratio was only weakly associated with BMI compared to other LVDF measures, supporting this suggestion and the findings of earlier studies.[193] Therefore, data suggests the E/A ratio should not be used alone to assess LVDF in paediatric OW/Ob.

### 3.4.3 Cardiometabolic health and LVDF

IR is associated with cardiovascular events,[301] and contributes to progressive declines in LVDF, independent of confounders.[302] The pathogenesis of IR associated with obesity includes abnormal adipokine/cytokine production, systemic inflammation, mitochondrial dysfunction, lipotoxicity, oxidative stress, hypoxia, and hyperinsulinemia, likely stemming from increased VAT depots[128, 129] and contributing to impaired LVDF. This study found that HOMA-IR was associated with numerous measures of LVDF in young people with OW/Ob, confirmed by the findings of the systematic review. Averaged E/e' ratios and other TDI measures were more strongly associated with HOMA-IR than PWD measures, reflecting the pattern of associations with BMI, but Fisher's z-test was unable to demonstrate statistically significant differences in the strength of these associations, probably due to the smaller dataset. Although the relationship with BMI and HOMA-IR were broadly similar, IVRT was more strongly related to HOMA-IR than it was to BMI.

As described by Nagueh,[20] "*myocardial relaxation is measured by the rate of LV systolic pressure decay during the IVR*". During this period, removal of $Ca^{2+}$ enables the detachment of actin-myosin cross-bridges and results in the decline of cellular tension and relaxation of the sarcomere units. This is an

energy-dependent process that is regulated by enzymes such as SERCA2a, the $Na^+/Ca^{2+}$ exchanger, and phospholamban.[20, 86] It has been shown that diastolic strain rate during IVR is more strongly linked with -dP/dt and τ (gold standard measures of LVDF – see Chapter 2) than during early filling.[20] Therefore, as energy is needed to enable actin-myosin decoupling, the stronger links of HOMA-IR with IVRT could be due to IR impacting myocardial energetics, prolonging IVRT.[379] However, due to the relative lack of data on HOMA-IR, which could have contributed to the results of this study, further work will be needed to fully understand the independent relationships of IR with LVDF.

The MetS was originally used to define the myriad of effects of IR.[120] As IR was the most commonly reported CMRF associated with LVDF in our results, and with no consensus on the definition of MetS in children, markers of IR (e.g. HOMA-IR) may be better suited to summarise metabolic health associated with LVDF in children with obesity. Further work should aim to decipher whether IR is more strongly associated with LVDF than childhood definitions of MetS or other forms of cardiovascular risk factor clustering. Furthermore, other methods that more accurately reflect IR, such as the gold-standard hyperinsulinaemic-euglycaemic clamp technique, or other methods such as oral glucose tolerance tests, should be used to assess whether LVDF is linked with IR derived by these methods.

The systematic review identified some studies reporting worse LVDF in children/adolescents with obesity and poor cardiometabolic health (e.g. obesity with MetS) compared to normal cardiometabolic health counterparts. Although poor metabolic health is more likely in individuals with obesity, its adverse effects on LVDF can be demonstrated regardless of weight class in adults.[122] Future work should examine the mechanisms and consequences of both obesity and cardiometabolic health on LVDF in younger people.

### 3.4.4 Exercise and LVDF

The health benefits of PA are well-known. Low PA and CRF are both associated with a higher risk of LVDD in adults.[380, 381] The limited evidence found here suggests that lower levels of CRF in childhood may also be linked with reduced LVDF, but more studies are needed before meaningful conclusions

can be drawn. Similarly, there have been no studies examining the link between PA and LVDF in children. Given that PA levels are decreasing in children and adolescents,[64, 159] further work should aim to study PA and its relationship with LVDF.

Results from adult exercise/lifestyle interventions on LVDF are inconclusive, with some reporting a return to normal function,[303] some reporting improved function,[382] and some reporting no change.[304] This could be because irreversible cardiovascular remodelling has occurred in some adult populations after long-term exposure to obesity and CMRFs. Although not directly comparable to humans, animal models show prevention of LVDD, induced by obesity and the MetS, by regular bouts of exercise.[383] A small number of studies identified in this study supports this, showing improvements of LVDF in children with obesity after an exercise or lifestyle intervention. The limited evidence found suggests a possible advantage of early intervention in children, prior to irreversible cardiovascular remodelling, but more data are needed before meaningful conclusions can be made.

Although only reported in two interventional studies, exercise was linked to a significant improvement in IS.[341, 349] Skeletal muscle is known to be an important repository for blood glucose, while exercise is known to improve IS in skeletal muscle as well as increasing total skeletal muscle mass.[384] If there is increased skeletal muscle IS, then a similar mechanism could exist in the myocardium, potentially providing a mechanism for improved LVDF. Such a mechanism has been identified in rats where isolated cardiomyocytes and isolated perfused hearts exerted a stronger contractile force to a given insulin concentration following a programme of swim training.[385] Given that HOMA-IR was associated with LVDF and that IR was the primary CMRF associated with LVDF in the systematic review, improved IS following an exercise programme may be an important way to improve LVDF. Interventions aimed at improving IR and LVDF are therefore worth further exploration.

### 3.4.5 Standardisation of group definitions

Previous studies do not report a standardised measure of obesity that can be compared between studies. This was confirmed with >20 definitions identified for normal-weight, overweight, and

obesity. This was a limitation also experienced by Koopman and Mertens in their 2014 review article of LVDF in children and adolescents.[73] When country-specific BMI z-scores are reported, authors should also report a standardized, global BMI z-score using common, easily accessible tools such as the WHO 2007 BMI z-scores.[52] This would allow for the direct comparison between studies and aid future attempts to statistically synthesise data on childhood and adolescent OW/Ob.

### 3.4.6 Strengths and limitations

This work has a number of strengths and potential limitations. Some studies were rejected from the meta-analyses due to insufficient reporting of BMI, HOMA-IR, age and sex. Nevertheless, the results of the systematic review, which was more inclusive, broadly reflected the findings of the meta-analyses, supporting our conclusions. The impact of group selection bias in the meta-analyses was limited by including the pairwise differences between groups within a study as a unique level in the multilevel model but, importantly, ignoring the authors' group definitions in favour of a continuous analysis, using the reported mean BMI instead. Linear regression analyses can be unduly influenced by non-normal distributions, but results did not differ meaningfully after the normalisation of distributions and with repeat analysis using robust z-scores.

Although some studies reported BMI z-scores, it was not possible to construct an analysis using BMI z-scores due to the heterogeneity in the normative data used to calculate them. Any study that defined groups using BMI z-scores and that were not included in the meta-analyses were included in the systematic review.

The marked heterogeneity in definition of normal-weight and OW/Ob required an analytical approach that ignored these group definitions. As a consequence, it was also not possible to analyse whether individual study findings were distributed evenly around the mean effect size and, therefore, less likely to be subject to publication bias. Thus, this study does not address possible publication bias statistically. However, it should be noted that only data on unpublished studies truly allow for publication bias to be determined and these are not available in this context.

## 3.5 Conclusions

This review provides the first evidence by meta-analysis that childhood and adolescent adiposity, as indexed by BMI, is associated with worse LVDF, and most strongly associated with septal e'/a' ratios. These findings should aid the development of paediatric guidelines for the assessment of LVDF, by highlighting the most sensitive measures for early detection of LVDF in children and adolescents with OW/Ob.

Increased levels of HOMA-IR were found to be associated with LVDF, which may be particularly useful for understanding the early pathogenesis of LVDD. Further work should address the longitudinal consequences of childhood obesity and cardiometabolic dysfunction with LVDF.

There was some evidence of better LVDF with higher CRF, while exercise/lifestyle interventions provided signs of improved LVDF post-intervention in children with obesity. There have also been no studies in this age-group that examined the link between PA and LVDF and so further work is needed to understand relationships of PA and CRF with LVDF and the benefit of early intervention.

# Chapter 4: General methods

The work in this thesis has been collected as part of a bigger study – the Oxfordshire Sedentariness, Obesity & Cardiometabolic Risk in Adolescents – a Trial of Exercise in Schools (OxSOCRATES) (NCT04118543). The study was approved by The University of Oxford Ethics Committee (Ref. R54302/RE006) and was in accordance with the Declaration of Helsinki.

In this chapter, a general overview of the OxSOCRATES Study as well as the methods of how the participants were recruited is presented.

## 4.1 OxSOCRATES Study

The OxSOCRATES Study aims to comprehensively characterise how early metabolic dysfunction affects cardiovascular health in adolescents. Reversibility of cardiovascular ill-health in the group with OW/Ob will be performed by RCT, comparing the effect of an 8-week online exercise intervention to a low-activity sham intervention. I was co-responsible for the collection and analysis of the pre-screening and baseline data collected from a sub-population of OxSOCRATES participants, as well as incorporating a cardiopulmonary exercise test (CPET). The inclusion and exclusion criteria for these participants are detailed below.

## 4.2 Inclusion criteria

Age and sex-appropriate BMI z-scores were calculated using WHO standards.[52] OW/Ob were defined as a BMI z-score >1 (overweight: >1 and ≤2; obesity: >2) and normal-weight as a BMI z-score ≥ -2 and ≤1. Adolescents with normal-weight and with high vigorous PA (VPA) (≥75$^{th}$ percentile; 18.7 minutes for boys and 14.5 minutes for girls) or low VPA (≤25$^{th}$ percentile; 8.7 minutes for boys and 6.5 minutes for girls) were recruited as a control group. Any normal-weight participants that were recruited before

classification of VPA cut-offs were included in non-group-based statistical analyses. Thresholds for normal-weight VPA were based on the OxSOCRATES pre-screening data.

## 4.3 Exclusion criteria

Participants that had contraindications for exercise determined by the Physical Activity Readiness Questionnaire;[386] safety issues due to behavioural/intellectual limitations, or medical conditions such as neuromuscular disorders, uncontrolled epilepsy and/or congenital heart disease; dairy allergy and/or Type-1 Diabetes (due to the ingestion of a dairy drink during the OxSOCRATES protocol); or had any contraindications for MRI were excluded.

## 4.4 Recruitment

Adolescents were recruited by two methods: (1) school-based recruitment following the OxSOCRATES pre-screening process; and (2) recruitment by poster and email advertisement through secondary schools and academic institutions in Oxford.

### 4.4.1 School pre-screening

#### *4.4.1.1 Pre-screening process*

The primary method of recruitment was from schools. Parents were informed by letter from their child's school that their child(ren) will be participating in a physical education (PE) lesson that included a fitness and health screening session. Anonymous data on fitness indicators and anthropometrics were collected to characterise the year-group. The schools retained a file linking the children's names to random identifiers that OxSOCRATES researchers did not have access to. Parents and children were given the opportunity to opt-out and adolescents who opted-out participated in the PE lesson but no data were collected.

#### *4.4.1.2 Recruitment*

The schools informed families about OxSOCRATES and were provided with the latest age-appropriate version of the participant information sheet as well as a consent form and a form to provide their contact details. When signed consents and contact details were returned to schools, teachers provided the OxSOCRATES Study team with the matched names to random identifiers from the pre-screening sessions, which meant the eligibility of those that expressed an interest in the study could be assessed. The names to unique identifiers were stored in a password-protected file on a secure University drive that only OxSOCRATES researchers had access to. If eligible, parent(s)/guardian(s) and their child(ren) were contacted to discuss the OxSOCRATES Study.

### 4.4.2 Poster and email online recruitment

A small number of participants were recruited by poster and email advertisement through secondary schools and academic institutions in Oxford. Schools and academic institutions circulated a poster to parents through their internal mailing system. Parents of children who expressed an interest in the study were emailed an online consent form and an online data collection form to provide their child's height, weight, and month and year of birth. If eligible, parent(s)/guardian(s) and their child were contacted to discuss the OxSOCRATES Study.

### 4.4.3 Sibling recruitment

Any siblings of participants that expressed an interest and that were eligible were also recruited.

## 4.5 Informed consent

All participants and parent(s)/guardian(s) were provided with the latest age-appropriate version of the participant information sheet. A telephone conversation was organised with each participant and their parent(s)/guardian(s) so that verbal explanations of the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol; and all known risks or benefits involved in taking part could be explained. Participants and/or their parent(s)/guardian(s)

were then asked to sign and date the latest informed consent/assent form(s). Participants that were willing and able to register their informed assent (<16 years old) or informed consent (≥16 years old) to participation and whose parent(s)/guardian(s), where appropriate, gave informed consent for participation of their child in the study were recruited.

## 4.6 Study visits

All participants completed a baseline assessment at Oxford Brookes University (OBU) and a baseline assessment at the Oxford Centre for Magnetic Resonance (OCMR). Study visits to OBU were mainly performed between 4-7 pm to avoid school hours and to ensure parent/guardian availability. However, some visits were performed at the weekend or during school holidays. All participants were asked to come hydrated, wearing light sports clothing, were asked to avoid exhaustive exercise at least 24 hours prior to attendance and to avoid eating large meals at least one hour prior to attendance. For study visits at OCMR, all participants were asked to abstain from caffeine, tobacco and alcohol use for at least 24 hours prior to attendance, abstain from exercise from 3 pm the previous day and consume a standardised meal (Margherita pizza) in the evening before attendance then fast overnight. When participants were booked in the afternoon, they were asked to consume bread or toast no later than six hours prior to scanning, with only a small quantity of butter or margarine and no other topping. At both visits, informed consent and assent were obtained from parent(s)/guardian(s) and participants, respectively.

## 4.7 Statistical analysis

All statistical analyses were completed using STATA (version 16.1, StataCorp, College Station, Texas). Statistical analyses unique to Study 2 and 3 are reported in sections 5.2.7 and 6.2.4, respectively. The following were used in both studies. Histogram plots were used to assess the normality of variables. Any non-parametrically distributed data were zero-skewed log-transformed by taking the natural log of the non-parametric variable after a correction factor had been applied (using the *-lnskew0-*

function). Data are represented as mean ± SD or median (IQR), depending on distributions. A p-value of <0.05, or when 95% CIs did not span zero, were considered statistically significant.

Following the completion of Aim 1 (section 1.8), the below analyses were planned for Aims 2-4 (section 1.8). Further detail is provided in the relevant data chapters.

**Aim 2: Determine the existence and extent of relationships between adiposity measures and LVDF in a population of adolescents with OW/Ob compared to those with normal-weight.** This aim was primarily answered in Study 1. Further analyses by multiple regression using LVDF and measures of adiposity, as well as confounders, were done to assess the strength of relationships. Based on the septal e'/a' results of Study 1, at least 14 participants (seven with OW/Ob and seven with normal-weight) were needed to achieve 80% power to detect a significant relationship of BMI with septal e'/a' ratios.

**Aim 3: Establish the existence and extent of relationship of different PA intensities, CRF and cardiometabolic health with LVDF in adolescents, independent of OW/Ob.** As PA can be modified on a day-to-day basis, whereas CRF and IR are more fixed, the primary aim was to determine the independent link of PA with LVDF. Multiple regression and mediation analyses of PA intensities, controlling for adiposity and confounders, were done to assess their independent relationship with different LVDF measures. As there have been no PA studies of this in adolescents, power for these analyses was determined *post-hoc* and detailed in the corresponding study. Similar multivariable analyses were performed with CRF and IR.

**Aim 4: Identify possible ways to prevent the development of impaired LVDF through PA.** As CRF is an important CVD risk factor and has been linked with LVDF in adolescents,[326] CRF from the OxSOCRATES pre-screening process was used to determine the extent to which each PA intensity is associated with CRF, independently of other PA intensities, and the duration of activity at each intensity associated with maximum CRF. Moving average models were optimally fitted to determine relationships between residualised PA variables and CRF. Threshold regression models determined

the duration of PA above which CRF improvement was minimal. To date, objectively assessed PA studies have reported the link of univariate associations between PA intensities and CRF (or using the combined metric of MVPA) and have not controlled for the potential intercorrelation between intensities either by multiple regression or by partial regression. Thus, power to detect the independent effect of PA intensities was determined *post-hoc* and detailed in the corresponding study.

# Chapter 5: Left ventricular diastolic function in adolescents with overweight/obesity – the link with physical activity, cardiorespiratory fitness, and insulin resistance (Study 2)

Study 1 identified that measures of OW/Ob are linked with LVDF in children and adolescents, but that there has been very little research to assess the link with PA. There has also been limited research assessing the links of CRF and IR with LVDF, once adjusted for adiposity. This chapter aimed to establish the existence and extent of relationships of different PA intensities, CRF and IR with LVDF in adolescents, independent of adiposity.

I have given an oral presentation of this work at the European Childhood Obesity Group 31st annual conference, which was published as a conference abstract.[387]

## 5.1 Introduction

### 5.1.1 Physical activity and LVDF

In adult studies, subjectively assessed PA is independently associated with heart failure outcomes, irrespective of BMI,[69, 388] and is more strongly associated with diastolic rather than systolic heart failure.[69]

In the 30-year follow-up of children from the Cardiovascular Risk in Young Finns Study, childhood levels of subjective PA were linked with adult LVDF (E/e' ratio), independent of child and adult adiposity, systolic BP, and other risk factors.[27] Although this study suggests that early intervention could prevent future adult LVDD, it did not assess the link with childhood/adolescent LVDF as a potential marker of change, and used subjective PA assessments.

In children and adolescents, there is a paucity of evidence of the link between PA and LVDF. Fussenich *et al*[389] assessed LVDF and objectively measured PA in children. They found that there was no difference in LVDF (assessed by the E/A ratio) between those who did and did not meet the WHO guidelines in 182 children aged 10-11 years. However, the E/A ratio may not be best-suited to assess LVDF in this age-group,[193] as shown in Study 1. Furthermore, the PA sampling rate was set at 5-second epochs, which may not capture the sporadic nature of PA in children and aolescents.[257] This is especially important for VPA where ~55% of VPA typically occurs during 2-seconds epochs, whereas only ~15% is completed during 5-second epochs.[257] Also, as children aged 10-11 years were assessed, the detrimental effect of low levels of PA may not have been detectable yet. However, irrespective of this, the authors did find that those who performed more 5-second VPA epochs were at lower risk of other CVD risk factors, such as adiposity, than those who performed less. They concluded that 17 minutes per day (min/day) of VPA is required to reduce CVD risk,[389] potentially highlighting the importance of VPA over other intensities, which is supported by a number of other child and adolescent studies.[46, 273, 390-395] Given the limitations of this study, further work is required to understand the extent of the link between PA and LVDF.

Two review articles support the case for VPA using objective measures of PA.[392, 393] Preliminary evidence in these reviews indicate that participating in longer durations of VPA is more strongly associated with some measures of cardiometabolic health than lesser intensities. The review by Gralla and colleagues[393] concluded that a minimum of 10 min/day of VPA may be sufficient to decrease adiposity and improve cardiometabolic risk factors in children and adolescents. However, the majority of these studies included in the reviews were cross-sectional, addressing associations, rather than RCTs. Furthermore, PA was assessed with a variety of different epoch lengths, which are known to influence the classification of PA intensities in children, with longer epochs classifying VPA as MPA and SB as LPA, and therefore highlighting the need for research with shorter epoch lengths.[396, 397]

A meta-analysis on childhood obesity has utilised a small number of RCTs (n=9) to examine the effect of HIIT (brief bursts of VPA) versus MICT on cardiometabolic health.[398] Results suggested that there are no differences between HIIT and MICT for anthropometric adiposity measures, glucose and lipid metabolism, and BP, although HIIT improved CRF to a greater extent.[398] However, there was large heterogeneity in the prescription of HIIT and MICT between studies. For example, some HIIT studies performed serial one-minute bouts at 100% maximal HR, interspersed with three-minute 50% maximal HR recoveries, whereas others performed two-minute bouts at 75-80% maximal HR with one-minute rests. Other studies defined MICT as 80% maximal HR, overlapping with HIIT prescriptions between some studies. There was also a lack of reported information on workload and attendance rates. Thus, review articles have concluded that more research is needed to determine whether VPA is more beneficial than MPA for CMRFs and cardiovascular structure and function, as well as highlighting the need for well-designed RCTs.[392, 393, 398]

Although performing more PA may directly improve cardiovascular health, it may also have an indirect effect by decreasing adiposity. Previous review articles have shown greater benefit of VPA over MPA for reducing adiposity in children and adolescents, but previous studies have typically assessed adiposity by anthropometrics, skin-fold thickness, BIA, and/or DEXA, which are each methodologically

limited (Chapter 2)[392, 393] and did not distinguish between VAT and SAT. Therefore, utilising MRI (the research gold-standard) is important to determine what intensity of PA is most important for reducing specific fat depots, especially VAT. The recent study by Medrano *et al*[46] has addressed this and found that only VPA is associated with VAT in children with overweight/obesity. Although MVPA was also linked, this was driven by VPA. As this was a cross-sectional study and as a control group with normal-weight was not included, more research will be required to confirm this. Therefore, as increased PA, mainly VPA, can lead to reductions in adiposity, it is unknown whether VPA has a direct effect on LVDF or whether measures of adiposity mediate this relationship.

Although research into PA and LVDF in adolescents is limited, a small number of studies have examined the link between LVDF and CRF.

### 5.1.2 Cardiorespiratory fitness and LVDF

In adult studies, CRF mediates the relationship of BMI with CVD outcomes. In the study by Kokkinos *et al*,[399] being in the highest compared to lowest tertile of CRF resulted in a 63% (normal-weight), 66% (overweight), and 73% (obese) lower risk of heart failure in men. However, there was no standardised age as to when participants completed the CRF test, which could have taken place any time between 1987-2017. Therefore, some could have completed the test closer to when they experienced a heart failure event, meaning that their CRF would have been impaired to a greater extent than if they completed the test 10-15 years prior, potentially biasing results. In another study, once adjusted for CRF, individuals with metabolically-healthy obesity had a similar prognosis for CVD outcomes as metabolically healthy normal-weight individuals, and a better prognosis than metabolically-unhealthy obese individuals.[148] However, much like the all-male study by Kokkinos *et al*,[399] this study had a large male bias (75.7%) and was also mostly Caucasian (98%). Although methodologically limited, these data suggest that high CRF may mediate CVD risk in individuals with OW/Ob, but less is known in adolescents, especially with regards to LVDF.

Study 1 identified two studies that have assessed the relationship between CPET derived CRF and LVDF.[326, 328] As both these studies were relatively small in size and did not account for adiposity in their analyses, more research is required to determine the independent link between CRF and LVDF.

### 5.1.3 Insulin resistance and LVDF

Large community studies show that increasing levels of IR lead to a progressive decline in LVDF, independent of confounders in adults.[302] Although commonly seen in individuals with obesity, IR might be independently linked with LVDF, possibly by: promoting LV hypertrophy; impairing glucose transportation into the cell; impairing intracellular signalling kinase pathways; impairing $Ca^{2+}$ handling; reducing metabolic flexibility; impairing mitochondrial function; and causing endothelial dysfunction, all of which can ultimately lead to reduced LV compliance, fibrosis and myocardial cell death,[106-109, 127-130, 136, 137] stiffening the LV.

Study 1 identified that IR was associated with impaired LVDF in adolescents, but the majority of these analyses were not adjusted for adiposity. Two studies[144, 323] report that adolescents with IR and OW/Ob had worse LVDF compared to insulin sensitive counterparts. However, adiposity was assessed by BMI z-score in these studies, which is a poor measure of adiposity in children/adolescents.[202-205] PA and CRF are also known to be independently linked with IR,[141, 143] highlighting the need for further work to understand the individual involvement of more precise measures of adiposity and IR in LVDF. Furthermore, although Study 1 found associations between fasting measures of IR and LVDF, more dynamic tests of IR are available and so investigation of how these are linked with adolescent LVDF are warranted (a discussion of these other tests is provided in Appendix 2).

### 5.1.4 Aims

Understanding the link between PA and LVDF may provide a mechanism to improve LVDF and/or prevent future LVDD in adolescents. Thus, this study will use the baseline, cross-sectional data from the OxSOCRATES study with the primary aim of establishing the existence and extent of relationships

of different PA intensities with LVDF, independent of the potential mediating effect of adiposity. Secondary aims of this study include assessing group-based differences in LVDF between those with OW/Ob, normal-weight with high VPA, and normal-weight with low VPA, as well as the extent of the independent relationships of adiposity, CRF, and IR with LVDF. Elevated BP is common in OW/Ob[103] and is known to be linked with reduced LVDF in children and adolescents,[131] and so ambulatory BP data will also be used to establish the extent of any independent relationships of LVDF with BP.

## 5.2 Methods

Participants were included as part of the baseline assessments of the OxSOCRATES Study (Chapter 4). Briefly, participants had their adiposity, anthropometrics, and CRF assessed at OBU and were set-up with a wrist-worn accelerometer for PA. Participants also had a visit to OCMR on another day and had echocardiography and blood samples completed. This study was written in accordance with the STROBE reporting guidelines.[400]

### 5.2.1 Adiposity

#### 5.2.1.1 *Anthropometrics*

Height to the nearest 0.1 cm and weight to the nearest 0.1 kg were measured using a portable Harpenden Stadiometer (Holtain Ltd, Crymych, UK) and a SECA medical 770 digital floor scale (SECA, Hamberg, Germany), respectively. Participants were instructed to remove their shoes and any hats and/or ponytails. During the school screening and OBU visits, participants wore light sports clothing and were instructed to remove any heavy clothing (e.g. jumpers) where appropriate. During OCMR visits, participants wore light scrubs. BMI was calculated using height and weight and was additionally reported as age- and sex- adjusted z-scores, using WHO 2007 reference data.[52] As age was not obtained during the school screening sessions, all children that completed the pre-screening process were given a standardised age for that academic year for the purpose of calculating estimates of sex-

and age-adjusted BMI z-scores to assess eligibility. For example, children in Year-9 (aged 13-14 years) were given an age of 13.5 years.

#### 5.2.1.2 *Bioelectrical impedance analysis*

During the visit to OBU, BIA was assessed using a Tanita MC-780 body composition monitor (Tanita Corporation, Tokyo, Japan) before their CPET. Participants were asked to step onto the scale with clean feet, ensuring correct placement of the heel and toes on the electrical pads. Participants took a firm grip of the handheld electrodes and placed their arms down straight by their side. We ensured that participants' arms did not touch their body and that their inner thighs were not touching where possible. Total fat mass (kg) from the Tanita MC-780 was recorded, which is comparable to DEXA equivalents ($r=0.93$, $p<0.001$; ICC=0.88, 95% CI 0.84-0.91), whereas fat mass percentage equivalents are less strongly linked in adolescents ($r=0.78$, $p<0.001$; ICC=0.66, 95% CI 0.56-0.75).[401] Furthermore, fat mass measured by the Tanita MC-780 in adolescents is highly reproducible over three consecutive days (test-retest ICC=0.99),[401] supporting its use to measure fat mass in adolescents. The FMI was calculated by dividing BIA fat mass (kg) by height (m) squared. BIA fat-free mass was also used for indexing maximal oxygen consumption ($VO_2$max).

### 5.2.2 Physical activity

#### 5.2.2.1 *Data collection*

During the school pre-screening sessions, adolescents were provided with a small triaxial, wrist-worn accelerometer (Axivity AX3, Axivity Ltd., Melton Park, UK) and were asked to wear the accelerometer for seven consecutive days. The sampling frequency was set to 100Hz with a dynamic range of ±16g to avoid a ceiling effect on VPA. If participants did not complete the pre-screening process or had invalid PA (see below), then participants were provided with an accelerometer at the baseline OBU assessment.

Wrist-worn accelerometery has been shown to accurately predict children's energy expenditure,[402, 403] while the AX3 accelerometer has been shown to accurately detect various child postures and PA intensities,[404, 405] as validated by its successful use in large paediatric studies.[271] Many studies have traditionally used hip-worn accelerometry due to theoretical advantages in measuring whole-body movement.[270] Although hip- and wrist-mounted accelerometer measurements of MVPA correlate well, their correlation for LPA and ST is weaker, with wrist-worn devices tending to measure more PA and less ST.[270] This is probably because of decoupling of wrist and hip movements at lower intensities/ST.[270] We chose to use wrist-worn accelerometers in OxSOCRATES as this method increases wear compliance in children,[270] but this does limit comparison with studies using different body locations.

#### 5.2.2.2 *Data analysis*

A valid recording consisted of at least three weekdays and one weekend day. Each valid day required >6 hours of awake wear time (>50% awake time for most participants). To define the noise floor of stationary AX3 sensors, accelerometers were placed on a flat surface, with no interference for 24 hours. From this, non-wear was defined as periods where the SD and range of g for 1-second epochs varied by <0.018g and <0.11g, respectively, for at least 15 minutes.[262] Non-wear periods were excluded from analyses. Although an algorithmic process was used initially to identify day/night periods and non-wear time, all data were then screened manually and edited if necessary.

A bespoke MatLab script, written by Dr Alexander Jones, calibrated the three axes using an established method.[406] Briefly, this method exploits the fact that each time the sensor comes to rest at an arbitrary orientation with respect to gravity, gravitational acceleration (1 g) will add a point to a spherical, 3-dimensional point cloud with radius 1 g. If sufficient points accrue with sufficient dispersion around the sphere, as is usually the case in a 7-day recording, it can be determined whether the cloud is spherical or ellipsoid. In the latter case, one or more accelerometer axes are over- or under-estimating the force of gravity and can be calibrated to correct this. After calibration, the signal was filtered using

a 4th order Butterworth bandpass filter (0.2-15Hz) and EN minus one (ENMO) was calculated and summed into 1 second epochs, which is referred to as bandpass-filtered followed by EN (BFEN). As VPA may be more important than lesser intensities of PA for cardiovascular health,[273, 389-395] BFEN was used as this was shown to better classify VPA (mean bias = -0.5%) than other metrics in the target age-group and shown to be statistically equivalent to vigorous activities assessed by indirect calorimetry.[407] Previously validated thresholds for BFEN were used to determine the average duration of daily PA in each intensity: 0.1g for light PA (LPA), 0.314g for MPA, and 0.998g for VPA.[168, 407] PA below the LPA threshold was categorised as sedentary time (ST).[168] These BFEN thresholds are both sensitive and specific for classifying different intensities of PA with indirect calorimetry (ST: sensitivity=0.97, specificity=0.88; LPA: not applicable [ST and MPA cut points established the boundaries for LPA]; MPA: sensitivity=0.91, specificity=0.87; VPA: sensitivity=0.95, specificity=0.85).[168]

Other metrics of PA processing were calculated, using established methods, and were also included in analyses to ensure that using BFEN did not bias results. PA bands were defined using published thresholds relevant to each metric.[262] The metrics were ENMOz (ENMO with negative values rounded to zero; thresholds: LPA, 0.1g; MPA, 0.192g; VPA, 0.696g),[408, 409] HFEN (a 4$^{th}$ order Butterworth high-pass filter [0.2Hz] applied to each of the three axes followed by EN; thresholds: LPA, 0.1g; MPA, 0.314g; VPA, 0.998g),[168] and HFEN+ (HFEN plus ENMO of the three axes after a 4$^{th}$ order Butterworth low-pass filter [0.2Hz]; thresholds as per HFEN). Signals were also translated to ActiGraph counts for comparability with other studies, using a validated method.[410]

An individual's PA in a given intensity category is correlated with their PA at other intensities, preventing robust comparison of the effects of different intensities on health outcomes. This can be addressed by constructing variables that contain only the variance at each intensity that is independent of all other intensities (i.e. zero correlated). This was done using partial multivariable linear regression modelling[411] to generate a set of residualised variables for MPA, LPA, and ST, which together with their base variable (unadjusted VPA), are referred to as rPA variables (see Appendix 3

for details). To check that the directionality of this partial modelling did not influence the results significantly, this process was repeated in the opposite direction, starting with ST as the base variable, to generate another set of rPA variables. Standard multiple regression with all unadjusted PA intensities was also used to confirm the findings of partial models

### 5.2.3 Cardiorespiratory fitness

#### 5.2.3.1 *Data collection*

At OBU, participants completed a breath-by-breath analysis of $VO_2$peak on an ergometer bike (Lode Corival CPET, Groningen, The Netherlands) using the Godfrey protocol (a steady-state test with stepwise increases in work rate).[285] Reliability of measuring $VO_2$max by a cycle ergometer in youths with obesity is high with a 0.98 test-retest intraclass correlation coefficient and variability of only 5.7%.[412] Room temperature was set between 16-20°C. $O_2$ consumption and $CO_2$ production were assessed using a Cortex MetaLyzer 3B (Cortex Biophysik GmbH, Leipzig, Germany). Two minutes of unloaded pedalling (phase 1) was followed by an increase in workload each minute of either 15 or 20 watts for participants ≤150 cm and >150 cm tall, respectively (phase 2). The OMNI Scales for rating perceived exertion (RPE) for both legs and breathing[413, 414] were recorded during unloaded pedalling, after alternate workload increments, and at the end of the test. The test was terminated when the participant reached volitional exhaustion or was unable to maintain a cadence of 60 revolutions per minute (RPM) despite verbal encouragement. Once the test had finished, participants completed three minutes of low resistance (25 watt) cycling (phase 3). As per other studies of $VO_2$peak in children,[415] at least two of the following criteria had to be met for successful completion of the test:

1. HR >180 beats per minute;
2. a respiratory exchange ratio (RER) >1.06;
3. whether the ventilatory threshold was reached;
4. RPE for legs and breathing >8;
5. subjective signs of exhaustion such as sweating, heavy breathing and/or red-faced.

#### 5.2.3.2 *Data analysis*

For 20mSRT results, the level and stage were transformed into sex-specific CRF z-scores. These were calculated for all participants based on results from the sample of boys (n=143) and girls (n=124) with normal-weight from the pre-screening.

**Classification of peak values – CPET**

Raw data for $VO_2$, HR, and RER were classified into 15-second median epochs and filtered using a zero-phase, 8$^{th}$ order Butterworth low-pass filter (cut-off 0.5Hz). The maximum value of the filtered data during phase 2 of the CPET was used to determine $VO_2$peak, peak HR, and peak RER. Peak work rate was determined as the work rate when the exercise test was terminated. The average RPM of the 15-second epochs during phase 2 of the CPET were also reported.

**Dealing with sub-maximal tests – CPET**

When participants did not complete a maximal test, $VO_2$peak was estimated using their submaximal data. For individuals who achieved a maximal test, data within the HR zone of 155-165 bpm were averaged and used to determine submaximal parameters. A multiple regression model using height, weight, and these submaximal estimates of $VO_2$ and $VCO_2$ was found to predict 83% of the variance of actual $VO_2$peak ($r^2$=0.83; Figure 5.1) in 97 participants with reasonable precision and minimal bias. The resultant regression equation was:

$$\text{Estimated } VO_2\text{peak} = 0.0148(\text{height[cm]}) - 0.00517(\text{weight[kg]}) + 2.652(VO_2 \text{ subpeak[L/min]}) - 1.639(VCO_2 \text{ subpeak[L/min]}) - 1.520$$

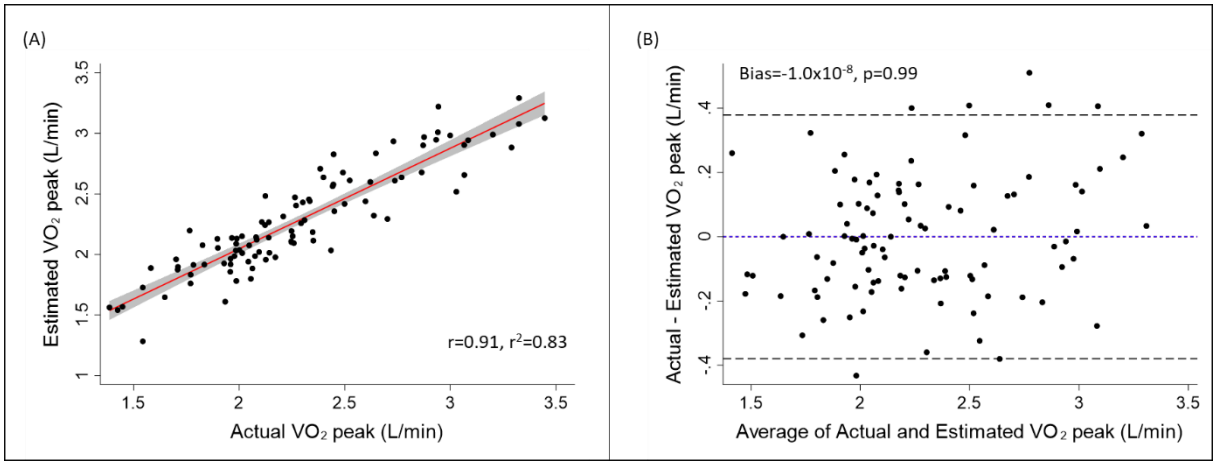


**Figure 5.1: Estimated peak oxygen consumption ($VO_2$) versus actual $VO_2$.**

Regression plot (A) and Bland-Altman plot (B).

The reported CRF measures were peak work rate, peak HR, and $VO_2$peak (L/min). $VO_2$peak standardised for body mass ($VO_2peak_{kg}$ [mL/kg/min]), and $VO_2$peak standardised FFM ($VO_2peak_{FFMkg}$ [$mL/kg^{FFM}$/min]) were also calculated using $VO_2$peak, body mass, and FFM (determined by BIA), respectively. $VO_2peak_{kg}$ has been described as a "performance" measure of maximal CRF (a "*measure of the immediately available oxidative energy which can be supplied to move a kilogram of body weight from one point to another*"),[416] whereas $VO_2peak_{FFMkg}$ has been described as a "physiological" measure of CRF, since fat mass does not contribute to the work being performed.[417] Therefore, both measures are included in all statistical analyses.

### 5.2.4 Echocardiography

At OCMR, echocardiography was completed following standard guidelines[19, 85] using a General Electric Vivid i portable ultrasound system with a 3S-RS transducer (GE Healthcare, Chicago, United States of America). Participants were positioned in the left lateral decubitus position. Up to ten cardiac cycles for each LVDF measure were recorded and averaged to account for respiratory variation. I undertook the majority of the echocardiograms for the population of OxSOCRATES participants in this thesis. Images were reviewed/analysed offline using Horos (Horosproject.org). If signal spikes or feathering could not be avoided when acquiring wave forms, then they were ignored during data analysis. Septal

TDI metrics were used as the LVDF measures of interest based on the findings from Study 1. Details of how each LVDF measure were obtained are provided below.

#### 5.2.4.1 *Pulsed-wave Doppler*

In the apical four-chamber view, colour flow imaging was used to optimally align the PWD with mitral inflow. The PWD sample volume (1-3 mm axial size) was placed between the tips of the mitral valve leaflets. Low velocity portions of the spectrum were filtered. The amount of Low Velocity Reject was indicated by the green vertical bar at the right end of the baseline (Appendix 2, Figure 2.1). A high sweep speed was used to accurately record the E-wave, A-wave, DT, and E/A ratios (Appendix 2, Figure 2.1).

#### 5.2.4.2 *Colour M-mode Doppler*

In the apical four-chamber view, using colour flow imaging, the sample volume was placed through the centre of the mitral inflow, ensuring a vertical column from LV base to apex. Then, in colour M-mode Doppler, the baseline scale of the colour flow was lowered beyond the Nyquist limit so that the central, high velocity blood jet was blue. From this, the slope method was used to measure mitral-to-apical Vp (Appendix 2, Figure 2.2).[418, 419]

#### 5.2.4.3 *Continuous-wave Doppler*

In the five-chamber view, using continuous-wave Doppler, the sample volume was partially placed (~1 cm) into the LV outflow tract to simultaneously display aortic ejection and mitral inflow. Low velocity portions of the spectrum were filtered. The amount of Low Velocity Reject was indicated by the green vertical bar at the right end of the baseline (Appendix 2, Figure 2.3). Maximal sweep speed (200 mm/s) was used to accurately identify IVRT from the cessation of aortic outflow to the onset of mitral inflow (Appendix 2, Figure 2.3).

#### 5.2.4.4 *Tissue Doppler imaging*

In the apical four-chamber view, the PWD sample volume (5-10 mm), using the TDI preset, was placed at the lateral and septal basal regions of the LV myocardium to cover mitral annulus excursion in both systole and diastole. Waveforms were optimised so that there was minimal angulation (<20°) between myocardial motion and the plane of the ultrasound beam. A high sweep speed was used to accurately record septal, lateral, and averaged e' and a' peak velocities and to calculate e'/a' ratios (Appendix 2, Figure 2.4). E/e' ratios were calculated using E-wave peak velocities and septal, lateral, and averaged e' peak velocities.

#### 5.2.4.5 *Reproducibility*

To determine intra-observer reliability, 10 LVDF datasets were randomly selected, and I completed the analysis twice (n=20). Intra-observer reliability was assessed by intraclass correlation coefficients (ICC) using a two-way mixed effects model.[420] Excellent reliability was defined as an ICC >0.90, good reliability as an ICC 0.75-0.90, moderate reliability as an ICC 0.50-0.75, and poor reliability as an ICC <0.50.[420]

### 5.2.5 Insulin resistance and sensitivity

#### 5.2.5.1 *Blood samples*

As discussed in Chapter 4, participants came fasted to the study visit at OCMR. Participants were cannulated by a paediatric consultant and 20mL of blood was drawn and syringed into fluoride blood tubes for glucose analysis and serum-separating tubes for insulin analysis. These were gently inverted 10 times. Cannulas were flushed with ~5mL of sodium chloride (0.9%) saline solution. Samples were left at room temperature for 30 minutes before being centrifuged at 3700 rpm for 15 minutes at 4°C. Samples were then separated and placed in a fridge at 4°C.

Roughly 45-60 minutes after the baseline blood draw, participants consumed a liquid meal challenge (300mL single cream [54g fat; 62% saturates] and 88.9g maltose syrup [equivalent to 75g glucose])

flavoured with six drops of natural food flavourings (Foodie Flavours, Hertfordshire, UK). This meal was developed for use during cardiovascular MRI assessments and was shown to be well tolerated by adolescents in the pilot study run by Dr Alexander Jones.

At two and four hours post-meal challenge, 15-22.5mL of blood was drawn from the participant after a 1-2mL discard blood draw to remove any residual saline flush in the cannula and was processed as above. At the end of the study visit, samples were removed from the fridge and stored in a -80°C freezer until glucose and insulin assays were performed.

#### 5.2.5.2 *Glucose and insulin assays*

A description of the glucose and insulin assays performed by the Biochemistry laboratory at the John Radcliffe Hospital, headed by Professor Timothy James, are provided in Appendix 2.

Due to the known limitations of assessing IR and IS (detailed in Appendix 2) it was decided to run only one interim-assay during OxSOCRATES. The blood samples were transferred on the 29th March 2022. Therefore, only a sub-population had IR and IS results.

#### 5.2.5.3 *Fasting insulin resistance*

HOMA2-IR was used to compare the findings of this study to Study 1. This is an updated version of HOMA-IR to account for variations in peripheral and hepatic glucose resistance.[421] This metric has been shown to correlate well (r=0.81) with the hyperinsulinaemic-euglycaemic clamp technique in adolescents with OW/Ob.[422] The HOMA2 Calculator (version 2.2.4 – https://www.dtu.ox.ac.uk/homacalculator/) was used to calculate HOMA2-IR.

#### 5.2.5.4 *Dynamic insulin sensitivity*

IS, determined by an oral-glucose-tolerance-test (OGTT), provides more information than fasting measures because of the serial sampling employed during an OGTT, mimicking the normal physiology in response to a meal. The Matsuda index[423] was used to estimate IS as it has good correlations with

the hyperinsulinaemic-euglycaemic clamp technique in paediatric studies (r=0.74-0.78)[422, 424] and was calculated as follows:[423]

$$Matsuda\ IS\ index = 10000 / \sqrt{(G_0 \times I_0 \times G_{twm} \times I_{twm})}$$

$G_0$ indicates fasting glucose concentration (mg/dL); $I_0$, fasting insulin concentration (µU/mL); $G_{twm}$, time-weighted mean glucose concentration during OGTT (mg/dL); $I_{twm}$, time-weighted mean insulin concentration during OGTT (µU/mL).

Time-weighted mean values were determined by calculating integrals for the area-under-the-curve from baseline, two-hour, and four-hour time points using the trapezoid rule and then dividing by the total duration from the baseline to the four-hour time point to account for variations in duration between experiments.

### 5.2.6 Ambulatory blood pressure monitoring

At the end of the visit to OBU, adolescents were provided with an ambulatory BP monitor and appropriately sized cuff (Oscar 2, SunTech Medical Inc., Morrisville, USA) placed on the non-dominant arm. They were asked to wear the device for a minimum of 24-hours. The monitor was set to inflate every 20-minutes during the day and every 30-minutes at night, as per current recommendations.[425] The switch to 30-minute inflations were based on each participants' expected sleep and wake times.

A bespoke Python script, written by Dr Alexander Jones, removed any erroneous readings before data were averaged into wake, sleep, and averaged systolic and diastolic BPs. The script also calculated BP z-scores, adjusted for sex and age or height, based on the reference data from Wuhl *et al*.[426]

### 5.2.7 Statistical analysis

Differences in categorical variables between groups were assessed using Chi squared tests. Differences in continuous measures between the normal-weight and OW/Ob groups were tested using independent t-tests or Mann-Whitney U tests, depending on distributions. Differences between

the two normal-weight groups and OW/Ob groups were tested using ANOVA and Bonferroni post-hoc test or Kruskal-Wallis H tests and Dunn's post-hoc test, depending on distributions. The Kruskal-Wallis H tests and Dunn's post-hoc test was used to compare Tanner (puberty) stages between multiple groups. Differences in LVDF z-scores (calculated using mean and SD from participants with normal-weight and VPA >25$^{th}$ percentile) between the OW/Ob and normal-weight groups were tested using independent t-tests.

**Primary analyses**

The primary aim was to determine the existence and extent of relationships of different PA intensities with LVDF, independent of the potential mediating effect of adiposity. Linear regression was used to determine the univariate associations of different PA intensifies with LVDF measures, adjusted for age, sex, and Tanner score. These models were repeated with the inclusion of either BMI z-score or FMI. All unadjusted PA and rPA intensities (see Chapter 4) were included in multiple regression analyses to determine relationships that are truly independent of all other intensities (i.e. zero correlated).

As septal e'/a' ratios were found to be the LVDF measure most strongly related with adiposity in Study 1, and as VPA may be the most important PA intensity for cardiovascular health,[392, 393, 398] the primary outcome was the strength of relationship of VPA with septal e'/a' ratios independent of adiposity measures, other PA intensities, and confounders. Differences in septal e'/a' ratios across quartiles of VPA, were assessed using the Jonckheere-Terpstra test. These quartiles were constructed using partial multivariable linear regression modelling[411] so that they were independent of other PA intensities, age, sex and Tanner scale. These were repeated after adjustment for BMI z-score or FMI.

To determine the direct effect of PA and mediating effect of adiposity on LVDF, mediation analysis by SEM was performed. SEM was used as it has been shown to outperform traditional, regression based approaches to mediation analysis,[427] and allows for both the direct and indirect paths to be estimated simultaneously. One of the assumptions of mediation analyses is that there is a temporal precedence to the paths. Therefore, as PA leads to a reduction in adiposity[392] and increased adiposity in childhood

leads to future reductions in LVDF,[27] PA intensities that were found to be independently associated with LVDF in multiple regression analyses were used as the independent variable, either BMI z-score or FMI as the mediator variable, and LVDF measures as the dependent variable, adjusting for age, sex and Tanner score. As PA can lower BP in adolescents,[398] similar models were constructed but with ambulatory BP z-scores added as an additional mediator (multiple mediation). As an increase in adiposity is known to increase BP,[103] another path from adiposity to BP z-score was also added.

To determine mediation or non-mediation (significance of the indirect effect), bootstrapping using 10,000 resamples was performed to calculate bias-corrected accelerated (BCa) 95% CIs.[428-430] BCa 95% CIs were chosen as these correct for any bias and/or skewness in the distribution of the bootstrap estimates, and have greater statistical power than other bootstrap methods.[431-434] However, as it has been reported that bias-corrected and BCa 95% CIs can lead to inflated Type 1 error rates,[431-434] percentile 95% CIs were also reported.[431, 433] If all three methods of bootstrap resampling (percentile, bias-corrected, and BCa) report significant findings, then it can be presumed that results are not due to Type 1 error. This technique was used in preference to the causal-steps with Sobel test method, which may be statistically flawed and low in power.[432, 435]

**Secondary analyses**

Secondary aims of this study were to assess group-based differences in LVDF between those with OW/Ob and those with normal-weight, who were additionally split into normal-weight low or high VPA (Chapter 4). Group-based differences were determined as described above. Secondary aims were also to determine the existence and extent of the relationship of CRF, IR, and ambulatory BP with LVDF once adjusted for adiposity. Linear regression was used to determine the univariate associations of either adiposity, CRF, or IR with LVDF measures, adjusted for age, sex, and Tanner score. These models were repeated with the inclusion of either BMI z-score or FMI as covariates. As PA is the primary modifiable determinant of CRF, CRF models were repeated with the inclusion of PA.

Sensitivity analyses were performed with HR interbeat intervals added as an additional covariate in all analyses as it has been reported to be related with measures of LVDF.[19, 436] Further detail is provided in Appendix 2.

### 5.2.8 Power calculations

There have been no previous studies examining the association of PA with LVDF in adolescents with/without OW/Ob. Therefore, the required sample size to detect the independent association of PA with LVDF was determined *post-hoc* using G*Power (version 3.1.9.7). A multiple regression model of septal e'/a' ratios (dependent variable), and all PA intensities (VPA, MPA, LPA, ST), BMI z-score, and confounders (age, sex, and Tanner score) as independent variables, was fit as the primary outcome model, with the aim of measuring power for the independent effect of VPA. The results were inputted into G*Power using the "*F test – Linear multiple regression: Fixed model, $r^2$ increase*" function.

## 5.3 Results

### 5.3.1 Participant characteristics

A flowchart of the included participants is given in Figure 5.2. Some participants had missing data for a select number of LVDF measures due to the fusion of early and late stages of diastole.

Table 5.1 reports the group differences in participants characteristics, PA, and CRF. Although, there was no statistical difference in the ratio of male-to-females between any groups (adjusting for Bonferroni correction), the normal-weight low VPA group had a lower percentage of male participants. The lack of difference is likely due to the small sample size of the normal-weight low VPA group. Age, Tanner score, and height did not differ between groups. Anthropometrics, fat-mass, and FMI were highest in the OW/Ob group. No differences in these were found between the normal-weight groups.

Participants with normal-weight had lower awake, sleep, and averaged systolic and diastolic BPs than the OW/Ob group, primarily driven by those with normal-weight and high VPA. Although only

significant in some BP measures, those with normal-weight and high VPA had lower BPs than counterparts with low VPA, and those with normal-weight and low VPA had similar or marginally lower BPs than those with OW/Ob.

Participants with normal-weight performed more VPA and MPA, and performed less LPA and ST than the OW/Ob group. Normal-weight low VPA participants performed less MPA and LPA and were more sedentary than high VPA counterparts. The low VPA group had similar levels of PA and ST as those with OW/Ob.

Participants with OW/Ob were less fit than the normal-weight group. The normal-weight high VPA group had the highest $VO_2peak_{kg}$, then the normal-weight low VPA group, and then the OW/Ob group. This trend persisted when using $VO_2peak_{FFMkg}$, although differences between the normal-weight low VPA and OW/Ob groups became marginally non-significant (p=0.054). The normal-weight high VPA group had lower resting heart rates than both the low VPA and OW/Ob groups, while the two latter groups had similar resting heart rates.

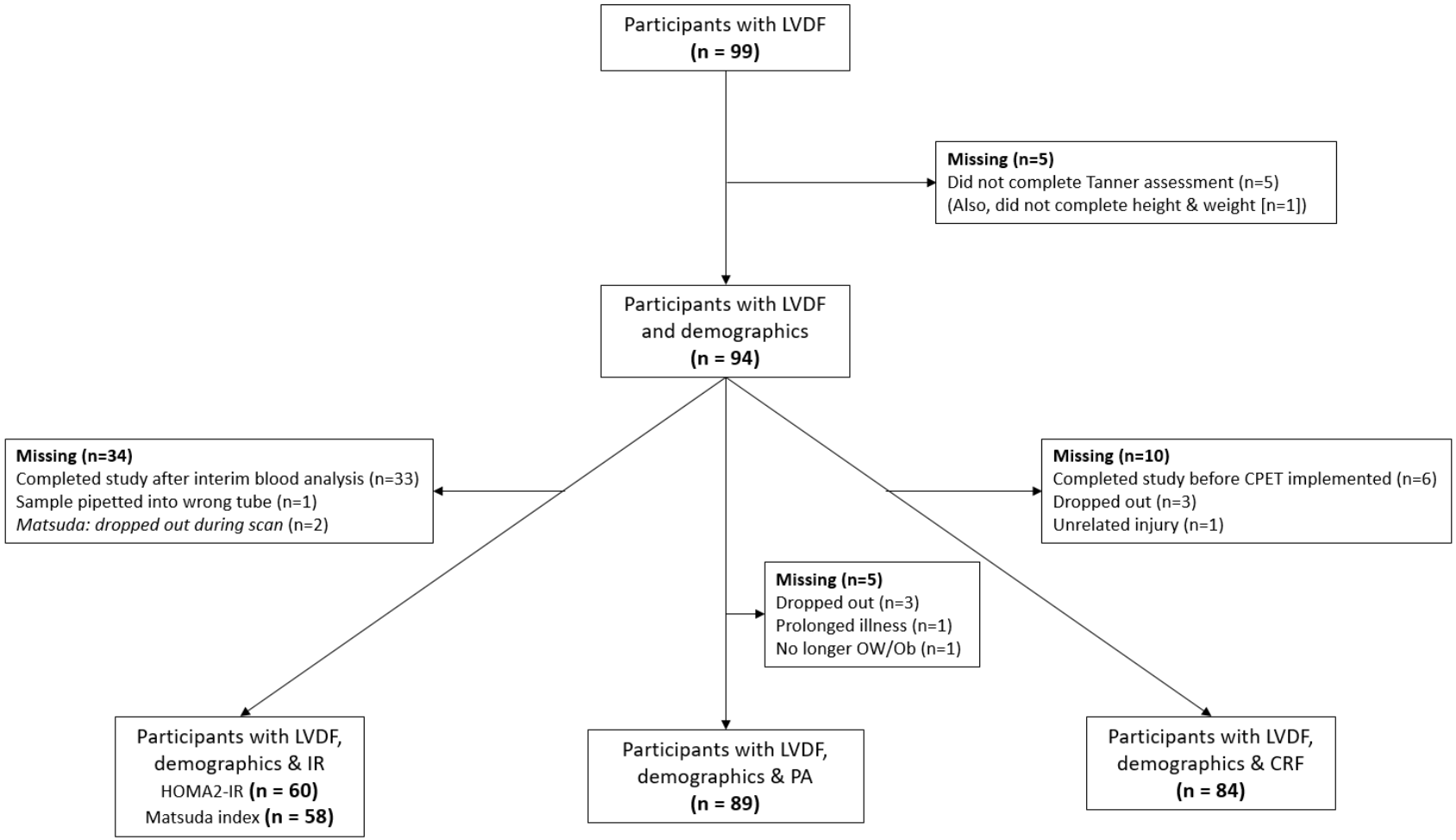


**Figure 5.2: Flow diagram of the number of adolescents included in the study.**

CPET indicates cardiopulmonary exercise test; CRF, cardiorespiratory fitness; HOMA2-IR, homeostasis model assessment of insulin resistance version 2; IR, insulin resistance; LVDF, left ventricular diastolic function; OW/Ob, overweight and obesity; PA, physical activity.

**Table 5.1: Participant characteristics**

| Measure | n | All NW [a] (Group 1) | NW High VPA (Group 2) | NW Low VPA (Group 3) | OW/Ob (Group 4) | *P*-value | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 1 vs 4 | 2 vs 3 vs 4 | 2 vs 3 | 3 vs 4 | 2 vs 4 |
| **N (n)** | 99 | 60 | 33 | 12 | 39 | - | - | - | - | - |
| **N (male %)** | 99 | 58 | 64 | 33 | 62 | 0.75 | 0.16 | 0.21 | 0.26 | 0.99 |
| **Age (years)** | 99 | 14.2 ± 1.0 | 14.2 ± 0.9 | 14.4 ± 1.6 | 14.3 ± 0.6 | 0.86 | 0.67 | 0.99 | 0.99 | 0.99 |
| **Tanner score** | 94 | 4 (3,4) | 4 (3,4) | 4 (3,5) | 4 (3,5) | 0.53 | 0.24 | 0.064 | 0.22 | 0.13 |
| **Height (cm)** | 98 | 165.2 ± 8.7 | 163.4 ± 9.3 | 168.3 ± 4.8 | 166.7 ± 7.8 | 0.38 | 0.12 | 0.26 | 0.99 | 0.25 |
| **Weight (kg)** | 98 | 52.8 ± 6.7 | 51.6 ± 7.3 | 54.9 ± 3.8 | 78.5 ± 12.9 | **<0.001** | **<0.001** | 0.99 | **<0.001** | **<0.001** |
| **BMI (kg/m²)** | 98 | 19.3 ± 1.4 | 19.3 ± 1.4 | 19.4 ± 1.1 | 28.2 ± 3.7 | **<0.001** | **<0.001** | 0.99 | **<0.001** | **<0.001** |
| **BMI z-score** | 98 | -0.1 ± 0.6 | -0.05 ± 0.6 | -0.1 ± 0.5 | 2.2 ± 0.6 | **<0.001** | **<0.001** | 0.99 | **<0.001** | **<0.001** |
| **Fat mass (kg)** | 89 | 9.3 (7.5, 11.3) | 9.3 (7.0, 10.7) | 11.8 (9.5, 13.2) | 25.2 (20.5, 29.5) | **<0.001** | **<0.001** | 0.09 | **<0.001** | **<0.001** |
| **FMI (kg/m²)** | 89 | 3.6 (2.8, 4.1) | 3.6 (2.6, 4.2) | 4.3 (3.1, 4.8) | 9.3 (7.6, 11.4) | **<0.001** | **<0.001** | 0.12 | **<0.001** | **<0.001** |
| **ABPM (mmHg)** | | | | | | | | | | |
| *Awake systolic* | 92 | 120.4 (117.0, 126.1) | 120.4 (115.4, 125.6) | 124.2 (117.7, 124.7) | 126.6 (120.3, 131.1) | **0.012** | **0.032** | 0.28 | 0.11 | **0.005** |
| *Awake diastolic* | 92 | 65.6 (61.9, 69.8) | 64.9 (61.7, 69.8) | 68.4 (65.3, 72.0) | 68.0 (63.7, 72.5) | **0.042** | **0.042** | **0.027** | 0.35 | **0.013** |
| *Sleep systolic* | 92 | 104.9 (98.4, 109.8) | 102.9 (98.2, 108.6) | 106.9 (98.2, 109.8) | 110.7 (104.2, 116.8) | **0.002** | **0.006** | 0.43 | **0.022** | **0.001** |
| *Sleep diastolic* | 92 | 51.4 (48.3, 55.8) | 51.4 (49.0, 54.8) | 52.9 (51.3, 58.9) | 55.4 (52.7, 57.2) | **<0.001** | **0.008** | 0.09 | 0.19 | **<0.001** |
| *Average systolic* | 92 | 116.2 (112.4, 121.8) | 115.9 (112.4, 121.1) | 118.8 (110.7, 120.8) | 123.1 (116.2, 129.2) | **0.004** | **0.007** | 0.34 | **0.041** | **0.001** |
| *Average diastolic* | 92 | 62.2 (59.1, 65.7) | 61.0 (58.7, 65.2) | 65.3 (61.9, 67.6) | 64.7 (61.7, 69.2) | **0.006** | **0.007** | **0.019** | 0.49 | **0.001** |
| **PA (mins)** | | | | | | | | | | |
| *VPA* | 94 | 18.8 (10.1, 23.9) | 23.8 (20.4, 26.5) | 5.0 (4.8, 6.0) | 7.5 (4.5, 12.0) | **<0.001** | **<0.001** | **<0.001** | 0.13 | **<0.001** |
| *MPA* | 94 | 181 ± 34 | 196 ± 30 | 159 ± 27.8 | 149 ± 44 | **<0.001** | **<0.001** | **0.012** | 0.99 | **<0.001** |
| *LPA* | 94 | 226 (207, 245) | 241 (214, 251) | 211 (196, 226) | 195 (177, 225) | **<0.001** | **0.001** | **0.032** | 0.20 | **<0.001** |
| *ST* | 94 | 498 (434, 532) | 474 (430, 518) | 535 (504, 574) | 539 (504, 581) | **0.004** | **0.002** | **0.011** | 0.43 | **<0.001** |
| **CRF** | | | | | | | | | | |
| *$VO_2$peak (L/min)* | 87 | 2.4 ± 0.5 | 2.5 ± 0.5 | 2.2 ± 0.5 | 2.2 ± 0.4 | **0.045** | **0.028** | 0.22 | 0.99 | **0.039** |
| *$VO_2$peak (mL/kg/min)* | 87 | 45.1 ± 7.6 | 47.2 ± 5.8 | 38.9 ± 7.7 | 28.1 ± 5.5 | **<0.001** | **<0.001** | **0.001** | **<0.001** | **<0.001** |
| *$VO_2$peak ($mL/kg^{FFM}$/min)* | 87 | 55.0 ± 7.8 | 57.4 ± 6.3 | 49.0 ± 7.4 | 43.2 ± 6.7 | **<0.001** | **<0.001** | **0.003** | 0.054 | **<0.001** |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Peak work rate (watts)* | 87 | 192 ± 41 | 199 ± 40 | 183 ± 44 | 165 ± 33 | **0.002** | **0.002** | 0.72 | 0.58 | **0.001** |
| *RPE lungs (0-10)* | 87 | 8 (6, 9) | 8 (6, 9) | 7 (6, 8) | 8 (5, 8) | 0.39 | 0.78 | 0.36 | 0.45 | 0.24 |
| *RPE legs (0-10)* | 87 | 9 (7, 10) | 8 (7, 9) | 7 (7, 9) | 9 (7, 9) | 0.77 | 0.76 | 0.27 | 0.23 | 0.43 |
| *Resting heart rate (bpm)* [b] | 87 | 77.2 (67.1, 84.6) | 71.7 (81.4) | 80.9 (79.0, 87.4) | 81.5 (74.8, 94.1) | **0.018** | **0.004** | **0.016** | 0.45 | **0.0008** |
| *Peak heart rate (bpm)* | 87 | 191 ± 11 | 190 ± 10 | 187 ± 11 | 191 ± 11 | 0.83 | 0.51 | 0.99 | 0.74 | 0.99 |

Data are represented as mean ± standard deviation or median (interquartile range) depending on distributions. BMI indicates body mass index; CRF, cardiorespiratory fitness; FMI, fat-mass index; LPA, light physical activity; MPA, moderate physical activity; NW; normal-weight; OW/Ob, overweight and obesity; PA, physical activity; RPE, rating of perceived exertion; ST, sedentary time; $VO_2$, oxygen consumption; VPA, vigorous physical activity. [a], there were 15 normal-weight participants that were included in the study before we had means to determine VPA, which were subsequently classified as intermediate VPA as they had neither low nor high VPA. [b], resting heart rate was taken during the echocardiogram, not before the cardiopulmonary exercise test. Five participants did not complete the Tanner assessment, three of whom did have data on left ventricular diastolic function, PA, and CRF. One participant completed echocardiography but did not have height and weight measured due to severe needle phobia reaction, leading them to withdraw before height and weight could be measured.

### 5.3.2 Group differences in LVDF

There were no differences in blood flow Doppler metrics of LVDF between any groups (Table 5.2). Impaired LVDF in the OW/Ob groups was identified in all septal TDI measures and in some averaged TDI measures, but these were driven primarily by septal TDI (Table 5.2). The largest differences in TDI z-scores between normal-weight and OW/Ob groups were seen with septal TDI (Appendix 2, Table 2.1). Most notable was that there was a 1.01 (95% CI: 0.60, 1.43) lower mean z-score in septal e' peak velocities in those with OW/Ob compared to normal-weight counterparts.

Participants in the normal-weight low VPA group had similar LVDF to the OW/Ob group. There were no statistical differences between the two normal-weight groups, except for averaged e'/a' ratios, but there was a statistical trend towards significance ($P<0.1$) in some TDI measures (Table 5.2), apparently driven by lower e' velocities in the low VPA group.

**Table 5.2:** LVDF differences between groups

| Measure | n | NW All [a] | NW High VPA | NW Low VPA | OW/Ob | *P*-value | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | All NW vs. OW/Ob | NW low vs. NW high vs. OW/Ob | NW low vs. NW high | NW low vs. OW/Ob | NW high vs. OW/Ob |
| **E-wave (cm/s)** | 99 | 88.5 ± 13.3 | 89.3 ± 14.4 | 85.0 ± 11.4 | 88.5 ± 12.2 | 0.99 | 0.61 | 0.99 | 0.99 | 0.99 |
| **A-wave (cm/s)** | 99 | 57.9 ± 13.4 | 57.8 ± 13.6 | 59.1 ± 10.6 | 61.7 ± 14.3 | 0.18 | 0.48 | 0.99 | 0.99 | 0.70 |
| **E/A** | 99 | 1.6 ± 0.4 | 1.6 ± 0.3 | 1.5 ± 0.2 | 1.5 ± 0.4 | 0.22 | 0.34 | 0.68 | 0.99 | 0.66 |
| **DT (ms)** | 96 | 167.7 ± 30.8 | 174.5 ± 29.3 | 156.3 ± 26.5 | 161.8 ± 26.3 | 0.33 | 0.08 | 0.17 | 0.99 | 0.18 |
| **IVRT (ms)** | 99 | 72.4 ± 10.1 | 72.7 ± 11.3 | 74.5 ± 6.7 | 72.4 ± 9.5 | 0.99 | 0.81 | 0.99 | 0.99 | 0.99 |
| **Vp (cm/s)** | 97 | 71.4 ± 18.3 | 71.7 ± 16.7 | 65.5 ± 12.9 | 74.4 ± 40.2 | 0.62 | 0.66 | 0.99 | 0.99 | 0.99 |
| **e' (cm/s)** | | | | | | | | | | |
| *Septal* | 99 | 13.1 ± 1.9 | 13.3 ± 1.5 | 12.4 ± 2.2 | 11.3 ± 1.7 | **<0.001** | **<0.001** | 0.36 | 0.15 | **<0.001** |
| *Lateral* | 98 | 16.9 ± 3.4 | 17.8 ± 2.7 | 15.2 ± 4.5 | 16.2 ± 3.3 | 0.32 | **0.032** | 0.062 | 0.99 | 0.12 |
| *Averaged* | 98 | 15.0 ± 2.4 | 15.5 ± 1.6 | 13.8 ± 3.2 | 13.7 ± 2.1 | **0.008** | **0.001** | 0.054 | 0.99 | **0.002** |
| **a' (cm/s)** | | | | | | | | | | |
| *Septal* | 99 | 6.3 ± 1.1 | 6.1 ± 1.0 | 6.6 ± 0.9 | 6.8 ± 1.3 | **0.041** | **0.045** | 0.64 | 0.99 | **0.041** |
| *Lateral* | 97 | 6.2 ± 1.1 | 6.2 ± 1.1 | 6.4 ± 0.9 | 6.4 ± 1.4 | 0.37 | 0.71 | 0.99 | 0.99 | 0.99 |
| *Averaged* | 97 | 6.2 ± 0.9 | 6.1 ± 0.9 | 6.5 ± 0.8 | 6.6 ± 1.1 | 0.09 | 0.14 | 0.72 | 0.99 | 0.16 |
| **e'/a'** | | | | | | | | | | |
| *Septal* | 99 | 2.1 ± 0.5 | 2.2 ± 0.5 | 1.9 ± 0.4 | 1.7 ± 0.4 | **<0.001** | **<0.001** | 0.095 | 0.66 | **<0.001** |
| *Lateral* | 97 | 2.8 ± 0.8 | 3.0 ± 0.7 | 2.4 ± 0.8 | 2.6 ± 0.7 | 0.24 | **0.046** | 0.085 | 0.99 | 0.16 |
| *Averaged* | 97 | 2.5 ± 0.5 | 2.6 ± 0.5 | 2.2 ± 0.5 | 2.2 ± 0.5 | **0.007** | **0.001** | **0.032** | 0.99 | **0.002** |
| **E/e'** | | | | | | | | | | |
| *Septal* | 99 | 6.8 ± 1.2 | 6.8 ± 1.2 | 7.0 ± 1.2 | 7.9 ± 1.2 | **<0.001** | **<0.001** | 0.99 | 0.067 | **<0.001** |
| *Lateral* | 98 | 5.5 ± 1.4 | 5.1 ± 1.0 | 6.1 ± 2.2 | 5.6 ± 1.0 | 0.55 | **0.039** | 0.053 | 0.74 | 0.22 |
| *Averaged* | 98 | 6.2 ± 1.2 | 5.9 ± 0.9 | 6.6 ± 1.6 | 6.8 ± 0.9 | **0.006** | **0.004** | 0.25 | 0.99 | **0.003** |

Data are represented as mean ± standard deviation. A-wave indicates late diastolic peak mitral inflow velocity; a', late diastolic tissue peak velocity; DT, E-wave deceleration time; E-wave, early diastolic peak mitral inflow velocity; e', early diastolic tissue peak velocity; E/e', E-wave/e' ratio; e'/a', e'/a' ratio; IVRT, isovolumetric relaxation time; NW,

normal-weight, OW/Ob, overweight and obesity; Vp, mitral propagation velocity; VPA, vigorous physical activity. DT data were missing for three participants because of fused E- and A-waves; Vp data was missing for two participants because of difficulties being able to accurately define the Vp slope; lateral e' and a' data was missing for two and three participants, respectively, because of fused e' and a' waves.

### 5.3.3 Associations of adiposity with LVDF

There were no associations of any blood flow Doppler measures with adiposity. Associations with TDI measures are reported in Table 5.3. Adiposity measures were most strongly associated with septal TDI measures and were associated with averaged measures, but these were driven by septal TDI.

**Table 5.3: Associations of obesity measures with LVDF**

| Measure | **BMI z-score** b (95% CI) | r | **FMI** [a] b (95% CI) | r |
|---|---|---|---|---|
| **e' (cm/s)** | | | | |
| *Septal* | **-0.71 (-1.01, -0.42)** | **-0.43** | **-1.33 (-1.90, -0.78)** | **-0.46** |
| *Lateral* | -0.41 (-0.99, 0.18) | -0.15 | -0.91 (-2.01, 0.20) | -0.18 |
| *Averaged* | **-0.55 (-0.94, -0.17)** | **-0.28** | **-1.11 (-1.18, -0.38)** | **-0.32** |
| **a' (cm/s)** | | | | |
| *Septal* | **0.29 (0.10, 0.48)** | **0.29** | **0.41 (0.05, 0.76)** | **0.24** |
| *Lateral* | 0.15 (-0.06, 0.36) | 0.15 | 0.31 (-0.08, 0.69) | 0.18 |
| *Averaged* | **0.21 (0.05, 0.37)** | **0.26** | **0.34 (0.05, 0.63)** | **0.25** |
| **e'/a'** | | | | |
| *Septal* | **-0.19 (-0.26, -0.11)** | **-0.45** | **-0.31 (-0.44, -0.17)** | **-0.44** |
| *Lateral* | -0.11 (-0.24, 0.01) | -0.18 | -0.23 (-0.47, 0.003) | -0.22 |
| *Averaged* | **-0.15 (-0.23, -0.06)** | **-0.34** | **-0.27 (-0.43, -0.10)** | **-0.34** |
| **E/e'** | | | | |
| *Septal* | **0.42 (0.22, 0.61)** | **0.39** | **0.72 (0.34, 1.10)** | **0.37** |
| *Lateral* | 0.11 (-0.11, 0.33) | 0.10 | 0.21 (-0.21, 0.63) | 0.11 |
| *Averaged* | **0.25 (0.08, 0.43)** | **0.28** | **0.45 (0.11, 0.79)** | **0.27** |

All analyses were adjusted for age, sex, and Tanner score. a' indicates late diastolic tissue peak velocity; BMI, body mass index; e', early diastolic tissue peak velocity; E/e', E-wave/e' ratio; e'/a', e'/a' ratio; FMI, fat mass index; 95% CI, 95% confidence interval. [a], zero-skewed, log adjusted.

### 5.3.4 Primary analyses – associations of physical activity with LVDF

Univariate regression analyses of LVDF measures with unadjusted PA (Model 1) and confounders, found that there were no relationships with any blood flow Doppler measures.

VPA was associated with all measures of TDI, apart from lateral a' velocities (Appendix 2, Table 2.2). The strongest relationship was with septal e'/a' ratios (r=0.44, 95% CI: 0.27, 0.75). There were modest associations of MPA and LPA with septal e' velocities and ST with some TDI measures in Model 1. When all unadjusted PA intensities (Model 2) or rPA intensities (Model 3) were included, VPA remained associated with all TDI measures, apart from lateral a' velocities in Models 2 & 3, whereas

other PA/rPA intensities were unrelated. VPA was most strongly associated with septal e'/a' ratios in these models (r=0.44-0.53).

Participants in the upper-middle and upper quartiles of VPA had higher mean septal e'/a' ratios than those in the lowest quartile, adjusted for lesser intensities and confounders (Figure 5.3[A]). Back transformation of the adjusted, zero-skewed, logged VPA variable, identified that those in the upper-middle- and upper-quartiles of VPA performed a median of 16.3 min/day of VPA on average (16.3, IQR: 14.8, 19.4).

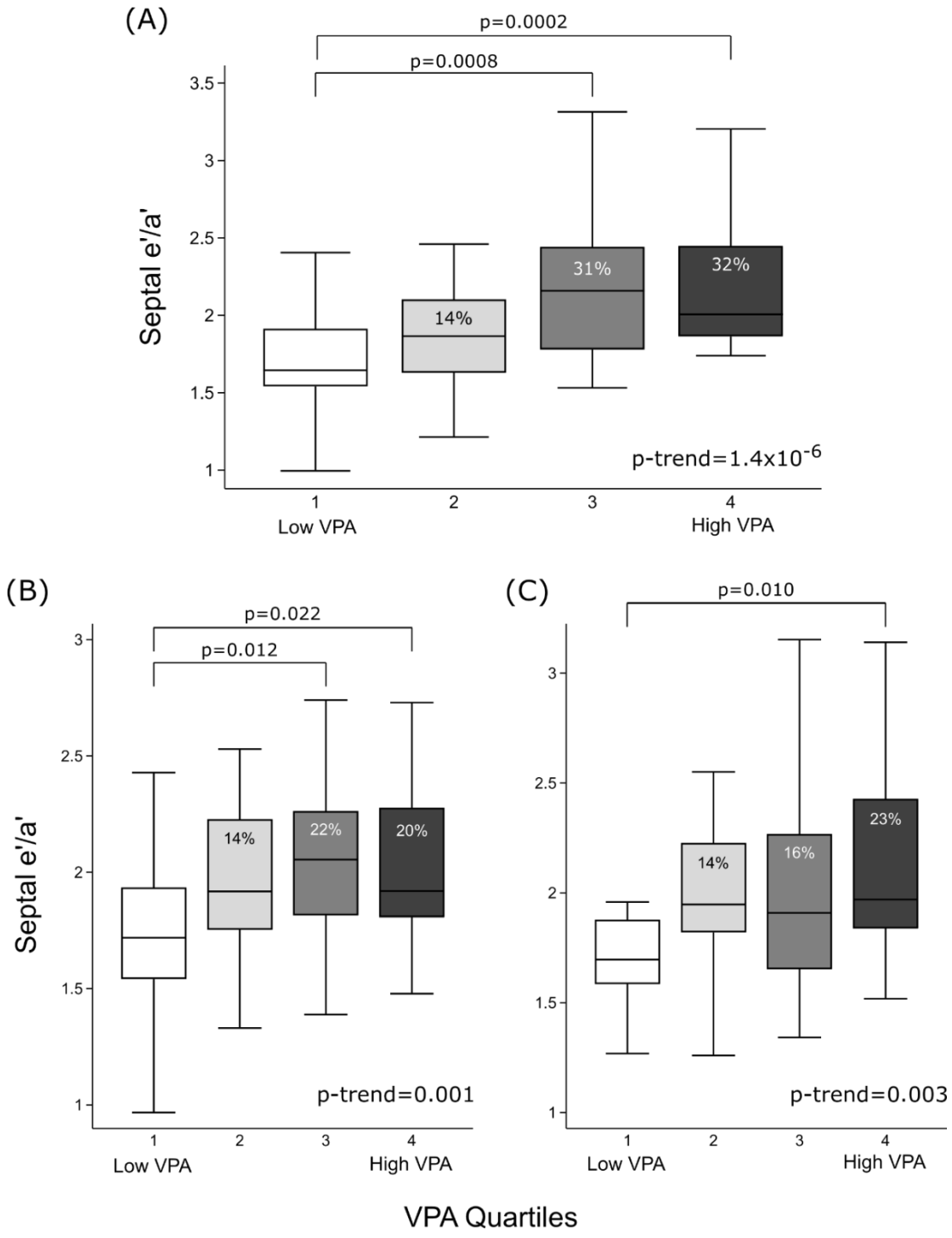


**Figure 5.3: The independent effect of increasing vigorous physical activity (VPA) on septal early-to-late peak myocardial velocity ratios (e'/a').**

Quartiles of VPA and septal e'/a' ratios were adjusted for other physical activity intensities, age, sex, Tanner score (A). Quartiles of VPA were additionally adjusted for body mass index z-scores (B) or fat mass index (C). Percentages represent the mean difference from the lowest quartile. Differences between all quartiles were tested by the Jonckheere–Terpstra test and between quartile differences by independent t-tests with Bonferroni correction.

#### 5.3.4.1 *Physical activity and adiposity – fully adjusted multiple regression*

VPA was associated with TDI measures, independently of BMI z-score and unadjusted PA intensities (Model 4) or rPA intensities (Model 5) (Appendix 2, Table 2.3). This finding remained when FMI was used instead of BMI z-score in the models (Appendix 2, Table 2.3; Models 6 & 7, respectively).

The primary outcome to assess whether septal e'/a' ratios were independently related with VPA are reported in Figure 5.3 and Appendix 2, Table 2.3. Participants in the upper-middle and upper quartiles of VPA had higher mean septal e'/a' ratios than those in the lowest quartile (Figure 5.3[B]), adjusted for confounders and BMI z-scores. Similar results were found when FMI replaced BMI z-scores (Figure 5.3[C]). Back transformation of the adjusted, zero-skewed, logged VPA variable, identified that those in the upper quartile of VPA performed a median of 18 min/day of VPA on average (BMI z-score: 18.1 [IQR: 16.2, 24.5]; FMI: 18.0 [IQR: 16.7, 21.0]).

BMI z-score and FMI were independently associated with septal e' velocities, e'/a' ratios, and E/e' ratios in all models (Appendix 2, Table 2.3).

The best model fits were with septal e' velocities ($r^2$=0.35-0.36) and septal e'/a' ratios ($r^2$=0.31-0.34).

The above models were re-run for septal e'/a' ratios, but with replacement of BFEN PA with other PA metrics, which did not meaningfully alter the findings (Appendix 2, Table 2.4).

#### 5.3.4.2 *Achieved power*

A multiple regression model with septal e'/a' ratio as the dependent variable and all PA intensities, BMI z-score, and confounders as independent variables, was fitted as the primary outcome model. The $r^2$ of the model was 0.34 (n=89; residual variance: 1–0.34=0.66) and the partial $r^2$ for VPA (variance explained by VPA) was 0.09 (p=0.006). The achieved power to detect the independent effect of VPA was 93%. This was repeated but with the replacement of BMI z-score with FMI (n=81; power=80%). Both calculations were repeated with rPA intensities and the achieved power was 91% with BMI z-score and 91% with FMI.

Only VPA was used in subsequent mediation analyses as this was the only intensity of exercise that was reliably associated with LVDF measures in multiple regression analyses.

#### 5.3.4.3 *Vigorous physical activity and adiposity – mediation analyses*

VPA, adjusted for confounders, was moderately associated with BMI z-scores (r=-0.42, $p=1.1x10^{-5}$) and FMI (r=-0.48, $p=3.7x10^{-3}$), so SEM mediation analyses were performed to determine either the direct or indirect effect (through adiposity) of VPA on septal e'/a' ratios (Figure 5.4). BMI z-score and FMI partially mediated this effect, explaining 36% and 31% of the total effect of VPA, respectively, but there remained a significant direct effect of VPA. The 95% bootstrapped percentile, bias-corrected, and BCa CIs of the indirect effect did not span the null, providing confidence that results were not due to Type 1 error.

#### 5.3.4.4 *Vigorous physical activity, adiposity, and ambulatory blood pressure – multiple mediation analyses*

In fully adjusted multiple regression, VPA (r=0.26–0.30, p=0.012–0.037), FMI (r=-0.20-0.21, p=0.065–0.090), and 24-hour systolic (r=-0.21–0.22, p=0.037–0.065) or diastolic (r=-0.19–0.20, p=0.066–0.089) BP z-scores were independently associated (some as trends [p<0.10]) with septal e'/a' ratios. Therefore, SEM multiple mediation analyses were done, similar to above, but with the addition of BP z-scores as an additional mediator and a path between FMI and BP (Figure 5.5). Systolic and diastolic BP z-scores (adjusted for height [Figure 5.5 (A and B)] or age [Figure 5.5 (C and D)]) reduced the mediating effect of FMI to borderline non-significance. Instead, there were significant, or borderline significant, paths from FMI to BP z-scores (path d) and BP z-scores to septal e'/a' ratios (path b2). There remained a significant direct effect of VPA.

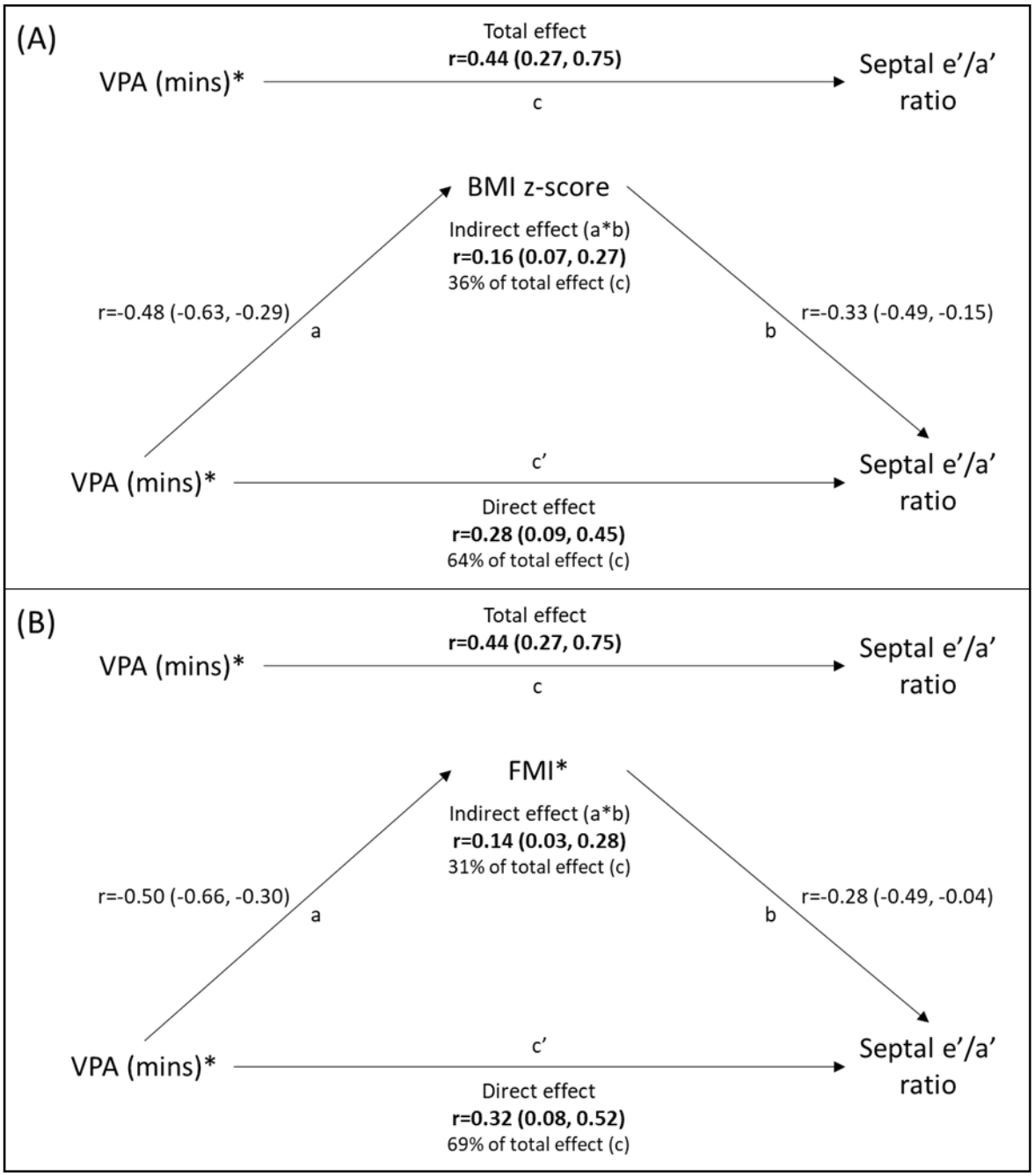


**Figure 5.4: Mediating effect of adiposity on the relationship between vigorous physical activity (VPA) and septal early-to-late myocardial velocity ratios (e'/a').**

All analyses were adjusted for age, sex, and Tanner score. The 95% confidence intervals (CIs) for the a, b, and c' paths are bootstrapped, bias-corrected accelerated 95% CIs. BMI indicates body mass index; FMI, fat mass index. *, zero-skewed, log transformed.

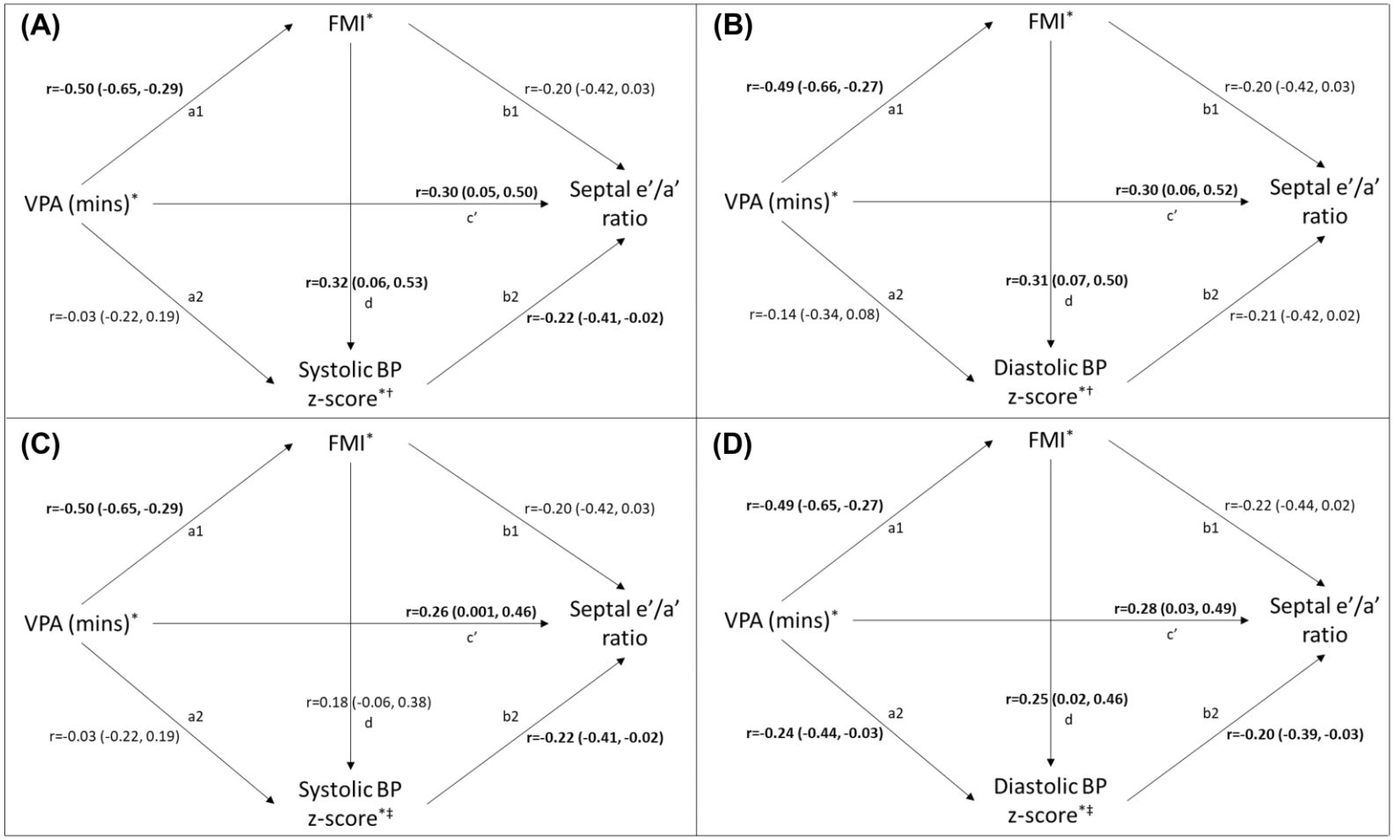


**Figure 5.5: Mediating effect of adiposity and 24-hour blood pressure (BP) z-scores on the relationship between vigorous physical activity (VPA) and septal early-to-late myocardial velocity ratios (e'/a').**

All analyses were adjusted for age, sex, and Tanner score. The 95% confidence intervals (CIs) are bootstrapped, bias-corrected accelerated 95% CIs. FMI indicates fat mass index. *, zero-skewed, log transformed; †, z-score adjusted for height; ‡, z-score adjusted for age.

### 5.3.5 Secondary analyses – cardiorespiratory fitness

#### 5.3.5.1 *Associations of cardiorespiratory fitness with LVDF*

$VO_2peak_{kg}$ and $VO_2peak_{FFMkg}$ were associated with A-wave peak velocities, adjusted for confounders, and $VO_2peak_{FFMkg}$ was associated with DT. There were no other relationships with other blood flow Doppler measures (Appendix 2, Table 2.5). $VO_2peak_{kg}$ was associated with all TDI measures, apart from lateral a' velocities. $VO_2peak_{FFMkg}$ was associated with all TDI measures, apart from lateral and averaged a' velocities, and lateral e'/a' ratios. Similar to PA, CRF was most strongly associated with septal TDI measures, with the strongest relationships with septal e'/a' ratios (r=0.47-0.49).

#### 5.3.5.2 *Cardiorespiratory fitness and adiposity – fully adjusted multiple regression*

In fully adjusted models with adiposity, $VO_2peak_{kg}$ and $VO_2peak_{FFMkg}$ were independently associated with A-wave velocities. There were relationships of $VO_2peak_{kg}$ with DT, adjusted for FMI (r=0.50, b=1.43 [95% CI: 0.25, 2.61]), and $VO_2peak_{FFMkg}$ with DT, adjusted for BMI z-score and FMI (r=0.29, b=0.97 [95% CI: 0.05, 1.89]; r=0.33, b=1.10 [95% CI: 0.17, 2.03], respectively).

TDI results are reported in Appendix 2, Table 2.6. $VO_2peak_{kg}$, adjusted for BMI z-score (Model 8) or FMI (Model 9), was independently associated with a number of TDI measures. $VO_2peak_{FFMkg}$, adjusted for BMI z-score (Model 10) or FMI (Model 11), was independently associated with a number of TDI measures.

Interbeat intervals were associated with CRF, independent of FMI and confounders ($VO_2peak_{kg}$: r=-0.60, p=0.003; $VO_2peak_{FFMkg}$: r=-0.40, p=0.003).

#### 5.3.5.3 *Cardiorespiratory fitness, adiposity and physical activity – fully adjusted multiple regression*

Multiple linear regression with septal e'/a' ratios as the dependent variable and $VO_2peak_{kg}$, all rPA intensities, FMI, and confounders as independent variables, was performed. VPA was the only variable

associated with septal e'/a' ratios (r=0.30, b=0.33 [95% CI: 0.03, 0.64]). Similar results were found when $VO_2peak_{FFMkg}$ was used instead (r=0.29, b=0.32 [95% CI: 0.01, 0.63]).

### 5.3.6 Secondary analyses – insulin resistance sub-study

In a sub-population of adolescents (n=60 [n=53 with FMI data), IR measures were associated with A-wave velocities, IVRT, and septal and averaged TDI measures in univariate analyses adjusted for confounders (Appendix 2, Table 2.7; Model 12).

Adiposity was associated with HOMA2-IR (BMI z-score: r=0.54, b=0.23 [0.13, 0.32]; FMI: r=0.55, b=0.38 [0.19, 0.57]) and the Matsuda index (BMI z-score: r=-0.55, b=-0.18 [-0.25, -0.11]; FMI: r=-0.60, b=-0.33 [-0.47, -0.19]). Once accounting for this relationship in fully-adjusted models, HOMA2-IR remained associated with A-wave velocities, septal a' velocities, septal e'/a' ratios, and septal and averaged E/e' ratios, and the Matsuda index with A-wave velocities and IVRT (Appendix 2, Table 2.7; Models 13 & 14).

### 5.3.7 Secondary analyses – ambulatory blood pressure

There was a negative relationship of diastolic BP with peak E-waves (r=-0.28, p=0.013) and E/A ratios (r=-0.23, p=0.043). Sleep systolic BP was negatively associated with E/A ratios (r=-0.26, p=0.031) and there was a positive trend with peak A-waves (r=0.23, p=0.060). Sleep diastolic BP was negatively associated with peak E-waves (r=-0.26, p=0.018), E/A ratios (r=-0.34, p=0.002), and there was a positive trend with peak A-waves (r=0.19, p=0.084). When adjusted for adiposity (FMI), relationships persisted between awake diastolic BP and peak E-waves; sleep systolic BP and E/A ratios; and sleep diastolic BP and peak E-waves and E/A ratios. Results were similar when BP z-scores were used instead.

There were numerous associations of TDI measures with awake and sleep systolic and diastolic BPs (Appendix 2, Table 2.8). The strongest relationships were with peak e' velocities (r≤-0.41), with many of these relationships persisting after adjustment for adiposity (FMI).

In all analyses (sections 5.3.3-5.3.7), inclusion of interbeat intervals did not meaningfully alter the results.

### 5.3.8 Echocardiography reliability

ICCs for intra-observer reliability are reported in Appendix 2, Table 2.9. Excellent reliability was reported for the majority of LVDF measures, good reliability for septal, lateral, and averaged peak a' velocities, and moderate reliability for DT and Vp.

## 5.4 Discussion

In this study, greater routine average daily VPA was shown to be linked to better LVDF in adolescents, independent of adiposity and lesser PA intensities. Other intensities were not related with LVDF when VPA was accounted for. This suggests that regular VPA may be required to protect against the adverse effects of adiposity on LVDF, with the best effects seen in the quartile of adolescents achieving only 15-20 minutes of average VPA per day in total. Furthermore, this study confirms the results of Study 1, showing that the strongest association between adiposity and LVDF is with septal TDI, which was also found with PA, CRF and IR measures. Of the septal measures, septal e'/a' ratios were typically most strongly associated, further supporting this measure as the best for identifying early LVDF impairments in adolescents.

### 5.4.1 Adiposity and LVDF

In agreement with the findings of Study 1, excess adiposity was found to be linked with impaired LVDF. As Study 1 only assessed adiposity by BMI, this study included adiposity assessed by FMI. Fat mass determined by the Tanita MC-780, which was used in this study, is similar to fat mass derived by DEXA.[401] Other research that has assessed adiposity by DEXA has established an adverse link of higher adiposity with impaired LVDF.[326, 346, 351] Therefore, this study adds to the literature and the findings of Study 1 that excess adiposity in adolescents is linked with reduced LVDF.

The cause of LVDF impairment in adolescents with excess adiposity is incompletely understood and probably multifactorial. In a study by Rayner *et al*,[33] myocardial energetics, myocardial triglyceride content, and LV concentric remodelling fully mediated the effect of VAT on LVDF in 80 adults. These candidate mechanisms are discussed below as possible mediators of the link between impaired LVDF and OW/Ob in adolescents.

***Free-fatty acid metabolism and energy availability***

An increase in FFAs in the circulation of people with obesity leads to greater FFA uptake by cardiomyocytes and a subsequent shift to FFA metabolism,[112] which reduces adenosine-triphosphate yield and mitochondrial efficiency.[33, 81] As diastole is more sensitive than systole to disruptions in energy availability,[88, 89] functional consequences of reduced energy availability typically manifest with impaired LVDF before other functional impairments appear. Thus, LVDF can be considered an early marker of this process. Adults with obesity have reduced energy availability, evidenced by a reduced phosphocreatine/adenosine-triphosphate ratio.[33] Although this ratio is linked with LVDD,[33] myocardial energy availability at rest is typically maintained in adults with obesity by an increase in the creatine-kinase shuttle reaction rate, transferring adenosine-triphosphate from the mitochondria to the myofibrils.[90] However, this shuttle cannot keep up with increases in myocardial work, resulting in reduced energy availability, systolic function impairments, and exercise intolerance during stress.[90] This supports the two studies in Study 1, which showed that baseline differences in LVDF between adolescents with and without obesity are exaggerated during exercise.[310, 333] Phosphocreatine/adenosine-triphosphate ratios have not been studied in adolescents, which is warranted as this mechanism may partly explain the early impairments in LVDF found in this thesis.

***Myocardial steatosis***

Myocardial steatosis is another possible mechanism that has not been well studied in adolescents. Over-spill of lipids into the myocardium first results in the storage of lipids as triacylglycerol,[33] but this storage is limited, resulting in the creation of toxic reactive lipid species.[112] These toxic lipid species

can lead to mitochondrial and cellular dysfunction, cardiac fibrosis, cardiomyocyte apoptosis, and ultimately LVDD.[33, 81, 112] There has been some preliminary evidence to suggest that obesity is associated with myocardial steatosis in a small population of adolescent males (n=22),[437] but this deserves further work as this mechanism may partly explain the link of adiposity with LVDF in this study.

***Myocardial concentric remodelling***

Adolescent obesity is also associated with LV hypertrophy,[75, 336] which is linked with an increase in LV transmural stiffness (discussed in section 1.3.3).[92] As changes to LV structure are evident in children and adolescents with OW/Ob, geometric remodelling of the LV may contribute to early LVDD aetiology by changes in the passive stiffness of the myocardium.[72-75, 92] The increased volume overload (preload) seen with increasing levels of obesity, without a concomitant decrease in peripheral resistance (afterload),[100] causes excessive stretch of cardiomyocytes and has been demonstrated to be a potent activator of the expression of genes that cause hypertrophy, independent of neuronal or humoral factors.[105] Humoral stimuli can also result in pathological LV hypertrophy,[106] with IR and hyperinsulinaemia in obesity triggering hepatic angiotensin II synthesis, as well as activation of insulin-like growth factor-1 receptors, stimulating LV hypertrophy.[106-109] Obesity is also associated with systemic inflammation, and inflammatory markers can directly induce LV hypertrophy and myocardial fibrosis.[106, 110] There is a linear relationship between adiposity and LV hypertrophy, independent of hypertension status,[111] and this is linked with increased passive stiffness[92] and reduced LVDF.[33] Mechanistically, this could be caused by alterations in the phosphorylation of titin and/or changes to the degree of myocardial fibrosis,[94, 95, 124-126, 438-441] as discussed in Section 1.3.3.

Greater BP, or greater LV afterload, is commonly seen in obesity.[103] In this study, it was identified that greater 24-hour ambulatory BP is associated with reduced LVDF, independent of adiposity. Furthermore, multiple mediation analyses suggest that increased adiposity, potentially due to decreased VPA, increases BP that is subsequently linked with reduced LVDF, independent of a

significant direct effect of VPA and an indirect effect of adiposity. This suggests that increased BP in OW/Ob could be an important mechanism for reduced LVDF in adolescents. In response to greater BPs, new sarcomere units are added in parallel to increase the cardiomyocyte cross-sectional area (LV hypertrophy) to generate greater systolic force and contractility to overcome the increased afterload.[102] However, this comes at the cost of increased LV stiffness and reduced LVDF.[92, 104] Indeed, a recent systematic review and meta-analysis identified that paediatric hypertension is associated with worse LVDF.[131] However, two studies identified in Study 1 found that there were no differences in LVDF between obese groups with or without hypertension.[133, 134] Thus, more research, specifically RCTs such as OxSOCRATES, will be required to fully understand any causal mechanism of raised BP on LVDF in adolescents.

### 5.4.2 Septal LVDF

In support of Study 1, global measures of LVDF, such as IVRT, were not associated with greater adiposity. It could be that the earliest pathological changes in LVDF, may be localised, compensated for by other elements of diastolic function elsewhere in the LV, and preferentially affecting particular myocardial fibre groups/directions in the heart, meaning that global measures are unaffected. Furthermore, blood flow and timing metrics are more susceptible to LV loading conditions than TDI measures,[185, 191, 196] potentially reducing their capability to detect any underlying relationships.

The earliest pathological changes in LVDF may be most prevalent in the LV septum.[75, 336] The theoretical wall stress in the septum in response to changes in LV pressure is greater than in the lateral wall when taking into account an already compensated heart (change in cavity shape, size, or wall thickness).[442] This is because the longitudinal fibres of the basal septum have some of the greatest radii of the LV, and so based on Laplace's law (LV wall stress = [LV pressure x radius] / 2 x LV wall thickness), the basal septum will predisposed to a higher wall stress for a given pressure and wall thickness compared to the lateral wall.[442] As increased wall stress triggers hypertrophic remodelling pathways,[106, 443] and as obesity is associated with an increase in LV loading,[100, 444] it is therefore

unsurprising that hypertrophic remodelling of the septum is evident in childhood obesity, typically before lateral wall changes.[75, 336] As LV hypertrophy is linked with an increase in LV transmural stiffness,[92] septal remodelling could explain the stronger relationships of septal TDI measures than lateral equivalents with adiposity that were seen in this thesis. This is supported by adult hypertensive studies whereby septal TDI measures were impaired to a greater extent than lateral equivalents and were related to the degree of septal hypertrophy, whereas lateral equivalents were unrelated with lateral wall hypertrophy.[445]

In this study, obesity measures were most strongly associated with septal e'/a' ratios. Of the two components of e'/a', e' was most strongly associated with adiposity, and this finding remained after adjustment for PA, CRF, or IR. As e' is inversely related with the time constant of LV relaxation, τ, my results likely represent a gradual reduction in septal myocardial relaxation with increasing adiposity in adolescents.[20] Therefore, given the preferential remodelling of septum in adolescents with OW/Ob[75, 336] and the findings of this study, septal e'/a' ratios might be considered the best marker to identify early impairments of LVDF in adolescents with obesity. However, as there is currently no normative data or guidelines on LVDF for adolescents, further research will be needed to determine whether the stronger link of adiposity with septal e'/a' ratios truly reflect early cardiac dysfunction. However, it may be possible to protect against these early impairments, independently from weight status, as measures of PA were associated independently with LVDF in this study.

### 5.4.3 Protective effect of high VPA and adverse effect of low VPA

Previous research has identified a lack of studies comparing the effect of MPA versus VPA on cardiovascular function.[392] In this study, results found that relatively short durations of daily VPA were linked with healthier LVDF in adolescents, independent of adiposity, lesser intensities of PA, and other confounders. Of note was that independent of other PA intensities and confounders, participants who were above the median level of VPA had ~31% higher septal e'/a' ratios than those in the lowest quartile. This strong effect persisted and was only weakened slightly when adiposity measures were

included. Furthermore, mediation analyses found that although adiposity partially mediates the effect of VPA on septal e'/a' ratios, there remains a significant direct effect of VPA, suggesting that VPA acts both directly and indirectly as a buffer against the adverse effect of adiposity on LVDF. This supports adult heart failure studies where high levels of subjectively reported PA led to an independent 19% lower risk of HFpEF,[69] as well as studies of cardiometabolic health in adolescents.[46, 157, 392, 393, 446, 447] However, some of the previous cardiometabolic studies in adolescents have shown no added benefit of shorter durations of VPA over longer durations of MPA.[392, 393] Multiple factors could explain this, but longer PA epoch sampling rates and not controlling for the intercorrelation between PA intensities could be important factors. Longer epoch lengths will average/smooth the accelerations to a greater extent than shorter durations, potentially diluting the intensity.[257] Those who do more MPA typically also do more VPA (Appendix 3, Table 3.1), and so not controlling for this might bias the results of univariate analyses, hiding the true effect of VPA or MPA.

There has also been only one study to address whether VPA improves LVDF to a greater extent than MPA in adolescents with OW/Ob. This study found that there were no additional benefits of HIIT on typical Doppler measures of LVDF or E/e' ratios.[332] Nonetheless, it appears that HIIT improved averaged e' and a' velocities to a greater extent than MICT, but unfortunately, the authors do not report statistics for the between group pre-to-post mean differences and there was also a high level of drop-out, with only a small number (52%, n=17) completing the HIIT programme.[332] Therefore, further work is required to understand if VPA is truly more beneficial than lesser intensities for improving LVDF in adolescents, especially in those with OW/Ob.

A lack of VPA may facilitate reductions in LVDF independently from adiposity. Early signs of this were identifiable between the normal-weight high and low VPA groups. Although there were no statistically meaningful differences between these groups, there was a statistical trend towards significance in TDI measures of LVDF ($P<0.1$). It is likely that these differences will increase as these adolescents age. Thus, being a healthy weight may not be enough to protect from impairments of LVDF.

Although VPA was independently associated with LVDF in this study, the mechanisms behind this have not been well studied. Obesity places constant, excess demands on the heart, including during sleep[448, 449] and can result in obesity-related cardiomyopathy.[72-74, 450] PA, on the other hand, provides short periods of stress on the heart and is associated with exercise-induced physiological cardiac hypertrophy.[451, 452 451, 452] However, exercise-induced hypertrophy is not maladaptive, is not linked with adverse outcomes in most individuals, and is not associated with the cardiac fibrosis or cell death seen in pathological hypertrophy.[453-455] This highlights an apparent conflict where one hypertrophic process is known to be detrimental and another one is apparently beneficial. Exercise promotes cardiac cell proliferation and renewal, and can protect against pathological heart remodelling.[455] In animal studies, exercise programmes have been shown to reduce LV passive stiffness by altering phosphorylation patterns of titin,[456] decreasing collagen deposition, and increasing myocardial capillary density and mitochondrial mass,[457] all of which are probably implicated in impaired LVDF. There have been multiple reviews on the benefits of exercise to increase mitochondria turnover, improve mitochondrial morphology, enhance mitochondrial respiration, reduce inflammation and reactive oxygen species, and increase insulin sensitivity, all of which are important for producing energy in active LV relaxation.[455, 458] Furthermore, exercise in healthy and cardiac disease animal models have increased SERCA2a protein levels/activity, which underpin active LV relaxation by the removal of $Ca^{2+}$ from the cytosol into the sarcoplasmic reticulum.[455]

As MPA and LPA were not independently associated with LVDF, it could be that VPA has a greater capacity to alter the aforementioned pathways for LVDF.[390, 391] For example, HIIT interventions increase skeletal muscle $Ca^{2+}$ reuptake, whereas MICT interventions do not in adults with MetS.[391] In animal models, HIIT was linked with quicker time to peak $Ca^{2+}$ decay in cardiomyocytes, suggesting improved $Ca^{2+}$ removal, whereas there was no difference with MICT.[459] The causal pathways of these are not well understood, but as MPA is primarily aerobic activity, whereas VPA can be both aerobic and anaerobic, it could be that these pathways are more readily altered when the anaerobic threshold

is reached. Evidence in human skeletal muscles supports this and shows that higher exercise intensities elicit greater increases in intracellular signalling kinases that activate pathways to upregulate mitochondrial protein synthesis and biogenesis than lower intensities.[460] HIIT, but not MICT, also increases myocardial glucose oxidation and decreases fatty-acid oxidation,[390] which could protect against the increased FFA metabolism in obesity[112] that is known to reduce adenosine-triphosphate yield and mitochondrial efficiency.[33, 81] Furthermore, HIIT upregulated cardiac gene expression of lactate dehydrogenase, hexokinase, and vascular endothelial growth factor, whereas MICT did not.[390] Thus, reaching the anaerobic threshold to switch on genes and pathways associated with LVDF might explain why VPA apparently has greater benefits than lesser intensities found in this study. However, more research is required to fully elucidate this.

### 5.4.4 Protective effect of high cardiorespiratory fitness and adverse effect of low cardiorespiratory fitness

This study also found that CRF is independently linked with LVDF. When $VO_2$peak was adjusted for FFM, $VO_2peak_{FFMkg}$ remained independently associated with LVDF, suggesting that irrespective of adiposity status, higher CRF may protect from impaired LVDF, whilst lower CRF could worsen LVDF. This supports adult studies showing that CRF protects against cardiovascular outcomes in adults, independent of weight status,[148, 399] and adds to results from previous publications on adolescent CRF and LVDF.[326, 328]

There is a strong genetic underpinning of CRF, explaining approximately 50% of the CRF response to aerobic exercise that needs to be considered.[461-463] Those with beneficial genetics could have a greater flexibility, or greater capacity for change, in their metabolic pathways associated with CRF, whereby a lower intensity and/or duration of PA can improve their metabolic functioning more readily than those with less beneficial genetics. Such a mechanism could also be involved with LVDF, predisposing some to a greater chance of having a healthier LVDF profile. In the genetic association study by Bye *et al*,[461] the cumulative number of single-nucleotide polymorphisms, identified as being positively associated

with higher CRF, was also negatively associated with CVD risk factors such as VAT, BMI, waist-circumference, and cholesterol, as well as being shown to influence gene expression in the heart.[461] As there have been some studies indicating a genetic underpinning of LVDD,[464, 465] further research will be required to understand if PA equally improves LVDF and CRF in those with beneficial or less beneficial genetics.

Although CRF was linked with LVDF in this study, measures of CRF and PA are often used interchangeably in relation to CVD risk and their strong interdependency makes it difficult to determine whether it is one or both of these factors that is most important for cardiovascular health.[466, 467] Therefore, how PA and CRF, independent of each other, were associated with LVDF was studied. Results indicated CRF is not associated with septal e'/a' ratios once VPA and adiposity are included, suggesting that interventions to improve population VPA levels will be important to prevent future LVDD.

### 5.4.5 Insulin resistance and LVDF

Following the results of Study 1, the independent effect of IR with LVDF was studied. It was found that fasting and dynamic measures of IR were adversely associated with a number of LVDF measures in adolescents. Relationships persisted when adjusted for adiposity, supporting the results of two previous adolescent studies.[144, 323] IR is typically seen in obesity and is a result of multiple maladaptive responses, including abnormal adipokine/cytokine production, systemic inflammation, mitochondrial dysfunction, lipotoxicity, oxidative stress, hypoxia and hyperinsulinaemia.[81, 128, 129] At the cardiomyocyte level, IR can impair glucose transportation into the cell; impair intracellular signalling kinase pathways; impair $Ca^{2+}$ handling; reduce metabolic flexibility; impair mitochondrial function; and cause endothelial dysfunction, all of which can ultimately lead to fibrosis and myocardial cell death.[127-130, 136, 137] IR is also implicated in LV hypertrophy,[106-109] which could be an important factor in explaining the results of this study.

Animal studies have shown that myocardial IR occurs early in LV hypertrophy.[379] This reduces insulin-stimulated glucose use and impairs glucose transporter type 4 translocation, which is accompanied by reduced myocardial energetics and the development of LVDD.[379] During diastole, removal of $Ca^{2+}$ enables the detachment of actin-myosin cross-bridges and results in the decline of cellular tension and relaxation of the sarcomere units. This is an energy-dependent process that is regulated by enzymes.[20, 86] Thus, reduced myocardial energetics are linked with LVDD.[33, 90, 379] Furthermore, as LV hypertrophy is first identified at the LV septum in children with obesity,[75, 336] any accompanying septal IR probably contributes to the impaired septal LVDF identified in this thesis. Our IR analyses support this, whereby IR was independently linked with septal but not lateral LVDF measures. However, further research in adolescents will be needed to understand this potential casual role, especially since this study was done in a sub-population of OxSOCRATES participants.

### 5.4.6 Strengths and limitations

This work has a number of strengths and potential limitations. As discussed in Chapter 2, BMI z-scores and BIA may not be the most suitable methods to determine the degree of adiposity in adolescents. However, fat mass derived from the Tanita MC-780 performs well against DEXA equivalents in children and adolescents.[401] Furthermore, the FMI was used instead of fat mass percentage, which attempts to remove the residual correlation with FFM.[225, 226]

This study also used typical LVDF measures obtained from a standard echocardiogram of LVDF, meaning that these findings can be readily transferred into clinical practice and will therefore help develop recommendations to prevent impaired LVDF in adolescents. There are also other methods to assess LVDF, including strain rate imaging, which provide a more comprehensive analysis of LV relaxation (Chapter 2). Therefore, further work with other measures of LVDF is warranted. Future work might also consider utilising methods such as principal component analysis to reduce the complexity of echocardiography datasets when multiple intercorrelated measures of LVDF are collected. Principal component analysis does this by identifying new variables that are linear combinations of the original

measures to capture the maximum amount of variance within the dataset, potentially identifying the "best" measures of LVDF.

There is no universally accepted metric to analyse PA derived from accelerometry.[262] As a team, we took care in our data processing to calibrate accelerometery and ensure accurate, manually curated, identification of wear periods. We also used BFEN in our analyses, as this was shown to classify VPA better than other metrics in our target age group.[407] However, analyses were repeated using the variety of other PA metrics favoured in the literature, which did not meaningfully alter results. For example, results could be due to BFEN being more sensitive for measuring VPA than for MPA.[407] However, other metrics of PA processing produced similar results, including ENMOz, which is more sensitive for assessing MPA than BFEN.[407]

Although the primary outcome was powered in this study, SEM mediation analyses were probably underpowered. With the effect sizes generated in this study for the "a" and "b" paths (Figure 5.4), it has been suggested that 116 participants would be needed for 80% power to detect the mediated effect using bias-corrected 95% CIs.[432] Therefore, these results should be treated as exploratory.

## 5.5 Conclusions

This study is the first to address the relationship between PA and LVDF in adolescents with and without OW/Ob. VPA is the only intensity associated with LVDF, which remains once adjusted for adiposity. Irrespective of adiposity, adolescents in the upper quartile of VPA, who perform roughly 18 min/day of VPA, have ≥20% higher septal e'/a' ratios than those in the lowest quartile, suggesting that this duration of VPA may protect against impaired LVDF. Given that PA guidelines currently focus on a combined metric of MVPA, future well-designed RCTs will be needed to investigate the potential beneficial role of VPA over MPA on myocardial function, as well as the dose of VPA required.

This study also confirmed the results of Study 1 that adolescents with OW/Ob have lower LVDF than normal-weight equivalents, which is most apparent when using septal TDI. This may provide a more

targeted approach to identify the earliest impairments in adolescents, but future work to compare septal versus lateral TDI measures against reference norms, which are currently unavailable for adolescents, will be needed to confirm this.

This study also supports the preliminary results of Study 1 that higher CRF and lower IR are linked with better LVDF in adolescents, independently of adiposity. Future work in a larger population of adolescents will be needed to assess the independent effect of CRF once adjusted for adiposity and PA. Similar analyses will also be important for IR, which was not possible in this study given the small sample size of IR data.

Given the increasing prevalence of childhood obesity in the UK, especially since the COVID-19 pandemic,[9] we can expect more adolescents to develop early impairments of LVDF. If this is not addressed, LVDF will likely worsen as these individuals age, potentially predisposing them to future LVDD[27, 83] with a concomitant rise in the number of future HFpEF cases.[18] Therefore, given the results of this study and the lack of VPA recommendations, identifying a duration of VPA linked with reduced CVD risk is important in the primordial prevention of CVD.

# Chapter 6: Intensity and duration of physical activity and cardiorespiratory fitness (Study 3)

As CRF is an important CVD risk factor independently linked with LVDF, as reported in Study 2, this chapter set out to establish the intensity and duration of VPA that is linked with high levels of CRF.

I have published this work in *Pediatrics* as the first author,[468] which received a published commentary.[469] This study was also the subject of an international news report and was discussed in an online podcast from the American Academy of Pediatrics (see page IV).

## 6.1 Introduction

The health benefits of PA and CRF are well-known, including reduced risks of obesity, diabetes, hypertension, CVD, poor mental health, and all-cause mortality.[157, 470-474] Measures of PA and CRF are often used interchangeably in relation to CVD risk and their strong interdependency makes it difficult to determine whether it is one or both of these factors that is most important for cardiovascular health.[466, 467] Nevertheless, interventions that increase both are likely to be beneficial.

Improving CRF in young people is an important goal, given that adolescents and young adults with high CRF have a lower risk of developing CVD risk factors compared to low CRF counterparts.[475-478] Study 2 supports this, showing that adolescents with higher CRF have better LVDF, independent of adiposity. In the 2020 statement from the American Heart Association,[157] Raghuveer and colleagues

highlight this importance, discussing the health benefits of good CRF in youths and go on to argue the need for yearly CRF tests to identify youths who would benefit from early intervention.

Although genetic variation contributes to differences in CRF,[461, 462] the principal modifiable determinant of CRF is habitual PA. However, when trying to improve CRF, there are substantial inter-individual responses in CRF to a given PA programme.[463, 479] For example, Ross *et al*[479] identified that some people need to perform long durations of high intensity PA to increase CRF, whereas others only need to perform short durations of less intense PA to increase CRF by the same amount. Some people are also unable to improve their CRF level following a PA intervention and have been termed "CRF non-responders".[463, 479] However, all participants were able to improve their CRF when they adhered (≥90% adherence) to high amounts of high intensity PA,[479] supporting the notion that VPA may be more important than lesser intensities for improving CRF in adolescents.[46, 273, 389-395, 398, 480]

VPA may also be more practical and achievable than MPA. Wrist-worn accelerometery data from >90,000 adults in the UK Biobank cohort study revealed that adults would have to perform >927 minutes per week of MPA compared to only >40 minutes of VPA to reduce CVD risk.[273] Although both intensities were associated with improved outcomes, prior studies have not attempted to account for the intercorrelation between PA intensities. The authors of the UK Biobank study comment on this and state "*Future research may need to concentrate on the components of total volume of PA using validated measures of activity intensity in a large sample to unravel the [individual] contribution of [MPA and VPA]*."[273] Therefore, although existing evidence suggests that increasing habitual VPA is likely to yield the greatest benefit for CRF in adolescents, it is uncertain if this is truly the most beneficial exercise intensity, whether other intensities offer additional independent benefits for CRF, and how much PA at each intensity is required to achieve adequate CRF according to established norms. This has not been addressed in the recent WHO PA guidelines.

The updated WHO PA guidelines recommend that children and adolescents undertake MVPA for an average of 60 min/day to improve physical, mental and cognitive health.[173] Despite their

recommendation, ≥75% of adolescents failed to achieve these minimum activity levels .[159, 160] Although the WHO guidelines were based on several systematic reviews, the method by which the expert panel arrived at this specific PA target for children and adolescents was not specified. Furthermore, the data that were included in the synthesis of the WHO guidelines included a variety of studies that differentially assessed PA. Data were collected either subjectively or objectively, with additional variation within the questionnaires used or the wearable device worn. For example, objective PA data were included from multiple methods, including accelerometery, HR, and/or pedometery. Even when limited to accelerometery, studies assessed PA by either a uniaxial or triaxial accelerometer and by placing accelerometers on either the wrist or hip, for example. Such variations are known to influence PA results (see Chapter 4). Therefore, as the methodology varies substantially within the WHO guidelines and without examination of the individual components of MVPA, a more thorough analysis of PA is warranted to determine if there are potentially more attractive and/or manageable ways in which adolescents can improve their CRF.

Our group has recently shown that CRF, and other health-related fitness components, have declined in Oxfordshire adolescents from 2014 to 2019, which coincided with a reduction in the number of total PE hours across the UK.[481] I was a co-author of this study. The results were supported by other longitudinal youth studies.[482, 483] In addition, the number of children and adolescents meeting the WHO PA guidelines has worsened dramatically as a result of the COVID-19 pandemic.[64] Given this, the lack of robust guidelines for CRF in adolescents, and the link between CRF and LVDF shown in Study 2, recommendations about the intensity and duration of PA linked with good CRF are needed urgently and should be based on robust evidence. As guidelines are currently based on combined MVPA targets, this an important aim. Therefore, this study set out to establish the extent to which each PA intensity is associated with CRF, independently of other PA intensities, and the duration of activity at each intensity associated with maximum CRF.

## 6.2 Methods

Data were collected as part of the pre-screening process for OxSOCRATES. Briefly, students participated in the 20mSRT as part of an enhanced PE lesson, and were provided with a wrist-worn accelerometer to assess PA over seven-days, as detailed in Chapter 4. Data from a sub-population of participants that were recruited for the baseline/cross-sectional element of OxSOCRATES who completed a maximal CPET were also used, as detailed in Chapter 4. This study was written in accordance with the STROBE reporting guidelines.[400]

The primary aim was to determine the extent to which each PA intensity is associated with CRF, as determined by the 20mSRT, independently of other PA intensities, and the duration of activity at each intensity associated with maximum CRF, in a large population of adolescents from Oxford. To confirm these results, analyses were repeated, but with replacement of the 20mSRT with a maximal CPET in a sub-population of participants.

### 6.2.1 Participants

Participants aged 13-14 years from two secondary schools in Oxfordshire (UK) were studied as part of the pre-screening process in the 2018/19 and 2019/20 academic years. Recruitment of participants to OxSOCRATES is detailed in Chapter 4.

### 6.2.2 Anthropometrics and physical activity

Anthropometrics and PA were collected as detailed in Chapter 5.

### 6.2.3 Cardiorespiratory fitness

As part of the pre-screening process, adolescents completed the 20mSRT.[484] This involved adolescents shuttle running between two sets of cones placed 20m apart. The frequency of the sound signals increased such that adolescents started running at 8 km/h, increasing to 9 km/h after one minute and then increasing by 0.5 km/h every minute thereafter.[484] All adolescents were given verbal motivation

to encourage maximal effort and were informed that they must touch the line with their foot either before or as the sound signal was emitted. The level and stage for each participant were recorded when they failed to reach the 20m mark on three consecutive occasions or when they voluntarily terminated the test. The test took place inside school gymnasiums.

A sub-population completed a CPET as described in Chapter 5.

### 6.2.4 Statistical analysis

Participants with data on sex, BMI z-score, 20mSRT, and a valid recording of PA were included. Non-parametrically distributed data were transformed as detailed in Chapter 4. Differences in the number of boys and girls were assessed using Chi squared tests. Sex differences were tested using independent t-tests or Mann-Whitney U test and represented as mean ± SD or median (IQR), depending on distributions. Differences between PA metrics were assessed by ANOVA and Bonferroni post-hoc test or Kruskal-Wallis H tests and Dunn's post-hoc test, depending on distributions.

As PA intensities are correlated with each other (Appendix Table 3.1), rPA intensities were constructed, as detailed in Chapter 4, that were independent of all other intensities (i.e. zero correlated, Appendix Table 3.2). Standard multivariable regression with all the normal PA intensities was also used to confirm the findings of the partial models.

The association of each rPA variable with CRF, adjusting for sex, was tested in separate models and, given their mutually independent nature, in a fully adjusted model including all rPA variables. These results were compared with models using unadjusted PA variables.

To capture the underlying relationships between rPA and the CRF, a filtering mechanism was used to smooth the raw data, removing any "noise", and allowing the trends in the data to be revealed. This approach differs from more common statistical methods such as linear regression that fit a function with a predefined shape to the data. By contrast, a zero-phase moving average filter was used, which makes no assumption about the shape of the relationship and imposes no linearity constraint, to

determine relationships between each rPA variable and CRF. The Akaike information criterion (AIC) was used to determine the moving average window size that yielded the optimum goodness-of-fit of each model before overfitting occurred. The threshold at which CRF might no longer improve significantly with increasing PA was determined as the point where any positive increase began to level off. This was done using the Stata function *-threshold-.* This function extends linear regression modelling to allow coefficients to differ across regions of data determined by a threshold value. The threshold value was estimated by selecting the model with the smallest sum of square residuals value for all possible thresholds. Comparison was made with linear, 2$^{nd}$ order polynomial, and moving median models to demonstrate which approach best fitted the data. The duration of PA needed to achieve median CRF was determined as the point where the moving average model reached a CRF z-score of zero.

To support the results of the moving average model, sex-specific mean differences in VPA between participants ≤50$^{th}$ percentile (Group 1) and participants >50$^{th}$ percentile (Group 2) of CRF were assessed using independent t-tests. A range of CRF percentile thresholds were determined from our own 20mSRT data, and from the mean of 13- and 14-year old 20mSRT data from European and global reference ranges, for comparison.[289, 485]

The above analyses were repeated using CPET derived CRF from a sub-study of participants in OxSOCRATES. The AIC was not used to determine the moving average window size that yielded the optimum goodness-of-fit because of the relative lack of data, which caused spurious fitting when the suggested AIC value was used. Therefore, these analyses should be considered exploratory.

To ensure that any plateauing in the moving average model was not due to a reduced number of participants with high amounts of VPA, the models were re-run after randomly downsampling the data (using the Stata function -*sample*-) so that there were only six datapoints per five minutes of VPA.

In order to assess differences in CRF (20mSRT) between low and high amounts of PA, the upper and lower quartiles of each normal PA variable and of each rPA variable were assessed using independent t-tests.

### 6.2.5 Power calculations

The required sample size to detect the independent association of PA with CRF was determined *post-hoc* using G*Power (version 3.1.9.7). A multiple regression model of CRF z-scores determined by the 20mSRT (dependent variable) and all PA intensities (VPA, MPA, LPA, ST) and sex as independent variables, was fitted as the primary outcome model, with the aim of measuring power for the independent effect of VPA. The results were inputted into G*Power using the "*F test – Linear multiple regression: Fixed model, $r^2$ increase*" function.

## 6.3 Results

### 6.3.1 Participants characteristics

Of the 480 adolescents who participated in the health assessment, 339 had sufficient, valid data allowing for inclusion (Figure 6.1). Their key characteristics are reported in Table 6.1. Height, weight, BMI, and BMI z-score data were missing for two, five, five, and six participants, respectively. Boys were taller than girls but there were no sex differences in weight, BMI or BMI z-score. Boys performed better in the 20mSRT and also did more VPA. Girls had higher MPA, LPA, and ST measures. PA determined by other metrics are reported in Appendix 3, Table 3.3. Adolescents that did not have valid PA wear time had similar characteristics, but had lower z-score total lap results compared to those with valid PA (Appendix 3, Table 3.4). Although not directly assessed, schools covered a range of socioeconomic and ethnic groups (Appendix 3).

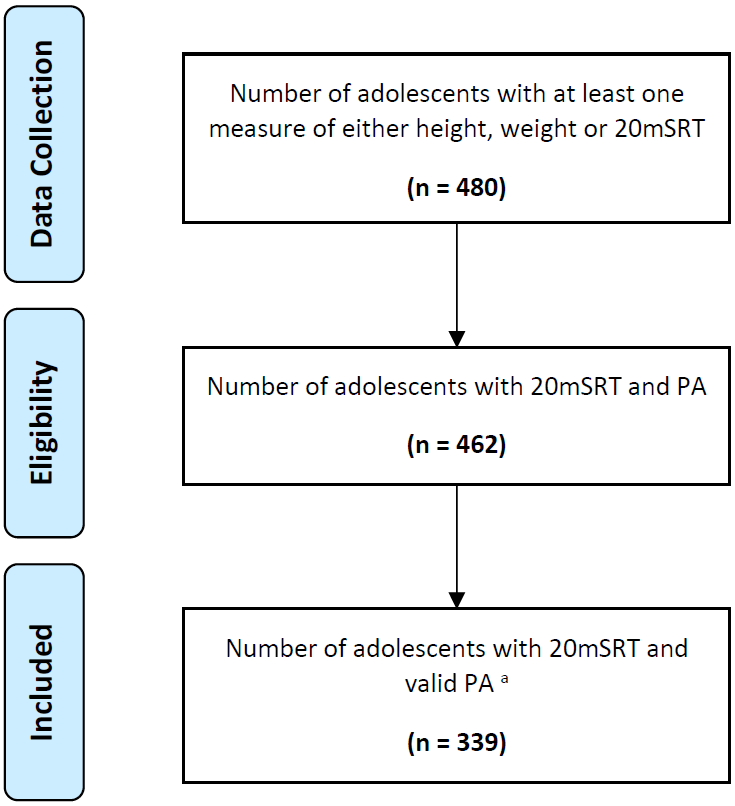


**Figure 6.1: Flow diagram of the number of adolescents included in the study.**

20mSRT indicates 20m shuttle run test; a, a valid week of physical activity (PA) consisted of at least 3 weekdays and 1 weekend day with each valid day requiring >6hrs of wear time.

**Table 6.1:** Participant characteristics

| Measure | All participants | Girls | Boys | *P*-value |
|---|---|---|---|---|
| N (%)[a] | 339 (100) | 169 (50) | 170 (50) | 1.00 |
| Height (cm)[b] | 165.2 ± 8.7 | 162.7 ± 6.9 | 167.6 ± 9.6 | $1.3x10^{-7}$ |
| Weight (kg)[b] | 56.0 ± 12.3 | 55.2 ± 11.5 | 56.9 ± 12.9 | 0.21 |
| BMI ($kg/m^2$)[b] | 20.5 ± 3.8 | 20.8 ± 3.8 | 20.1 ± 3.7 | 0.10 |
| BMI z-score (WHO)[b] | 0.3 ± 1.2 | 0.3 ± 1.2 | 0.3 ± 1.2 | 0.68 |
| 20mSRT (total laps)[c, d] | 44 (32, 62) | 38 (27, 54) | 54 (38, 76) | $2.4x10^{-9}$ |
| 20mSRT (z-score total laps)[c, d] | -0.4 (-1.0, 0.5) | -0.4 (-0.9, 0.5) | -0.4 (-1.1, 0.5) | 0.99 |
| Physical Activity | | | | |
| *VPA (mins)[c, d]* | 10.6 (7.2, 15.7) | 9.6 (6.6, 14.1) | 11.4 (8.0, 17.1) | $2.0x10^{-3}$ |
| *MPA (mins)[b]* | 153.2 ± 42.9 | 165.8 ± 40.5 | 140.6 ± 41.7 | $3.7x10^{-8}$ |
| *LPA (mins)[c, d]* | 202.5 (166.3, 228.8) | 205.1 (177.0, 231.2) | 198.6 (156.6, 226.0) | 0.03 |
| Sedentary Time (mins)[b] | 470.1 ± 99.5 | 489.9 ± 86.3 | 450.4 ± 107.7 | $2.3x10^{-4}$ |

Physical activity and sedentary time based on bandpass-filtered followed by Euclidean norm (BFEN). BMI, body mass index; LPA, light physical activity; MPA, moderate physical activity; VPA, vigorous physical activity; WHO, World Health Organization; [a], Chi squared test; [b], mean ± standard deviation; [c], median (interquartile range); [d], variable with non-normal distribution

### 6.3.2 Physical activity and cardiorespiratory fitness

Given the sex differences in PA shown in Table 6.1, all linear models were adjusted for sex. Linear regression of each PA intensity with the CRF measures (total laps and total laps z-score) found the strongest association with VPA and progressively weaker associations with MPA and LPA (Table 6.2, Model 1). Standard multivariable regression showed that only VPA was independently associated with CRF after adjustment for the other intensities (Table 6.2, Model 2). Further modelling using partial regression were then carried out to examine the independent effects of each intensity. This was done with the rPA variables individually (Table 6.2, Model 3) and in mutually adjusted models with the rPA variables calculated with either VPA (Table 6.2, Model 4) or ST (Table 6.2, Model 5) as the base variable. Only VPA was independently associated with CRF, irrespective of analysis approach. Having

thus demonstrated the benefit of the partial regression modelling approach, only rPA variables were used in subsequent analyses.

Model 2 was used as the primary outcome model with the aim of measuring power for the independent effect of VPA. The $r^2$ of the model was 0.17 (n=339; residual variance: 1–0.17=0.83) and the partial $r^2$ for VPA was 0.13 (variance explained by VPA). The achieved power to detect the independent effect of VPA was 99%.

In the moving average model, greater average daily VPA was associated with better CRF until 19 minutes of VPA, when the relationship plateaued ($r^2$=0.35; Figure 6.2). Median CRF was found in adolescents performing 14 (range 12-17) minutes of VPA daily. The range was estimated as the VPA values where the 95% confidence limits of CRF z-score crossed the median. Results did not change meaningfully when the awake wear time threshold was set at >10hrs (n=302; plateau=20 minutes; range 12-17; $r^2$=0.36). As in the linear models, other rPA measures were unrelated to CRF (Figure 6.3). Moving average models using other metrics of PA demonstrated similar results to BFEN (Appendix 3, Figures 3.1-3.5), although the median and plateau points differed, depending on the method used (Appendix 3, Table 3.5). The moving average model provided the best model fit of the data when compared to linear, 2$^{nd}$ order polynomial, and moving median models (Appendix 3, Figure 3.6). When analyses were repeated by sex, there were steep increases in CRF with increasing VPA up to 20 and 18 minutes in boys and girls, respectively, plateauing after that (Appendix 3, Figure 3.7). However, these models were not statistically different from each other (overlapping confidence limits). The presence of a plateau after downsampling the data indicates that the above results are not due to a reduced number of datapoints above 20 minutes of daily VPA (Appendix Figure 3.8).

To support the findings of the moving average model, independent t-tests assessed mean differences in VPA between individuals below (Group 1) and above (Group 2) a number of different median CRF thresholds (Table 6.3). Boys and girls in Group 2 undertook significantly more VPA than those in Group 1, regardless of threshold definition. There were no significant group differences in any of the other

rPA intensities. Similarly, CRF z-score differed when comparing the upper and lower quartiles of VPA, but not those of any other rPA measure (1.03 z-scores, 95% CI=0.75, 1.30; Figures 6.2 & 6.3).

**Table 6.2: Association of physical activity (PA) and residualised PA with cardiorespiratory fitness**

| | *r* | *B* | *95% CI* | *p* |
|---|---|---|---|---|
| **Total Laps** | | **Model 1** | | |
| *VPA* | 0.39 | 1.34 | 1.01, 1.66 | $5.5x10^{-15}$ |
| *MPA* | 0.21 | 0.12 | 0.06, 0.17 | $1.0x10^{-4}$ |
| *LPA* | 0.11 | 0.06 | $3.2x10^{-3}$, 0.11 | 0.04 |
| *ST* | $2.6x10^{-3}$ | $6.2x10^{-4}$ | -0.02, 0.03 | 0.96 |
| **Z-score Total Laps** | | | | |
| *VPA* | 0.41 | 0.06 | 0.05, 0.07 | $1.6x10^{-14}$ |
| *MPA* | 0.21 | $5.1x10^{-3}$ | $2.5x10^{-3}$, $7.8x10^{-3}$ | $1.7x10^{-4}$ |
| *LPA* | 0.11 | $1.2x10^{-3}$ | $1.7x10^{-6}$, $4.8x10^{-3}$ | 0.05 |
| *ST* | -0.01 | $-1.2x10^{-4}$ | $-1.3x10^{-3}$, $1.0x10^{-3}$ | 0.83 |
| **Total Laps** | | **Model 2** | | |
| *VPA* | 0.45 | 1.53 | 1.1, 2.0 | $9.2x10^{-12}$ |
| *MPA* | -0.07 | -0.04 | -0.14, 0.06 | 0.47 |
| *LPA* | -0.03 | -0.02 | -0.10, 0.10 | 0.70 |
| *ST* | 0.05 | 0.01 | -0.01, 0.04 | 0.36 |
| **Z-score Total Laps** | | | | |
| *VPA* | 0.47 | 0.07 | 0.05, 0.09 | $2.0x10^{-11}$ |
| *MPA* | -0.08 | $1.8x10^{-3}$ | $-6.4x10^{-3}$, $2.8x10^{-3}$ | 0.44 |
| *LPA* | -0.03 | $5.8x10^{-4}$ | $-4.3x10^{-3}$, $3.2x10^{-3}$ | 0.76 |
| *ST* | 0.04 | $3.7x10^{-4}$ | $-8.0x10^{-4}$, $1.5x10^{-3}$ | 0.53 |
| **Total Laps** | | **Model 3** | | |
| *VPA (base)* | 0.39 | 1.34 | 1.01, 1.66 | $5.5x10^{-15}$ |
| *rMPA* | -0.02 | -0.01 | -0.09, 0.06 | 0.74 |
| *rLPA* | $-3.6x10^{-3}$ | $-2.9x10^{-3}$ | -0.08, 0.08 | 0.95 |
| *rST* | 0.07 | 0.02 | -0.01, 0.05 | 0.16 |
| **Z-score Total Laps** | | | | |
| *VPA (base)* | 0.41 | 0.06 | 0.05, 0.07 | $1.6x10^{-14}$ |
| *rMPA* | -0.02 | $-7.1x10^{-4}$ | $-4.2x10^{-3}$, $2.8x10^{-3}$ | 0.69 |
| *rLPA* | $-6.9x10^{-3}$ | -2.3x10-$^{4}$ | $-3.9x10^{-3}$, $3.5x10^{-3}$ | 0.90 |
| *rST* | 0.06 | $7.4x10^{-4}$ | $-5.3x10^{-4}$, $2.0x10^{-3}$ | 0.25 |
| **Total Laps** | | **Model 4** | | |
| *VPA (base)* | 0.40 | 1.35 | 1.02, 1.67 | $6.3x10^{-15}$ |
| *rMPA* | -0.06 | -0.04 | -0.11, 0.03 | 0.24 |
| *rLPA* | $-2.3x10^{-3}$ | $-1.8x10^{-3}$ | -0.08, 0.07 | 0.96 |
| *rST* | 0.04 | 0.01 | -0.01, 0.04 | 0.36 |
| **Z-score Total Laps** | | | | |
| *VPA (base)* | 0.41 | 0.06 | 0.05, 0.08 | $1.5x10^{-14}$ |
| *pMPA* | -0.07 | $-2.0x10^{-3}$ | $-5.3x10^{-3}$, $1.2x10^{-3}$ | 0.22 |
| *pLPA* | $-4.3x10^{-3}$ | $-1.5x10^{-4}$ | $-3.6x10^{-3}$, $3.3x10^{-3}$ | 0.93 |
| *pST* | 0.03 | $3.7x10^{-4}$ | $-8.0x10^{-4}$, $1.5x10^{-3}$ | 0.53 |

| **Total Laps** | **Model 5** | | | |
|---|---|---|---|---|
| *rVPA* | 0.44 | 1.5 | 1.08, 1.92 | $1.2x10^{-11}$ |
| *rMPA* | -0.08 | -0.05 | -0.15, 0.06 | 0.41 |
| *rLPA* | 0.02 | 0.01 | -0.08, 0.10 | 0.82 |
| *ST (base)* | -0.04 | -0.01 | -0.03, 0.01 | 0.46 |
| **Z-score Total Laps** | | | | |
| *rVPA* | 0.46 | 0.07 | 0.05, 0.09 | $2.5x10^{-11}$ |
| *rMPA* | -0.09 | $-2.2x10^{-3}$ | $-7.1x10^{-3}$, $2.7x10^{-3}$ | 0.38 |
| *rLPA* | 0.02 | $6.0x10^{-4}$ | $-3.5x10^{-3}$, $4.7x10^{-3}$ | 0.77 |
| *ST (base)* | -0.05 | $-5.4x10^{-4}$ | $-1.6x10^{-3}$, $5.1x10^{-4}$ | 0.31 |

**Model 1**: total laps or z-score total laps (dependent variable) versus each physical activity (PA) intensity and sex (independent variables). **Model 2:** fully adjusted multiple regression of total laps or z-score total laps (dependent variable) versus all PA intensities and sex (independent variables). **Model 3**: total laps or z-score total laps (dependent variable) versus each residualised PA (rPA) intensity and sex (independent variables). **Model 4**: fully adjusted model of total laps or z-score total laps (dependent variable) versus all rPA intensities and sex (independent variables) with vigorous PA (VPA) as the base. **Model 5**: fully adjusted model of total laps or z-score total laps (dependent variable) versus all rPA intensities and sex (independent variables) with sedentary time (ST) as the base. *B* represents unstandardised correlation coefficients; CI, confidence intervals of *B*; LPA, light PA; MPA, moderate PA; *r*, standardised correlation coefficients; rLPA, residualised LPA; rMPA, residualised MPA; rST, residualised ST; rVPA, residualised VPA.

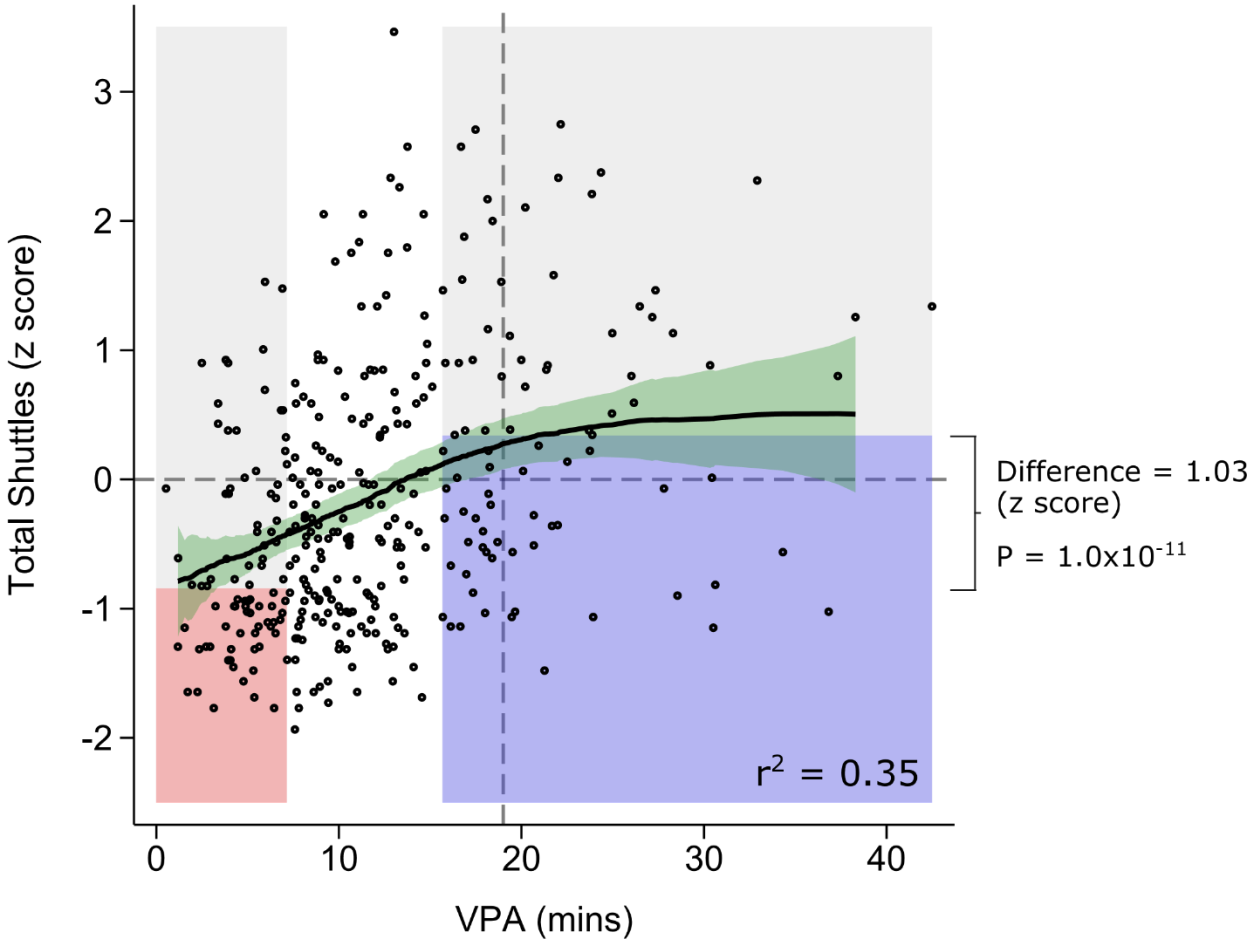


**Figure 6.2: Moving average model of the association of daily vigorous physical activity (VPA) with the z-score of total number of shuttles run (cardiorespiratory fitness - CRF).**

The black line indicates the best-fit moving-average non-linear relationship between VPA and CRF with 95% confidence intervals (green). The vertical grey dashed line indicates the point at which the moving-average model plateaus at about 20 minutes. The significant mean difference in CRF between the lowest (red) and highest (blue) quartiles of VPA is given. VPA based on bandpass-filtered followed by Euclidean norm (BFEN).

**Table 6.3: Sex-specific difference in VPA between participants either below or above median fitness**

| CRF Median Threshold | Group 1 VPA (mins) | Group 2 VPA (mins) | Δ (95% CI) | *P*-value |
|---|---|---|---|---|
| **Boys** | | | | |
| Total Laps | 10.7 ± 6.7 | 15.9 ± 8.3 | 5.3 (3.0, 7.5) | $9.4x10^{-6}$ |
| Z-score Total Laps | 10.8 ± 6.6 | 17.6 ± 8.2 | 6.8 (4.5, 9.1) | $1.8x10^{-8}$ |
| European Total Laps | 10.7 ± 6.7 | 15.8 ± 8.2 | 5.1 (2.8, 7.4) | $2.0x10^{-5}$ |
| Global Total Laps | 10.3 ± 7.0 | 15.4 ± 7.9 | 5.1 (2.7, 7.4) | $2.8x10^{-5}$ |
| **Girls** | | | | |
| Total Laps | 9.1 ± 5.2 | 12.3 ± 5.8 | 3.2 (1.5, 4.9) | $2.0x10^{-4}$ |
| Z-score Total Laps | 9.5 ± 5.1 | 12.5 ± 6.2 | 3.1 (1.3, 4.8) | $6.0x10^{-4}$ |
| European Total Laps | 8.1 ± 4.2 | 12.1 ± 6.0 | 3.9 (2.2, 5.7) | $1.0x10^{-5}$ |
| Global Total Laps | 8.2 ± 4.2 | 11.7 ± 6.0 | 3.6 (1.8, 5.4) | $1.5x10^{-4}$ |

Boys and girls were divided into either Group 1 (≤50$^{th}$ percentile) or Group 2 (>50$^{th}$ percentile) based on the median of the total number of laps completed on the 20m shuttle run test, of z-score total laps, of European normative data for total laps,[485] and of global normative data for total laps.[289] Data are represented as mean ± standard deviation. Vigorous physical activity (VPA) was based on bandpass-filtered followed by Euclidean norm (BFEN). CI represents confidence intervals of the mean difference between groups; CRF, cardiorespiratory fitness; Δ, mean difference (Group 2 – Group 1).

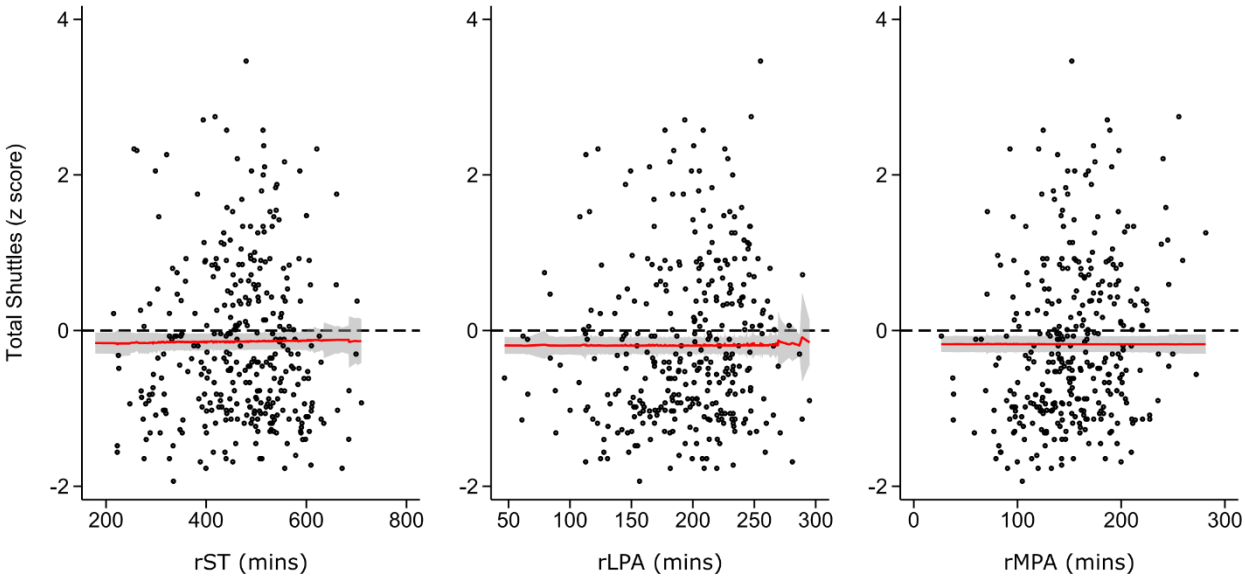


**Figure 6.3 Moving average models of the associations of residualised physical activity variables (rPA) with the z-score of total number of shuttles run (cardiorespiratory fitness).**

Physical activity variables were adjusted by partial multivariable regression modelling for the confounding effect of correlations between activity levels. rPA based on bandpass-filtered followed by Euclidean norm (BFEN). rLPA indicates residualised light physical activity; rMPA, residualised moderate physical activity; rST, residualised sedentary time.

### 6.3.3 Sub-study

To support the above findings, analyses were repeated using CRF in a sub-population of adolescents who completed wrist-worn accelerometery and a CPET (n=96). In moving average models, greater average daily VPA was similarly associated with better CRF until ~19 minutes of VPA, when the relationship plateaued (Appendix 3, Figure 3.9). Other rPA intensities were unrelated.

## 6.4 Discussion

Habitual PA is known to have many health benefits, including higher levels of CRF in all age-groups. This study showed greater average VPA per day was associated with better CRF in a large population of adolescents up to about 20 min/day. The association plateaued after this, with greater VPA durations associated with little or no further improvement in CRF. After intercorrelations between PA intensities were controlled for, only VPA was associated with CRF. As current guidelines set PA activity targets using a combined metric of MVPA that could be satisfied by undertaking only MPA and not VPA, this study provides grounds for clearer public health messaging on how to improve CRF in this population. Most adolescents fail to achieve the current recommendation of ≥60 minutes of MVPA per day on average.[159, 173] One possible reason is that this duration is quite long, requiring a daily time commitment that some may find difficult to maintain. A shorter target of 20 minutes might be easier to schedule daily and a focus on VPA would simplify messages about the intensity of activity that is likely to improve CRF.

These findings support those in adults where 15 min/day of VPA was shown to improve both CRF and glycaemic control,[395] and are also similar to findings in children and adolescents.[389, 393] In the study by Fussenich *et al*,[389] children who performed ≥17 min/day of VPA were at lower risk of CVD risk factors than those who performed less. In the review by Gralla *et al*,[393] it was concluded that a minimum of 10 min/day of VPA may be sufficient to decrease adiposity and improve cardiometabolic health markers. The current PA guidelines provide recommendations based on a combined metric of MVPA, which does not specify the duration of either MPA or VPA.[173] This study provides such evidence,

highlighting the importance of VPA over lesser intensity PA for CRF. Given these findings, the findings in Study 2, and those of others detailed above, 20 min/day of VPA may be sufficient to either prevent or reverse cardiometabolic and cardiovascular ill-health.

The results in this study are consistent with other cross-sectional studies in highlighting the benefits of VPA.[46, 392, 393] However, to my knowledge, no prior study in adolescents has attempted to examine the independent effects of different PA intensities, controlling for their intercorrelation. The approach in this thesis suggests that, although past reports generally acknowledge the inferiority of MPA or lesser intensities of PA, conclusions about MPA could have been erroneously drawn due to the confounding strong correlation between VPA and MPA. To put it simply, it is generally not possible to do more VPA routinely without increasing the amount of MPA that you do too.

There was no additional effect of MPA or lower intensity PA on CRF, supporting cross-sectional and longitudinal evidence that VPA improves adiposity and cardiometabolic health to a greater extent than MPA.[46, 392-394] It is, however, possible that other health-related measures that were not studied may improve with lower intensities of PA.[486] However, as shown in this study, it is important to first control for intercorrelations between PA intensities before examining associations with any health-related measures. Nevertheless, CRF remains an important predictor of cardiometabolic health outcomes.[475-478] It is notable too that earlier studies have generally found that only very high amounts of MPA are associated with improved health outcomes, possibly because MPA was acting as a weak proxy for VPA in such studies.[273] Furthermore, in studies of exercise interventions, VPA interventions were found to have similar adherence rates to those with lesser intensity, but with reduced time commitments, highlighting no apparent adherence disadvantage from focusing on VPA.[392]

Undertaking 20 min/day of VPA to improve CRF may provide the means for adolescents to improve their long-term health outcomes at scale.[475-478, 482, 483] However, this study design does not address causation in the associations shown. Although reverse causation may partially explain our findings,

prior knowledge on the relationship between exercise and CRF suggests this is unlikely to be a dominant explanatory factor.[480]

Future studies should also aim to evaluate how best to achieve a target of 20 min/day of VPA in large populations. In the Fit to Study trial by our group at OBU, implementation of a VPA intervention in >18,000 Year 8 students across 104 schools found no improvements in CRF, cognitive performance, or mental health. Furthermore, the intervention was planned to deliver 10 minutes of VPA, but students participated in VPA for an average of only 4 minutes per PE lesson, suggesting that it may be difficult to achieve similar structured VPA targets at scale.[487] Thus, it may be better to integrate 20 min/day of VPA into daily life, such as running up the stairs or cycling to school.

On average, girls undertook less PA and had lower CRF than boys, which is in-line with previous studies.[159, 488] However, there was no evidence that the models of the relationship between PA and CRF differed significantly according to sex, supporting the use of the combined model and unified recommendations for all.

Having high CRF is not only linked with LVDF (Study 2), but is also associated with a number of other important health-related measures, such as CMRFs, arterial stiffness, lung function, cognitive function, and mental health.[157] However, much like the declines in PA and increases in OW/Ob in children and adolescents,[9, 64] we identified in a serial cross-sectional study associated with the work in this thesis that CRF has been declining in Oxfordshire adolescents.[481] Therefore, if nothing is done to manage this problem, we can expect a marked rise in the burden of diseases, such as LVDD, as these children age. Setting good lifestyle habits early in life will therefore be important for reducing the burden of CVD and HFpEF, especially since OW/Ob and PA track from adolescence to adulthood.[23-26, 489, 490] Thus, these findings support the notion that primordial prevention techniques in adolescents with OW/Ob will be important for reducing the population burden of CVD.[15]

### 6.4.1 Strengths and limitations

This work has a number of strengths and potential limitations. The findings of this study are based on data from two Oxfordshire, UK schools and might, therefore, not be generalisable to broader populations. However, the sample was well-balanced by sex, covered a range of socioeconomic and ethnic groups, and had a wide distribution of BMI and CRF. Whilst it is acknowledged that this study needs replicating, importantly, there was no significant difference in shuttle run performance between our data and European and Global normative data, suggesting that our population is representative.[289, 485]

Recently the validity of the 20mSRT as an indirect assessment of CRF has been both questioned[491] and defended.[492] Although its utility and validity for ranking fitness in large populations of children is supported in numerous contemporary reviews,[291-293, 296] it is acknowledged that the 20mSRT might be an imperfect measure of CRF in adolescents. Therefore, the analysis was repeated using a sub-population of adolescents who completed a laboratory-based CPET to assess $VO_2$peak adjusted for FFM. This confirmed the results of the primary analyses (see Appendix 3, Figure 3.8), providing confidence that the results were valid and that the 20mSRT was representative of more physiological measures of CRF in this context.

The limitations of PA detailed in Study 2 also apply here. Analyses were repeated using the variety of other PA metrics favoured in the literature, which did not meaningfully alter the results. However, the time spent in different PA metrics, as well as the duration of VPA at the point where the relationship with CRF plateaued did inevitably differ slightly between metrics.

## 6.5 Conclusion

This study provides evidence that adolescents who undertake 20 min/day of VPA on average have maximal CRF, with little evidence of additional benefit from undertaking more VPA per day or from lesser intensities of PA. This modest duration and more specific intensity of PA may provide a better underpinning for future guidelines that currently recommend a longer duration of less specific activity

(MVPA) each day. Further work should aim to test whether interventions based on this new target offer significant improvements in adolescent cardiometabolic and cardiovascular health.

# Chapter 7: General discussion

## 7.1 Summary

This thesis has examined the link between adolescent OW/Ob, PA, CRF, and cardiometabolic health with LVDF. This was achieved first by pooling previous results in a large systematic review and meta-analysis, confirming that adolescent OW/Ob is associated with worse LVDF as well as identifying the measures of LVDF most strongly linked with OW/Ob. The literature search identified that there was a lack of data on PA and LVDF, and so data from the OxSOCRATES study was used to address this. It was found that VPA, independently from OW/Ob, is linked with LVDF, whilst lesser intensities are seemingly unrelated. Most notably, there was evidence that a modest duration of VPA improves LVDF significantly, with a ~30% improvement in septal e'/a' ratios either directly or indirectly by reducing adiposity. Given the recent call for more research to understand obesity-related cardiac dysfunction[40] and the continued rise in the prevalence of adolescent OW/Ob and physical inactivity,[9, 64] this work provides an important understanding of LVDF in adolescents with OW/Ob and provides the means to potentially reverse this through VPA.

## 7.2 Main findings

This thesis had three main hypotheses: 1) OW/Ob is associated with worse LVDF in adolescents; 2) PA, independent from OW/Ob, can protect against the adverse cardiac effects of OW/Ob; and 3) poor cardiometabolic health and low CRF are linked with worse LVDF in adolescents, independently from OW/Ob. This thesis provides strong evidence for these hypotheses whilst two other key themes were also identified. Firstly, septal TDI, primarily the septal e'/a' ratio, is most strongly linked with adiposity, PA, CRF, and IR in adolescents, and secondly; VPA of a modest duration on average, per day, may provide a better underpinning for future guidelines that currently recommend a longer duration of less specific activity each day. These findings and their importance are discussed in this chapter.

### 7.2.1 Overweight/obesity and LVDF

The key objective of this thesis was to determine whether OW/Ob is linked with LVDF in adolescents. In Study 1, a novel approach to meta-analysis using weighted, random-effects regression was undertaken to limit the impact of group selection bias, a pitfall of conventional, group-based meta-analyses, and allowed for the continuous relationships between the degree of adiposity and measures of LVDF to be studied. From this, it was concluded that adolescent OW/Ob is associated with impaired LVDF and this was further confirmed in Study 2, which used BMI z-scores and FMI instead of BMI to assess adiposity. As both BMI z-score and FMI measures are easily obtained during a routine clinical assessment, these measures are generalisable to help identify the beginnings of LVDD. These findings add to the ever-growing body of literature of the adverse health consequences of being overweight or obese in adolescence.

The early impairments found in this thesis likely worsen as these adolescents age, resulting in impaired LVDF in early adulthood.[27] This will continue to worsen throughout the life-course, evidenced by the increased risk for CVD outcomes in later life in young adults with impaired LVDF[83] and the high proportion of patients (84%) with HFpEF having OW/Ob.[18] Therefore, identifying those with the beginnings of LVDD is important and the findings in this thesis suggest that septal TDI may be ideally placed to do this. However, it is important to note that there are no current recommendations as to what change or "score" of LVDF constitutes impaired LVDF in adolescents. Longitudinal risk studies to address this will be of great importance.

### 7.2.2 Septal LVDF

Detection methods for LVDD in adults are well-established,[19] but it has been unclear which measures best detect the earliest stages of LVDD and would, therefore, be most suitable in adolescents, particularly in those with OW/Ob. Study 1 identified that OW/Ob in adolescents was most strongly associated with septal TDI measures of LVDF, which was replicated in Study 2, as well there being

strongest relationships of septal TDI with PA, CRF, and IR measures. Of the septal measures, the e'/a' ratio identified the largest group differences and strongest associations in Studies 1 and 2. These findings coincide with other studies,[185, 377, 445, 493-495] as well as with the original studies of LVDF that identified the e'/a' ratio as the best marker of early longitudinal compliance abnormalities,[185] capturing both the early reductions in septal myocardial relaxation (lower e') and the increased reliance on the LA to expand the LV septum (a'). This could be explained by preferential remodelling of the septum, identifiable in OW/Ob,[72-75, 336] early hypertension,[445, 495, 496] and hypertrophic cardiomyopathy[497] because of a greater susceptibility of the LV septum to wall stress.[442] Studies in sheep with no overt CVD have found that the LV septum is softer, having less collagen and a lower elastic modulus than the LV lateral wall.[498] In pathological states such as obesity, increased collagen I deposition, increased cross-link formation, and glycation lead to a fibrotic state, stiffening the LV and impairing LVDF.[98] However, non-fibrotic collagen is an important structural protein that when compressed can release energy to aid in LV passive relaxation.[97] Thus, compression of this greater collagen in the LV lateral wall[498] during systole would result in greater lateral wall passive relaxation, whereas the septum may have to lean more on active relaxation when confronted with increased wall stress. Obesity is known to impair active LV relaxation in adults,[33, 90] and so pathological remodelling might occur first in the septum because of a reduced capacity for passive relaxation and impaired active relaxation.

It could also be explained by septal TDI being less influenced by translational movement of the heart, as well as fewer Doppler beam angle errors when aligning the ultrasound beam with the septum, providing a better diagnostic utility than lateral equivalents.[499] Although there are other methods of assessing LVDF that may remove some of the limitations of echocardiography, such as MRI, echocardiography is the most widely used non-invasive tool. However, STE is another method of echocardiography that can assess regional myocardial deformation, and so further work to replicate the findings in this thesis using STE will be key, especially since Study 1 identified a handful of studies

that have reported differences in LV strain-rate in children and adolescents with OW/Ob.[326, 334, 340, 351, 368] Nevertheless, the findings of the differences between septal and lateral walls could have important clinical implications that could help develop recommendations to screen for early adolescent impairments in LVDF.

### 7.2.3 Cardiometabolic health and LVDF

Another key objective of this thesis was to determine whether poor cardiometabolic health and low CRF are linked with worse LVDF in adolescents, independently from OW/Ob. In Study 1, it was identified that IR (HOMA-IR) was the most commonly reported CMRF linked with LVDF and this was confirmed in Study 2. Study 2 further utilised an OGTT to measure dynamic IS to mimic the normal physiology in response to a meal. Both fasting and dynamic measures of IR/IS were associated with LVDF, even after controlling for the strong confounding relationship of adiposity. Interestingly, Studies 1 and 2 identified that IR is linked with IVRT (LV relaxation before mitral-valve opening [Figure 1.3]), which persisted in Study 2 after controlling for interbeat intervals. Therefore, IR may play an important role in this specific part of diastole and further work should aim to elucidate this.

This thesis was also able to demonstrate the link between CRF and LVDF, independent of adiposity, which was not addressed in the two studies identified in Study 1.[326, 328] CRF was assessed by both the 20mSRT and by CPET in this thesis, meaning that the results are both generalisable to large-scale fitness testing using the 20mSRT as well as being comparable to results from the gold-standard CPET.

Although a strong genetic underpinning confounds CRF,[461-463] studies suggest that VPA can improve CRF, albeit to different extents.[463, 479, 500] Typically, following standardised exercise programmes, some individuals are unable to increase their CRF and are termed “non-responders”.[463] However, the proportion of those who are classified as non-responders is much less when participants undertake a high-amount of high-intensity exercise compared to shorter durations, lower intensities, and/or have

lower adherence.[463, 479, 500] Thus, performing VPA may be most important to induce cardiovascular benefits.

### 7.2.4 The case for vigorous physical activity

Study 1 identified that there was a paucity of evidence on the link between PA and LVDF in adolescents with and without OW/Ob. This, therefore, comprised the primary aim of Study 2 where it was identified for the first time that VPA, but not other intensities, is associated with multiple measures of LVDF. Of note, it was found that independent of other PA intensities and confounders, participants who were above the median level of VPA had ~31% higher septal e'/a' ratios than those in the lowest quartile, which remained when OW/Ob and adiposity were adjusted for, albeit to a lesser extent (~20-23%). This was further confirmed in SEM bootstrap resampling mediation analyses, where a significant direct effect of VPA on LVDF was identified, independent of partial-mediation by adiposity. This suggests that improving VPA can lead to better LVDF by both a direct effect, through mechanisms that are not yet clear, and by an indirect effect of lowering adiposity, subsequently improving LVDF.

The analyses in Study 2 also took into account the effect of CRF on LVDF. VPA was the only risk factor that remained associated with LVDF once adiposity, CRF, all PA intensities, and confounders were included in one model. Thus, as VPA is easily modified, these findings represent an attractive way to prevent early impairments in LVDF through VPA.

The current WHO PA guidelines recommend that *"Children and adolescents should do at least an average of 60 min/day of MVPA, mostly aerobic, PA, across the week."*[173] However, the findings in this thesis suggest that these recommendations are not sufficient to prevent impaired LVDF and other CVD risk factors (summarised in Appendix 4), especially if adolescents undertake only MPA. Furthermore, most adolescents fail to achieve these recommendations,[159, 173] which could be because 60 minutes of MVPA is quite long, requiring a daily time commitment that some may find difficult to maintain. In this thesis, it has been identified that a shorter duration of more specific PA may be sufficient and possibly

optimal for some outcomes. This was identified first in Study 2 and subsequently confirmed in a large population of adolescents in Study 3, supporting other adolescent and adult studies.[389, 393, 395] However, although past reports generally acknowledge the inferiority of MPA or lesser intensities of PA, conclusions about MPA could have been erroneously drawn because of the confounding strong correlation between VPA and MPA. The partial multiple linear regression approach in this thesis accounted for this intercorrelation and found that MPA and other intensities are no longer associated in univariate analyses when rPA intensities are used, which was confirmed with standard multiple linear regression using unadjusted PA intensities. Therefore, as VPA and CRF have health benefits beyond LVDF, the findings of this thesis provide a strong grounding for specific VPA guidelines.

These results are timely as a recent UK Biobank study in ~72,000 adults found that an average of 15–20 min/week of VPA, independent of multiple confounders and lesser intensities, was associated with reduced risks of morbidity and mortality, with further reductions with longer durations of VPA, although there was a plateau in these relationships similar to Study 3.[501] This has been further supported by another recent UK Biobank study that reports improved cardiovascular, cancer, and all-cause mortality in those who perform more vigorous intermittent lifestyle PA (non-exercise VPA), independent of LPA, MPA, and structured VPA.[502] This suggests that setting good VPA habits in early life could have long-term benefits, especially since habitual PA tracks from adolescence into adulthood.[489, 490]

Although WHO strongly recommends that for adolescents "*Vigorous-intensity aerobic activities, as well as those that strengthen muscle and bone should be incorporated at least 3 days a week*", they state that "*There was insufficient evidence to determine whether specific health benefits vary by type or domain of PA.*"[173] The findings of 20 min/day of VPA in Study 3, as well as the results of Study 2, offer such evidence and provides grounds for clearer public health messaging in the primordial prevention of LVDD and reductions in CRF.

Therefore, in light of the findings in this thesis and supported by the other research cited throughout, it may be timely to change PA guidelines for overall health in children and adolescents to:

*"Children and adolescents should do at least an average of 20 min/day of VPA, across the week, within an overall MVPA target of 60 min/day on average."*

However, based on the findings in this thesis and on those of others,[46, 157, 392, 393, 446, 447] there is a strong case to recommend just VPA, of 20 min/day, to prevent against early cardiovascular impairments in adolescents, simplifying the message and thereby likely improving impact.

As performing 20 min/day may not be achievable by all, at least to begin with, then it might be reasonable to also recommend a lower limit of VPA that adolescents could start with. This approach has been taken in the guidelines for adults, where 75-150 min/week of VPA is recommended.[173] Although the review article by Gralla *et al* identified that children and adolescents who had better cardiometabolic health typically performed 10 min/day of VPA,[393] RCTs to compare different durations of VPA to identify clinically meaningful differences in those with poor cardiovascular health, such as those with OW/Ob, will be needed to identify this lower limit.

However, one of the main limitations potentially to VPA guidelines is that it is currently unclear how best to achieve a target of 20 min/day of VPA in large populations, especially in those with OW/Ob. Nevertheless, it is notable that a daily target need not be achieved in a single session and could, perhaps be easily achieved when broken down into shorter intervals of VPA. A short commute by bicycle instead of walking or even running for the bus could be significant contributors to a daily target and form part of a daily routine. Formal exercise sessions could contribute too, but may need to be well-designed to achieve good adherence. As discussed in Chapter 3, previous research has suggested that implementation of 10 minutes of VPA into school PE lessons is difficult, achieving only 4 minutes of VPA per lesson.[487] Conversely, in exercise interventions, VPA was found to have similar adherence rates to those with lesser intensities, but with reduced time commitments, highlighting no apparent disadvantage of focusing on VPA.[392] The difficulties found in previous studies could be due to the lack

of enjoyment in that specific mode of VPA. Data suggest that children and adolescents who enjoy exercise more report higher levels of PA and CRF[503-506] and so designing interventions around adolescent PA enjoyment could be important. Thus, it will be of interest to follow the progress of studies such as *Making a HIIT*,[507] which will co-design HIIT PE workouts with both teachers and pupils and will include measurements of student motivation, enjoyment, and self-efficacy. This will likely show that it is important to involve adolescents so that they adhere, engage, and continue to perform VPA once the programme has ended. This is probably even more important in adolescents with OW/Ob, as there may be greater barriers to exercise in this population.[508, 509]

## 7.3 Limitations

There are some limitations that should be considered in addition to those discussed in previous chapters. In this thesis, causality cannot be determined as all studies were cross-sectional by design and so RCTs and long-term cohort studies will be needed to support the findings in this thesis. However, some previous research using RCTs and long-term follow-ups support the claims made here.[27, 392]

PA was assessed using 7-days of wrist-worn accelerometery during or outside of a school week and during different months of the year. Seasonal changes in PA patterns, as well differences between leisure- and school-time PA, could lead to higher variability in the PA data collected. However, it was impractical to ensure all participants completed the PA assessment at the same time during the school-term/year. Although 7-days of PA, ensuring at least one weekend day, is recommended for research,[263, 510] longer wear-times or repeated measurements could have reduced the variability of assessing PA for just one week and at different times of the year, but this was not possible due to AX3 data storage limitations beyond 7-days and impracticalities of repeated measurements. As participants were informed that the AX3 was used to assess daily PA, some participants may have changed their daily PA because of this knowledge. However, this is a limitation experienced by all studies that prospectively assess PA, whether it be objective or subjective. As PA was reported as

average time per day, you would expect those who wore the AX3 for longer would have greater durations of PA at each intensity compared to those who worse it for less, possibly biasing results. However, results in Study 2 and 3 were unaltered when PA intensities were expressed as a percentage of total wear time instead (data not shown).

As discussed in Section 3.4.5, there are many definitions and reference datasets to classify overweight and obesity in children and adolescents. The WHO reference dataset was used in this thesis, meaning that our results can be compared globally as long as others also use this reference dataset. Using a different reference dataset, such as those for the UK, might have reallocated some individuals to either the normal-weight or OW/Ob group. However, the use of continuous analyses in this thesis meant the results would be unaffected by group allocation, although the results of some group-based testing might have been marginally altered. Furthermore, as obesity comprises greater amounts of adipose tissue compared to overweight, the inclusion of those with overweight might have decreased the group-based differences in LVDF but would have unlikely changed the results of continuous analyses.

## 7.4 Future directions

This thesis was able to identify reduced LVDF with increasing levels of adiposity and IR, and lower VPA and CRF, but was unable to determine the underpinning mechanisms. Further work in adolescents, including that of OxSOCRATES, utilising MRI and magnetic resonance spectroscopy data will be important to identify whether mechanism such as myocardial steatosis, energetics, and concentric remodelling are linked.

Echocardiography was used to measure LVDF in this thesis, so confirmation of these results using other methods such as LV strain and MRI mitral valve tracking will be important. This will also help in establishing whether septal LVDF is impaired before declines in other LV regions, or whether this is an artefact of TDI echocardiography. Furthermore, MRI whole-body composition data to quantify total

adiposity is seen as the research gold-standard,[50, 230] but this has not been done in adolescents. Dr Alexander Jones is currently coding a bespoke, automated algorithm to process MRI whole-body composition data, which will hopefully confirm the adiposity results in this thesis, as well providing further crucial information about the role of VAT and SAT on LVDF in adolescents.

Unfortunately, causality could not be addressed in this thesis. Well-designed RCTs, such as OxSOCRATES, will be needed to understand if VPA can reverse any impairments in LVDF in adolescents with OW/Ob, as well as investigating whether VPA programmes are more beneficial than MPA. If this is confirmed, RCTs should also investigate whether 20 min/day of VPA is sufficient. Results of this will be important to confirm these initial findings.

This thesis was unable to determine the role of genetics. Those with genetic variants that are beneficial for CRF may be able to improve their metabolic pathways linked with LVDF and CRF by performing lower amounts of less intense exercise, whereas others may have to perform more intense activity for longer. Future work to understand inter-individual responses of LVDF to exercise will be important to understand if a similar genetic underpinning is present in LVDF, as prescription of VPA may need to be tailored depending on an individual's genetic risk.[464, 465] Dr Alexander Jones and I have plans to utilise the UK Biobank data with the aim of addressing this.

Finally, primordial prevention of CVD is not limited to childhood and adolescence, but can begin before these children are born. Increasing evidence suggests that maternal obesity during pregnancy is associated with adverse health consequences for the offspring,[511-513] including impaired LVDF,[512, 514] extending into adult life.[515, 516] Long-term follow-ups are needed to determine whether RCTs of diet and exercise during pregnancy[517] can improve the health of adolescent offspring, as preliminary data in young children suggest a causal benefit.[512, 513] I have recently started a post-doctoral research position to address this, which will add to the findings of this thesis, and of OxSOCRATES, to determine effective ways to prevent CVD and hopefully continue to shift the mindset of healthcare professionals towards a goal of CVD prevention.

## 7.5 Conclusion

This thesis confirms that adolescent OW/Ob is associated with impaired LVDF, with the strongest relationships being with echocardiography measures that assess impairments in LV septal myocardial motion. As there are currently no paediatric guidelines to measure the beginnings of LVDD, implementation of these septal measures into general practice could help find those who are on a path towards LVDD and whom require early intervention to prevent further declines. A targeted approach through VPA, not simply focused on weight loss, has been identified, in theory, to prevent or reverse this adverse early phenotype. Future long-term cohort studies will be needed to definitively answer whether high levels of adolescent VPA is protective against future adverse health outcomes. If such studies report that VPA is indeed protective, as this thesis suggests, then this could have substantial implications for both public health strategies and in the method of healthcare delivery.

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# Appendix 1

## Study 1: Supporting methods

### Outcome measures

The primary outcome measures were: early mitral inflow peak velocity (E-wave); late mitral inflow peak velocity (A-wave); E-wave/A-wave ratio (E/A); E-wave deceleration time (DT); isovolumic relaxation time (IVRT); early diastolic tissue peak velocity (e'); late diastolic tissue peak velocity (a'); E-wave/e' ratio (E/e'); e'/a' ratio (e'/a').

Cardiometabolic risk factors included, but were not limited to: systolic and diastolic blood pressure; total triglycerides; total cholesterol; low-density lipoproteins; high-density lipoproteins; blood glucose; blood insulin; homeostatic model assessment of insulin resistance (HOMA-IR); haemoglobin A1C (HbA1c); and C-reactive protein (CRP).

### Combining septal and lateral wall tissue Doppler imaging measures

These formulas have been taken from the Cochrane handbook.[307]

| | **Septal** | **Lateral** | **Combined Groups** |
|---|---|---|---|
| Sample Size | $N_1$ | $N_2$ | $N_1 \times N_2$ |
| Mean | $M_1$ | $M_2$ | $\frac{N_1 M_1 + N_2 M_2}{N_1 + N_2}$ |
| SD | $SD_1$ | $SD_2$ | $\sqrt{\frac{(N_1 - 1){SD_1}^2 + (N_2 - 1){SD_2}^2 + \frac{N_1 N_2}{N_1 + N_2}\left({M_1}^2 + {M_2}^2 - 2M_1 M_2\right)}{N_1 + N_2 - 1}}$ |

Search Hedges

**PubMed.gov**

Date Searched: 11/07/2020

Number of results: 1237

Full Search Strategy:

((cardi*[title/abstract] OR heart[title/abstract] OR diastol*[title/abstract] OR ventric*[title/abstract]) AND (function*[title/abstract] OR dysfunct*[title/abstract] OR fail*[title/abstract])) AND (child*[title] OR adolesc*[title] OR teen*[title] OR youth*[title] OR paed*[title] OR pedia*[title]) AND (obes*[title/abstract] OR overweight[title/abstract] OR over-weight[title/abstract] OR adipos*[title/abstract] OR obesity[MeSH Terms])

**Cumulative Index to Nursing and Allied Health Literature (CINAHL)**

Date Searched: 11/07/2020

Number of results: 489

Full Search Strategy:

S1 – TX cardi* OR heart OR diastol* OR ventric*

S2 – TX function* OR dysfunct* OR fail*

S3 – TI child* OR adolesc* OR teen* OR youth* paed* OR pedia*

S4 – TX obes* OR overweight OR over-weight OR adipos*

S5 – S1 AND S2 AND S3 AND S4

**Cochrane Central Register of Controlled Trials, and ClinicalTrials.gov**

Date Searched: 11/07/2020

Number of results: 245

Full Search Strategy:

S1 – (child* OR adolesc* OR teen* OR youth* OR paed* OR pedia*):ti

S2 – (cardi* OR heart OR diastol* OR ventric* ):ti,ab,kw

S3 – (function* OR dysfunct* OR fail*):ti,ab,kw

S4 – (obes* OR overweight OR over-weight OR adipos*):ti,ab,kw

S5 – (obes* OR overweight OR over-weight OR adipos*):ti,ab,kw

S6 – S4 or S5

S7 – S1 AND S2 AND S3 AND S6

**Embase**

Date Searched: 11/07/2020

Number of results: 3267

Full Search Strategy:

((cardi* or heart or diastole* or ventric*) AND (function* or dysfunc* or fail*) AND (obes* or overweight or over-weight or adipos*)).mp AND (child* or adolesc* or teen* or youth* or paed* or pedia*).m_titl

**Web of Science**

Date Searched: 11/07/2020

Number of results: 2069

Full Search Strategy:

TITLE: (child* OR adolesc* OR teen* OR youth* OR paed* OR pedia*) AND TOPIC: (((cardi* OR heart OR diastol* OR ventric*) AND (function* OR dysfunct* OR fail*) ) AND (obes* OR overweight OR over-weight OR adipos*) )

## Title and abstract inclusion/exclusion

Exclude if there is no mention of;

- Child, children, adolescent, youth, young people, teenager *etc*.
- Cardiovascular system
    - Include if echocardiography (or echo, strain, Doppler, tissue Doppler imaging (TDI), pulsed wave Doppler, speckle tracking, myocardial-tracking, or similar) mentioned
    - Include if cardiovascular magnetic resonance imaging (CMR, MRI) mentioned
    - Include if mention “organ health”, “organ damage” or similar phrases
    - Include if cardiopulmonary mentioned
    - Include if right ventricle mentioned
    - Include if left atria mentioned
    - Exclude if no link to the heart (e.g. “vascular stiffness” or “endothelial function”)
- Overweight, obesity, BMI, adiposity, waist circumference *etc*.
    - Include if diabetes mentioned
    - Include if metabolic syndrome (MetS) mentioned

Exclude if mentioned;

- Congenital heart disease, cancer or any other disease/illness not related to obesity.

## Full-text inclusion/exclusion

To be included in the meta-analyses and systematic review BMI or IR meta-analysis, studies must have included:

(1) evaluation of children and adolescents (≤24 years of age) with OW/Ob that report BMI/Homeostatic Model Assessment for IR (HOMA-IR), age and sex; and

(2) correct methods to assess LVDF.

The IR meta-analysis was limited to using HOMA-IR due to the lack of data using other methods.

Exclusion criteria were as follows:

(1) study of other illness/disease;

(2) failure to report either BMI/HOMA-IR, age and/or sex;

(3) incorrect methods for assessing LVDF;

(4) substantial missing data;

(5) no clear description of inclusion/exclusion criteria; and

(6) suspected false reporting (e.g. reported standard deviations of 0).

To be included in the systematic review studies had to include:

(1) evaluation of children and adolescents (≤24 years of age) with a valid definition of childhood OW/Ob;

(2) report correct measures of LVDF; and

(3) concurrent evaluation with normal-weight or non-obese controls, and/or conducted analysis that studied the effect of CMRFs.

Exclusion criteria for the systematic review were as follows:

(1) study of other illness/disease (e.g. Type 2 diabetes);

(2) adult definition of obesity (if studies included subjects <18 years of age);

(3) failure to define obesity;

(4) significantly different age or sex between groups;

(5) incorrect methods for measuring LVDF;

(6) biased recruitment of subjects (e.g. recruitment of controls from relatives of study staff);

(7) no comparison control group;

(8) substantial missing data with no explanation as to why it is missing;

(9) inappropriate statistical analysis (e.g. no tests for normality);

(10) no clear description of inclusion/exclusion criteria; and suspected false reporting (e.g. reported standard deviations of 0).

### Full-text articles excluded – reasons

Forty-one full-text articles were excluded due to:

- Unsuitable for both quantitative and qualitative analysis (n=13)[199, 300, 376, 518-527]
- Study of other illness/disease (e.g. Type 2 diabetes) without normal overweight/obese (n=8)[528-535]
- Apparent duplicate data (n=4)[536-539]
- Reported results inconsistent/missing data (n=4)[540-543]
- Suspected false reporting (n=3)[544-546]
- Too old (>24 years of age) (n=3)[547-549]
- Incorrect method for assessing LVDF (n=2)[550, 551]
- No inclusion/exclusion criteria (n=2)[552, 553]
- Data in format that cannot be reviewed (n=1)[554]
- No overweight or obese (n=1)[555]

### Reporting of BMI, HOMA-IR, age and sex

To be included in the meta-analysis, studies had to report measures on BMI/HOMA-IR, age and sex. Where studies did not report age or sex for the control group but included a statement that controls were matched to the obese group, the control group was assumed to have the same age and sex distribution. Where studies did not report sex for either group but included a statement that groups were matched, then groups were assumed to have a 50/50 distribution of male and females.

### Transformation into standard units

**Median and interquartile range**

The methods provided by Shi *et al* were used to calculate the mean and SD from studies reporting median and interquartile range (IQR).[313] As it has been reported that this SD estimator may be sensitive to the skewness of the data (as is commonly the reason for reporting median [IQR]) a sensitivity analysis was completed to understand whether these transformations affected the outcomes. Results were not altered after the sensitivity analysis.

**Mean and confidence intervals**

The following formula has been taken from the Cochrane handbook.[307]

$$SD = \frac{\sqrt{N} \times (upper\ limit - lower\ limit)}{2 \times t}$$

The *t* value for a 95% confidence interval can easily be obtained in Excel by typing *=tinv(1-0.95,N-1)* where *N* is the study sample size.[307]

## Robust z-score calculation

Robust z-scores were calculated using the median (x̃) and the median absolute deviation (MAD) for each variable:

$$robust\ zscore\ = \frac{X_i - \tilde{x}}{MAD \times 1.4826}$$

The MAD is calculated as follows and is defined as the median of the absolute deviation from the median of the data points:

$$MAD = median_i(|X_i - median\ X_{1\ldots n}|)$$

## Fisher's z-test

Comparison of the strength of associations between standardized correlation coefficients (r) were calculated with the following formula and the difference (in terms of z-score) was read off from a two-sided z-table to provide a p-value:

$$Difference = \frac{|r| - |r_i|}{\sqrt{(N-3)^{-1} + (N_i - 3)^{-1}}}$$

The largest r coefficient was used for comparison to other r coefficients ($r_i$). Statistical significance was set at $P<0.05$.

## Study 1: Supporting results

### Systematic review of DEXA derived adiposity and LVDF

A number of studies have assessed the link between LVDF and adiposity by DEXA in adolescents,[326, 346, 351] supporting the findings of Study 1. In a population of 612 youth aged 10-24 years-old, total adiposity was negatively and positively associated with averaged e'/a' ratios (r=-0.28, P<0.01) and averaged E/e' ratios (r=0.37, P<0.01), respectively.[346] In a study of 44 adolescents aged ~15 years-old with and without obesity, early diastolic strain-rate was negatively associated with total adiposity (r=-0.3-0.4, P<0.05 [non-specific range of correlation coefficients]).[351] Dias *et al* similarly report that early diastolic strain-rate was negatively associated with total adiposity (r=-0.64, P=0.002) in 20 adolescents (12-16 years-old).[326]

### Systematic review of pulmonary vein flow velocities and diastolic strain

Similar measures to conventional transmitral flow velocities are used to assess left atrial pressure. These are systolic pulmonary vein peak velocity (S-wave), diastolic pulmonary vein peak velocity (D-wave), and the S wave/D wave ratio (S/D ratio).

Four studies provided data on S-wave.[308, 329, 344, 347] All four studies reported no difference in S-wave velocities in the overweight/obesity group. However, three studies reported a trend towards increased values in overweight/obesity group (P<0.1). Four studies provided data on D-wave.[308, 329, 344, 347] Two studies reported reduced D-wave velocities in the overweight/obesity group and two studies reported no difference. One study provided data on S/D ratio with no reported difference in the overweight/obesity group.[308] Further, work is needed to understand the utility of these measures of in children/adolescents with obesity.

Six studies reported data on diastolic strain or strain-rate by speckle-tracking echocardiography (STE) markers of myocardial motion.[326, 334, 337, 340, 351, 368] These speckles move with the myocardium and so measurement myocardial motion does not rely on the alignment of the motion vector with the

ultrasound beam as Doppler techniques do and may, therefore, be less prone to the limitations of other Doppler techniques.[556] Four studies reported reduced strain-rate in early diastole,[326, 334, 351, 368] whereas one study reported no difference.[337] One study reported increased strain-rate in late diastole in the overweight/obesity group,[334] whereas three studies reported no difference.[326, 351, 368] Three studies reported reduced longitudinal strain-rate.[340, 351, 368] Measures of early-to-late strain rate ratio were reduced in the overweight/obesity group in one study[334] and unaltered in another.[337] These measures hold promise and future studies should try to analyse myocardial motion by STE.

**Appendix 1, Table 1.1: Study characteristics**

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Ahmed, 2016 *315* | Total: 92<br><br>OB: 56<br>Con: 36 | OB: 30.17 ± 5.8<br>BMI z-score for age greater than 2 SDS (WHO)<br><br>Con: 16.37 ± 1.84<br>Non-OB | 5-15<br><br>OB: 9.13 ± 2.8<br>Con: nr - age matched to OB | OB: 51.2<br>Con: nr - sex matched to OB | e', a', e'/a' | Egypt | cross-sectional case-control, single centre | Yes/Yes | Yes<br><br>Unclear<br><br>[fasting insulin (μU/L) × fasting glucose (nmol/L)]/22.5 | fair |
| Akcaboy, 2016 *133* | Total: 83<br><br>OB: 27<br>Con: 21<br>Other: 35 | OB: 32.6 ± 3.3<br>BMI ≥ 95th percentile for age and sex (country specific - Turkey), OB normotensive<br><br>Con: 20.5 ± 2.6<br>cd<br><br>Other: 33.1 ± 4.7<br>OB hypertensive | 13-18<br><br>OB: 14.7 ± 1.5<br>Con: 15.4 ± 1.7<br>Other: 14.9 ± 1.4 | OB: 40.7<br>Con: 52.4<br>Other: 45.7 | E/A, E/e' | Turkey | cross-sectional case-control, single centre | Yes/Yes | Yes<br><br>Unclear<br><br>[fasting glucose (mg/dL) × fasting insulin (U/L)]/405 | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Akyol, 2013 [370] | Total: 201<br><br>OW/OB: 94<br>Con: 63<br>Other: 44 | OW/OB: 34.9 ± 9.3<br>Unclear - BMI > 90th percentile for age and sex (cd), OW/OB without MetS<br><br>Con: 20.1 ± 1.3 cd<br><br>Other: 38.4 ± 4.0 OW/OB with MetS | 9-18<br><br>OW/OB: 13.9 ± 3.7<br>Con: 13.3 ± 4.3<br>Other: 13.3 ± 4.1 | OW/OB with and without MetS (grouped as one for sex): 52.2<br>Con: nr | IVRT, e', a', e'/a' | Turkey | cross-sectional case-control, single centre | Yes/No | Yes<br><br>Overnight fast<br><br>[fasting insulin (mIU/L) × fasting glucose (mmol/L)]/22.5 | poor |
| Alkholy, 2016 [316] | Total: 122<br><br>OB: 82<br>Con: 40 | OB: 32.8 ± 4.6<br>BMI ≥ 95th percentile for age and sex (CDC)<br><br>Con: 18.7 ± 2.9 cd | 6-14<br><br>OB: 10.2 ± 2.8<br>Con: 10.6 ± 2.7 | OB: 57.3<br>Con: 55 | E-wave, A-wave, E/A, e' | Saudi Arabia | cross-sectional case-control, multi-centre | Yes/Yes | Yes<br><br>Unclear<br><br>[fasting insulin (μU/mL) × fasting glucose (mM)]/22.5 | fair |
| Alp, 2014 [317] | Total: 650<br><br>OB: 500<br>Con: 150 | OB: 29.59 ± 5.14<br>BMI > 95th percentile for age and sex (country specific - Turkey)<br><br>Con: 18.53 ± 2.7 cd | 6-17<br><br>OB: 11.92 ± 3.65<br>Con: 11.97 ± 2.78 | OB: 53.2<br>Con 54.7 | E-wave, A-wave, E/A, e', a', E/e', e'/a' | Turkey | cross-sectional case-control, single centre | Yes/Yes | Yes<br><br>Unclear<br><br>[fasting insulin (IU/mL) × fasting glucose (mmol/L)]/22.5 | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Aslan, 2019 [318] | Total: 300<br><br>OB: 98<br>Con: 102<br>Other: 100 | OB: 29.9 ± 3.6<br>BMI > 95th percentile for age and sex (WHO), OB without MetS<br><br>Con: 19.5 ± 2.3<br>cd<br><br>Other: 32.4 ± 3.5<br>OB with MetS | 12-17<br><br>OB: 13.9 ± 1.3<br>Con: 14.0 ± 1.3<br>Other: 14.0 ± 1.4 | OB: 40.8<br>Con: 49.0<br>Other: 40.0 | E-wave, A-wave, E/A, e', a' | Turkey | cross-sectional case-control, single centre | Yes/Yes | Yes<br><br>≥12hr fast<br><br>[fasting insulin (mU/ml) × fasting glucose (mmol/L)]/22.5 | fair |
| Battal, 2011 [319] | Total: 103<br><br>OB: 68<br>Con: 35 | OB: 31.0 ± 4.7<br>BMI ≥ 95th percentile for age and sex (cd)<br><br>Con: 18.8 ± 2.3<br>cd | 6-18<br><br>OB: 12 ± 1<br>Con: 12 ± 1 | OB: 35.3<br>Con: 40.0 | E-wave, A-wave, E/A, DT | Turkey | cross-sectional case-control, single centre | Yes/Yes | Yes<br><br>Unclear<br><br>[insulin (mU/L) × glucose (mmol/L)]/22.5 | poor |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Bjornstad, 2016 *320* | Total: 74<br><br>OB: 41<br>Con: 33 | OB: 2.0 ± 0.4 (BMI SDS)<br>BMI > 95th percentile for age and sex (CDC)<br><br>Con: 0.1 ± 0.7 (BMI SDS)<br>BMI > 5th percentile and < 85th percentile for age and sex | 12-19<br><br>OB: 14.4 ± 2.0<br>Con: 14.9 ± 2.1 | OB: 29<br>Con: 45 | E-wave, A-wave, E/A, DT, e', a', E/e' | USA | cross-sectional case-control, single centre | No/Yes | No | fair |
| Boyraz, 2013 *359* | Total: 201<br><br>OB: 95<br>Con: 63<br>Other: 43 | OB: 34.7 ± 8.3<br>BMI-SDS between 1.65-2.49 (IOTF)<br><br>Con: 20.1 ± 1.3 cd<br><br>Other: 37.4 ± 4.1<br>BMI-SDS between 2.50-2.99, severely OB | 9-18<br><br>OB: 13.8 ± 3.9<br>Con: 13.3 ± 4.3<br>Other: 13.1 ± 4.1 | OB and other: 52.2<br>Con: nr - sex-matched | DT, IVRT, e', a', e'/a' | Turkey | cross-sectional case-control, single centre | Yes/No | Yes<br><br>Overnight fast<br><br>[fasting insulin (mIU/L) × fasting glucose (mmol/L)]/22.5 | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Brar, 2019 *361* | Total: 78<br><br>OB: 25<br>Con: 39<br>Other: 14 | OB: 34.± 7<br>BMI ≥ 95th percentile for age and sex (cd)<br><br>Con: 19.2 ± 4<br>BMI < 85% percentile for age and sex<br><br>Other: 37.5 ± 8.8<br>OB with dysglycemia | 12-18<br><br>OB: 16 ± 1.9<br>Con: 15.8 ± 1.9<br>Other: 16 ± 1.9 | OB: 28<br>Con: 41<br>Other: 64.3 | E/A, E/e' | USA | cross-sectional case-control, single centre | Yes/Yes | No | fair |
| Chinali, 2008 *321* | Total: 446<br><br>OW/OB: 111<br>Con: 335 | MetS: 37.0 ± 8.1<br>92.8% OB<br><br>No MetS: 25.9 ± 6.4<br>37.1% OB | ≤ 20<br><br>MetS: 17.6 ± 1.5<br>No MetS: 17.3 ± 1.4 | MetS: 44.1<br>No MetS: 48.1 | E/A, DT, IVRT, | USA | cross-sectional case-control, mutlti-centre | No/Yes | No | fair |
| Corica, 2020 *322* | Total: 79<br><br>OW and OB: 59<br>Con: 20 | OW and OB: 2.2 ± 0.5 (BMI SDS)<br>BMI z score > 1 (WHO)<br><br>Con: -0.3 ± 0.8 (BMI SDS)<br>BMI z score ≤ 1 | 5-16<br><br>OW and OB: 9.8 ± 2.9<br>Con: 8.6 ± 2.9 | OW and OB: 55.9<br>Con: 50.0 | E/A, e', E/e' | Italy | cross-sectional case-control, single centre | No/Yes | No | good |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Cozzolino, 2015 *323* | Total: 98<br><br>OB: 33<br>Con: 30<br>Other: 35 | OB: 33 ± 9<br>BMI > 95th percentile for sex and age (cd)<br><br>Con: 24 ± 1<br>cd<br><br>Other: 33 ± 10<br>OB with insulin resistance | 10-16<br><br>OB: 12.7 ± 2.1<br>Con: 12.6 ± 2.0<br>Other: 12.9 ± 2.1 | OB: 42.9<br>Con: 50.0<br>Other: 45.9 | E-wave, A-wave, E/A, e', a', E/e', e'/a' | Italy | cross-sectional case-control, single centre | Yes/Yes | Yes<br><br>Overnight fast<br><br>[fasting insulin (pmol/L) x fasting glucose (mmol/L)]/22.5 | fair |
| Dahiya, 2015 *144* | Total: 69<br><br>OW/OB: 35<br>Con: 34 | OW/OB: 2 ± 0.8 [BMI SDS (median ± IQR)]<br>BMI equivalent to ≥ 25 at age 18 (IOTF)<br><br>Con: 0.03 ± 0.7 [BMI SDS (median ± IQR)], non-OW and OB | 10-19<br><br>OW/OB: 14.9 ± 2.3<br>Con: 15.3 ± 1.8 | OW/OB: 40.0<br>Con: 61.8 | A-wave, E/A, DT, IVRT, e', E/e' | Australia | cross-sectional case-control, single centre | No/Yes | Yes<br><br>Overnight fast (10-12hrs)<br><br>[fasting insulin (U/mL x fasting glucose (mmol/L)/22.5] | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Dhuper, 2011 [324] | Total: 343<br><br>OB: 213<br>Con: 130 | OB: 36.5 ± 0.53<br>BMI > 95th percentile for age and sex (CDC)<br><br>Con: 19.73 ± 0.21<br>BMI < 85th percentile for age and sex | nr<br><br>OB: 13.8 ± 0.2<br>Con: 13.8 ± 0.2 | OB: 50<br>Con: 61 | E/A, E/e' | USA | cross-sectional case-control, single centre | Yes/Yes | No | fair |
| Di Bonito, 2009 [325] | Total: 195<br><br>OB: 165<br>Con: 30 | OB: 27 ± 4<br>BMI ≥ 95th percentile for age and sex (country specific - Italy)<br><br>Con: 17 ± 2<br>BMI < 85th percentile for age and sex | 6-16<br><br>OB: 10 ± 3<br>Con: 10 ± 3 | OB: 50.4<br>Con: 49.2 | E/A, IVRT, e'/a' | Italy | cross-sectional case-control, single centre | No/Yes | Yes<br><br>Unclear<br><br>[fasting insulin (U/L) × fasting glucose (mmol/L)]/22.5 | fair |
| Di Bonito, 2010 [371] | Total: 799<br><br>OW/OB: 131<br>Con: 668 | MetS: 29 ± 5<br>99% OW or OB<br><br>No MetS: 24 ± 6<br>70% OW or OB | 6-16<br><br>OW/OB: 10 ± 3<br>Con: 10 ± 3 | OW/OB: 47<br>Con: 50 | E/A, IVRT, e'/a' | Italy | cross-sectional case-control, single centre | Yes/Yes | No | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Di Salvo, 2008 *362* | Total: 320<br><br>OB: 160<br>Con: 160 | OB: 31 ± 4<br>BMI > 97th percentile for age and sex (IOTF)<br><br>Con: 18 ± 3<br>cd | 6-15<br><br>OB: 12 ± 3<br>Con: 12 ± 3 | OB: 44<br>Con: 44 | E-wave, A-wave, E/A, S wave, D wave, DT, IVRT, E/e' | Italy | cross-sectional case-control, single centre | Yes/No | Yes<br><br>Overnight fast (>12hrs)<br><br>Not reported | fair |
| Dias, 2017 *326* | Total: 20<br><br>OB: 9<br>Con: 11 | OB: 31.7 ± 3.5<br>BMI > 95th percentile for age and sex (IOTF)<br><br>Con: 18.5 ± 2.3<br>BMI 5th-85th percentile for age and sex | 12-16<br><br>OB: 13.1 ± 1.0<br>Con: 13.5 ± 1.2 | OB: 33<br>Con: 73 | E/A, DT, IVRT, e', a', E/e', diastolic strain | Australia | cross-sectional case-control, single centre | Yes/Yes | Yes<br><br>Unclear<br><br>Not reported | fair |
| Dusan, 2015 *134* | Total: 148<br><br>OB: 54<br>Con: 30<br>Other: 49 | OB: 30.0 ± 3.8<br>BMI > 95th percentile for age and sex (IOTF)<br><br>Con: 20.5 ± 3.0<br>cd<br><br>Other: 29.4 ± 3.2<br>OB with hypertension | 9-19<br><br>OB: 14.1 ± 2.3<br>Con: 15.0 ± 2.3<br>Other: 14.1 ± 2.0 | OB: 72.2<br>Con: 60.0<br>Other: 67.3 | E-wave, A-wave, E/A, DT, IVRT | Serbia | cross-sectional case-control, single centre | Yes/Yes | Yes<br><br>Overnight fast (12hrs)<br><br>[fasting glucose (mmol/L) x fasting insulin (mU/L)]/22.5 | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Eklioglu, 2016 *364* | Total: 198<br><br>OB with pre-Diab: 81<br><br>OB without pre-Diab: 117 | OB with pre-Diab: 29.99 ± 9.03<br><br>BMI > 95th percentile for age and sex (CDC)<br><br>OB without pre-Diab: 28.05 ± 5.19<br><br>(see above) | 6-18<br><br>OB with pre-Diab: 11.84 ± 2.95<br><br>OB without pre-Diab: 11.88 ± 2.97 | OB with pre-Diab: 43.0<br><br>OB without pre-Diab: 45.3 | e', a', E/e' | Turkey | cross-sectional case-control, single centre | Yes/No | Yes<br><br>Overnight fast<br><br>[fasting glucose x fasting insulin]/22.5 | poor |
| El Saiedi, 2018 *327* | Total: 62<br><br>OB: 32<br>Con: 30 | OB: 30.6 ± 4.2<br>BMI ≥ 95th percentile for age and sex (country specific - Egypt)<br><br>Con: 19.1 ± 2.3<br>BMI 5th-85th percentile for age and sex | 6-19<br><br>OB: 10.8 ± 3.1<br>Con: 10.4 ± 3.0 | OB: 50.0<br>Con: 46.5 | E-wave, A-wave, E/A, e', a', E/e' | Egypt | cross-sectional case-control, single centre | Yes/Yes | No | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Franssen, 2019 *328* | Total: 58<br><br>OB: 29<br>Con: 29 | OB: 31.6 ± 4.2<br>BMI > 95th percentile for age and sex (IOTF)<br><br>Con: 19.5 ± 2.4<br>cd | 11-17<br><br>OB: 13.4 ± 1.1<br>Con: 14.0 ± 1.5 | OB: 51.7<br>Con: 55.2 | E-wave, A-wave, E/A, DT, e', E/e' | Belgium | cross-sectional case-control, single centre | Yes/Yes | Yes<br><br>Fast >10hrs<br><br>[fasting glucose (mg/dl) × fasting insulin (µU/ml)]/405 | fair |
| Ghanem, 2010 *329* | Total: 80<br><br>OB: 50<br>Con: 30 | OB: 28.4 ± 8.3<br>BMI > 95th percentile for age and sex (cd)<br><br>Con: 17.3 ± 2.8<br>cd | 6-18<br><br>OB: 11.2 ± 2.9<br>Con: 11.5 ± 3.4 | OB: 48.0<br>Con: 43.3 | E/A, S wave, D wave, DT, IVRT | Saudi Arabia | pre-post | Yes/Yes | No | poor |
| Harris, 2012 *557* | Total: 116<br><br>OB: 61<br>Con: 55 | OB: 32.6 ± 4.4<br>BMI ≥ 95th percentile for age and sex (cd)<br><br>Con: 21.2 ± 4.7<br>BMI ≤ 95th percentile for age and sex | ≤ 18<br><br>OB: 13.8 ± 2.3<br>Con: 13.8 ± 4.0 | OB: 35.8<br>Con: 28.6 | E-wave, A-wave, E/A, e' | Canada | cross-sectional case-control, single centre | Yes/No | No | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Hirschler, 2006 [330] | Total: 84<br><br>OB: 40<br>Con: 16<br>Severely OB: 28 | OB: 27.07 ± 4.19<br>BMI ≥ 95th percentile for age and sex (CDC)<br><br>Con: 15.83 ± 1.39<br>BMI < 85th percentile for age and sex<br><br>OW: 21.04 ± 2.40<br>BMI ≥ 85th percentile for age and sex | 14-21<br><br>OB: 16.9 ± 2.0<br>Con: 16.6 ± 1.9<br>OW: 16.9 ± 2.2 | OB: nr<br>Con: nr<br>OW: nr | E/A | Argentina | cross-sectional case-control, single centre | Yes/Yes | Yes<br><br>Fast (12-14hrs)<br><br>[fasting insulin (µ U/L) x fasting glucose (mmol/L)]/22.5 | fair |
| Hui, 2019 [331] | Total: 88<br><br>OB: 44<br>Con: 44 | OB: 36.0 ± 6.6<br>BMI > 95th percentile for age and sex (CDC)<br><br>Con: 20.0 ± 3.3<br>BMI z-score ≤ 2 | 8-18<br><br>OB: 13.7 ± 2.9<br>Con: 13.6 ± 2.9 | OB: 47.7<br>Con: 47.7 | E-wave, A-wave, E/A, IVRT, e', E/e' | Canada | cross-sectional case-control, single centre | Yes/Yes | No | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Ingul, 2010 [333] | Total: 20<br><br>OB: 10<br>Con: 10 | OB: 33.5 ± 4.3<br>BMI z score > 2 for age and sex (WHO)<br><br>Con: 20.4 ± 3.0<br>BMI z score ≤ 2 for age and sex | 13-16<br><br>OB: 14.8 ± 1.2<br>Con: 14.9 ± 1.3 | OB: 60<br>Con: 60 | E/A, DT, IVRT, e', E/e' | Norway | pre-post | Yes/Yes | No | fair |
| Ingul, 2018 [332] | Total: 199<br><br>OB: 99<br>Con: 100 | OB: nr<br>BMI > 95th percentile for age and sex (CDC)<br><br>Con: nr<br>BMI 5th-85th percentile for age and sex | 7-16<br><br>OB: 12.0 ± 2.3<br>Con: 11.5 ± 2.4 | OB: 53.5<br>Con: 50 | E-wave, A-wave, E/A, DT, IVRT, e', a', E/e' | Australia and Norway | controlled intervention | No/Yes | No | fair |
| Ippisch, 2008 [353] | Total: 38<br><br>OB: 38 | Morbidly OB: 60 ± 9<br>BMI > 99th percentile for age and sex (CDC) | 13-19<br><br>OB: 16 ± 1 | OB: 23.7 | E-wave, A-wave, E/A, e', a', E/e', e'/a' | USA | pre-post | Yes/Yes | No | good |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Kamal, 2012 [366] | Total: 120<br>OB: 70<br>Con: 50 | OB: 34.0 ± 3.8<br>BMI > 95th percentile for age and sex (cd)<br>Con: 21.6 ± 1.9<br>BMI < 95th percentile for age and sex | 12-15<br>OB: 14.0 ± 0.6<br>Con: 14.0 ± 0.9 | OB: 21.4<br>Con: 26.0 | e', e'/a' | Egypt | cross-sectional case-control, single centre | Yes/No | No | fair |
| Karaagac, 2019 [375] | Total: 34<br>OB: 34 | OB: 28.6 ± 4.3<br>BMI ≥ 95th percentile for age and sex (CDC) | nr<br>OB: 10.8 ± 2.3 | OB: 47.1 | E-wave, A-wave, e', a', E/e' | Turkey | pre-post | Yes/Yes | No | poor |
| Kibar, 2013 [354] | Total: 110<br>OB: 30<br>Con: 50<br>OW: 30 | OB: 32.9 ± 2.0<br>BMI ≥ 30 kg/m2<br>Con: 19.7 ± 1.6<br>BMI 17-24.9 kg/m2<br>OW: 27.2 ± 1.2<br>BMI 25-30 kg/m2 | 10-16.5<br>OB: 13.3 ± 2.0<br>Con: 13.2 ± 1.8<br>OW: 13.2 ± 2.1 | OB: 46.5<br>Con: 46.5<br>OW: 54.0 | IVRT, e', a', E/e', e'/a' | Turkey | cross-sectional case-control, single centre | Yes/No | Yes<br>Unclear<br>[fasting insulin (mIU/mL) x fasting glucose (mmol/L)]/22.5 | poor |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Kibar, 2015 *334* | Total: 110<br>OW/OB: 60<br>Con:50 | OW/OB: 30.1 ± 3.3<br>BMI > 95th percentile for age and sex (country specific – Turkey)<br>Con: 19.7 ± 1.6<br>cd | 10-16<br>OW/OB: 13.9 ± 2.3<br>Con: 13.2 ± 1.8 | OW/OB: 46.5<br>Con: 46.5 | Diastolic strain | Turkey | cross-sectional case-control, single centre | No/Yes | No | fair |
| Kinik, 2006 *352* | Total: 58<br>OB: 30<br>Con: 28 | OB: 26.9 ± 4.2<br>BMI ≥ 95th percentile for age and sex (CDC)<br>Con: 17.1 ± 1.6<br>cd | 4-17<br>OB: 10.8 ± 3.3<br>Con: 10.2 ± 3.0 | OB: 43.4<br>Con: 50.0 | E-wave, A-wave, E/A, e', a', e'/a' | Turkey | cross-sectional case-control, single centre | Yes/No | No | poor |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Koopman, 2012 [358] | Total: 48<br><br>OW/OB: 21<br>Con: 27 | OW/OB: 32.4 ± 4.9<br>OW BMI 85th - 94th percentiles, OB BMI ≥ 95th percentile for age and sex (CDC). 20/21 were OB<br><br>Con: 18.9 ± 2.3<br>BMI < 85th percentile for age and sex | 10-18<br><br>OW/OB: 14.2 ± 2.0<br>Con: 13.9 ± 2.3 | OW/OB: 78.6<br>Con: 81.0 | E-wave, A-wave, e', a', E/e', diastolic strain | Canada | cross-sectional case-control, single centre | Yes/No | No | poor |
| Korkmaz, 2016 [335] | Total: 158<br><br>OB: 79<br>Con: 79 | OB: 31.23 ± 3.85 (different age groups combined)<br>BMI > 95th percentile for age and sex or > 2 BMI z score (country specific - Turkey)<br><br>Con: 20.45 ± 1.76 cd | 10-16<br><br>OB: nr<br>Con: nr | OB: 40.5<br>Con: 51.9 | E/A | Turkey | cross-sectional case-control, single centre | No/Yes | No | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Labombarda, 2013 *336* | Total: 64<br><br>OB: 32<br>Con: 32 | OB: 30.2 (20.8 - 42.6) [median (IQR)]<br>BMI > 97th percentile for age and sex<br><br>Con: 18.06 ± 2.41 cd | 5-17<br><br>OB: 12.8 ± 2.1<br>Con: 12.8 ± 2.1 | OB: 46.9<br>Con: 46.9 | E-wave, A-wave, E/A, DT, e', E/e' | France | cross-sectional case-control, single centre | Yes/Yes | Yes<br><br>Unclear<br><br>[fasting glycaemia (mmol/L) × insulinaemia (mmol/L)]/22.5) | fair |
| Levent, 2005 *369* | Total: 75<br><br>OB: 25<br>Con: 25<br>Other: 25 | OB: 26.9 ± 2.7<br>BMI > 95th percentile for age and sex (cd)<br><br>Con: 17.4 ± 3.2 cd<br><br>Other: 31.9 ± 4.4<br>OB hypertensive | nr<br><br>OB: 11.9 ± 1.5<br>Con: 12.1 ± 1.8<br>Other: 13.8 ± 2.4 | OB: 52<br>Con: 52<br>Other: 56 | E-wave, A-wave, E/A, DT, IVRT | Turkey | cross-sectional case-control, single centre | Yes/No | No | poor |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Lorch, 2007 *337* | Total: 168<br><br>OB: 33<br>Con: 115<br>OW: 20 | OB: 32.4 ± 8.2<br>BMI ≥ 95th percentile for age and sex (CDC)<br><br>Con: 19.7 ± 2.4<br>BMI 5th - 84th percentile for age and sex<br><br>OW: 24.3 ± 2.4<br>BMI 85th - 94th percentile for age and sex | 10-18<br><br>OB: 13.3 ± 2.2<br>Con: 13.9 ± 2.3<br>OW: 13.8 ± 2.4 | OB: 75.8<br>Con: 49.6<br>OW: 45.0 | E/A, IVRT, a', e'/a', diastolic strain | USA | cross-sectional case-control, single centre | Yes/Yes | No | fair |
| Mangner, 2014 *338* | Total: 101<br><br>OW/OB: 61<br>Con: 40 | OW/OB: 30.8 ± 5.3<br>OB > 1.88 SD score (97th percentile), OW > 1.28 SD score (90th percentile) (country specific - German)<br><br>Con: 19 ± 2.6<br>cd | 8-21<br><br>OW/OB: 13.5 ± 2.7<br>Con: 14.1 ± 2.8 | OW/OB: 45.9<br>Con: 50.0 | E/A, DT, IVRT, e', a', E/e' | Germany | cross-sectional case-control, single centre | Yes/Yes | Yes<br>Overnight fast<br>Not reported | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Marcovecchio, 2016 *339* | Total: 45<br>OB: 30<br>Con: 15 | OB: 28.7 ± 5.1<br>BMI > 95th percentile for age and sex (country specific - Italy)<br>Con: 19.4 ± 2.4<br>BMI 5th-85th percentile for age and sex | 6-17<br>OB: 11.5 ± 2.4<br>Con: 12.8 ± 3.1 | OB: 40.0<br>Con: 66.7 | E-wave, A-wave, E/A, DT, IVRT, e', a', E/e' | Italy | cross-sectional case-control, single centre | Yes/Yes | Yes<br>Unclear<br>[fasting insulin (mU/L)×fasting glucose (mmol/L)]/22.5 | good |
| Mehta, 2004 *355* | Total: 116<br>OW/OB: 25<br>Con: 91 | OW/OB: 30.4 ± 6.5<br>BMI ≥ 25 kg/m2<br>Con: 20.2 ± 2.5<br>BMI < 25 kg/m2 | 10-18<br>OW/OB: 14.4 ± 2.1<br>Con: 13.8 ± 1.9 | OW/OB: 80.0<br>Con: 54.0 | E, A, E/A, e', a', E/e', e'/a' | USA | cross-sectional case-control, single centre | Yes/No | No | poor |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Mehta, 2009 *215* | Total: 49<br>OB: 17<br>Con: 32 | OB: 28.9 ± 5.4<br>WC > 90th percentile for age, sex and race (country specific - USA)<br>Con: 19.8 ± 4.0<br>WC ≤ 90th percentile for age, sex and race | 3-19<br>OB: 13.3 ± 3.2<br>Con: 13.3 ± 3.9 | OB: 76.5<br>Con: 59.4 | E-wave, A-wave, E/A, DT, e', a', E/e', e'/a' | USA | cross-sectional case-control, single centre | Yes/No | No | fair |
| Metwalley, 2018 *373* | Total: 120<br>OB: 60<br>Con: 60 | OB: 3.6 ± 0.8 [BMI SDS – Egypt]<br>BMI > 95th percentile for age and sex (IOTF)<br>Con: 0.3 ± 1.1<br>Non-obese | nr<br>OB: 9.8 ± 2.2<br>Con: 10.6 ± 1.7 | OB: 56.7<br>Con: 60.0 | E/A, DT, IVRT | Egypt | cross-sectional case-control, single centre | No/No | Yes<br>Overnight fast (>12hrs)<br>[fasting insulin (µU/mL) x fasting glucose (mmol/L)]/22.5 | poor |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Naylor, 2008 *374* | Total: 23<br>OB: 13<br>Con: 10 | OB: 32.5 ± 1.9<br>BMI > 95th percentile for age and sex (IOTF)<br>Con: 30.2 ± 2.6<br>BMI > 95th percentile for age and sex (not undertaking intervention) | nr<br>OB: 12.2 ± 0.4<br>Con: 13.6 ± 0.7 | OB: 53.8<br>Con: 40.0 | E/A, DT, IVRT, e', a', E/e' | Australia | pre-post | Yes/Yes | No | fair |
| Obert, 2012 *340* | Total: 61<br>OB: 37<br>Con: 24 | OB: 36.0 ± 5.1<br>BMI > 97th percentile for age and sex (cd)<br>Con: 20.8 ± 2.7<br>cd | nr<br>OB: 14.0 ± 1.5<br>Con: 13.6 ± 0.7 | OB: 29.7<br>Con: 37.5 | E-wave, A-wave, E/A, DT, IVRT, e', a', E/e', diastolic strain | France | cross-sectional case-control, single centre | Yes/Yes | Yes<br>Unclear<br>Not reported | fair |
| Obert, 2013 *341* | Total: 48<br>OB: 28<br>Con: 20 | OB: 36.0 ± 4.6<br>BMI > 97th percentile for age and sex. BMI z score >3 indicated severe obesity (cd)<br>Con: 21.1 ± 2.6<br>cd | nr<br>OB: 14.2 ± 1.5<br>Con: 14.9 ± 1.6 | OB: 32.1<br>Con: 40.0 | E-wave, A-wave, E/A, IVRT, e', E/e', e'/a', diastolic strain | France | pre-post | Yes/Yes | Yes<br>Unclear<br>Not reported | good |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Ozcetin, 2012 *308* | Total: 78<br><br>OB: 42<br>Con: 36 | OB: 27.83 ± 2.53<br>BMI > 95th percentile for age and sex (cd)<br><br>Con: 19.03 ± 2.54<br>cd | 8-16<br><br>OB: 10.12 ± 2.12<br>Con: 9.78 ± 1.78 | OB: 42.9<br>Con: 38.9 | E-wave, A-wave, E/A, S wave, D wave, S/D, e', a', E/e', e'/a' | Turkey | cross-sectional case-control, single centre | Yes/Yes | No | fair |
| Ozdemir, 2010 *342* | Total: 168<br><br>OB: 106<br>Con: 62 | OB: 28.1 ± 3.6<br>BMI z score > 2 (WHO)<br><br>Con: 18.1 ± 1.9<br>BMI z score ≤ 1 | nr<br><br>OB: 11.44 ± 2.30<br>Con: 11.75 ± 2.20 | OB: 55.7<br>Con: 58.1 | E/A, IVRT | Turkey | cross-sectional case-control, single centre | Yes/Yes | Yes<br><br>Fast >12hrs<br><br>Not reported | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Pacifico, 2014 *343* | Total: 126<br><br>OB: 54<br>Con: 18<br>Other: 54 | OB: 2.0 (1.95-2.15) [BMI SDS, mean (95% CI)]<br>BMI > 95th percentile for age and sex (IOTF)<br><br>Con: 0.47 (-0.13-1.0)<br>cd<br><br>Other: 2.1 (2.0-2.21)<br>OB with NAFLD | nr<br><br>OB: 12.6 (11.3-13.8) [mean (95% CI)<br>Con: 12.5 (11.3-13.8)<br>Other: 12.6 (11.3-13.8) | OB: 44.4<br>Con: 55.6<br>Other: 55.6 | E-wave, A-wave, E/A, DT, IVRT, e', a', E/e', e'/a' | Italy | cross-sectional case-control, single centre | No/Yes | Yes<br><br>Overnight fast<br><br>[fasting insulin (µU/mL) x fasting glucose (mmol/L)]/22.5 | fair |
| Porcar-Almela, 2015 *344* | Total: 130<br><br>OB: 49<br>Con: 42<br>Other: 39 | OB: 3.06 ± 0.58 (BMI SDS)<br>BMI z score > 2 (WHO)<br><br>Con: -0.04 ± 0.05<br>cd<br><br>Other: 5.49 ± 1.16<br>BMI z score > 4 | 7-16<br><br>OB: 11.45 ± 2.4<br>Con: 11.1 ± 2.7<br>Other: 10.6 ± 3.2 | OB: 59.2<br>Con: 50.0<br>Other: 48.7 | E-wave, A-wave, E/A, S wave, D wave, DT, IVRT, e', E/e' | Spain | cross-sectional case-control, single centre | No/Yes | Yes<br><br>Unclear<br><br>[fasting insulin (IU/L) x fasting glucose (mmol/L)]/22.5 | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Saltijeral, 2011 *309* | Total: 72<br><br>OB: 30<br>Con: 42 | OB: 30.93 ± 6.67<br>BMI z score > 2 (country specific - Spain)<br><br>Con: 19.37 ± 3.02<br>cd | nr<br><br>OB: 13.25 ± 2.68<br>Con: 13.90 ± 2.56 | OB: cd<br>Con: cd | E-wave, A-wave, E/A, e', a', E/e', e'/a' | Spain | cross-sectional case-control, single centre | No/Yes | No | fair |
| Sanchez, 2015 *368* | Total: 58<br><br>OB: 34<br>Con: 14<br>Other: 10 | OB: 32 (30-38) [median (IQR)]<br>BMI of ≥ 95th percentile for age and sex (CDC)<br><br>Con: 20 (17-23)<br>BMI 5th-85th percentile for age and sex<br><br>Other: 41 (30-53)<br>OB with abnormal LVMI and RWT | 12-18<br><br>OB: 14 (13-16) [median (IQR)]<br>Con: 15 (13-17)<br>Other: 14(12-15) | OB: 61.8<br>Con: 57.1<br>Other: 70.0 | Diastolic strain | USA | cross-sectional case-control, single centre | Yes/Yes | Yes<br><br>Overnight fast (>12hrs)<br><br>22.4/[glucose (mg/dL) x insulin (µU/mL)] | good |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Saritas, 2010 *367* | Total: 70<br><br>OB: 50<br>Con: 20 | OB: 26.0 ± 4.5<br>BMI > 97th percentile for age and sex (IOTF)<br><br>Con: 17 ± 2.6<br>cd | 66-166 months<br><br>OB: 125.58 ± 28.66 (months)<br>Con: 121.30 ± 40.33 | OB: nr<br>Con: nr | IVRT, e', a' | Turkey | cross-sectional case-control, single centre | Yes/No | No | poor |
| Schuster, 2009 *310* | Total: 35<br><br>OB: 10<br>Con: 17<br>Other: 8 | OB: 23.3 ± 1.8<br>BMI > 97th percentile for age and sex, 1st Degree (country specific - France)<br><br>Con: 17.6 ± 0.6<br>cd<br><br>Other: 29.0 ± 2.0<br>BMI > 97th percentile for age and sex, 2nd Degree | 10-12<br><br>OB: 11.7 ± 0.6<br>Con: 11.6 ± 1.1<br>Other: 11.4 ± 1.0 | OB: 100<br>Con: 100<br>Other: 100 | E-wave, A-wave, E/A, DT, IVRT, e', a', E/e' | France | cross-sectional case-control, single centre | Yes/Yes | No | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Schustero va, 2013 *350* | Total: 44<br>OW/OB: 21<br>Con: 23 | OW/OB: nr<br>BMI ≥ 85th percentile for age and sex<br>Con: nr<br>BMI < 85th percentile for age and sex | nr<br>OB: 13.5 ± 1.2<br>Con: 13.5 ± 1.1 | OB: nr<br>Con: nr | E-wave, A-wave, E/A, DT, IVRT | Slovakia | cross-sectional case-control, single centre | No/Yes | No | poor |
| Sert, 2013 *345* | Total: 248<br>OB: 83<br>Con: 68<br>Other: 97 | OB: 29.7 ± 2.8<br>BMI ≥ 95th percentile for age and sex (country specific - Turkey)<br>Con: 19.2 ± 2.3 cd<br>Other: 30.2 ± 2.6<br>OB with NAFLD | 12-17<br>OB: 13.3 ± 1.3<br>Con: 13.5 ± 1.3<br>Other: 13.2 ± 1.4 | OB: 51.8<br>Con: 51.5<br>Other: 52.6 | E-wave, A-wave, E/A, e', a', e'/a' | Turkey | cross-sectional case-control, single centre | Yes/Yes | Yes<br>Overnight fast (≥12hrs)<br>fasting insulin concentration [mU/ml] x fasting glucose concentration [mmol/L]/22.5 | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Shah, 2011 *346* | Total: 612<br><br>OB: 223<br>Con: 232<br>Other: 157 | OB: 37.6 ± 6.9<br>BMI ≥ 95th percentile for age and sex (CDC)<br><br>Con: 21.3 ± 2.4<br>BMI < 85th percentile for age and sex<br><br>Other: 39.1 ± 7.1<br>OB with Type 2 Diabetes | 10-24<br><br>OB: 18.1 ± 3.2<br>Con: 17.8 ± 3.5<br>Other: 17.9 ± 3.2 | OB: 29.1<br>Con: 38.4<br>Other: 34.4 | E-wave, A-wave, E/A, E/e', e'/a' | USA | cross-sectional case-control, single centre | Yes/Yes | No | good |
| Shah, 2015 *363* | Total: 447<br><br>OB: 182<br><br>Severely OB: 265 | OB: 32.5 ± 2.9<br><br>BMI ≥ 100-119% of the 95th percentile for age and sex (CDC)<br><br>Severely OB: 42.7 ± 6.9<br><br>BMI ≥ 120% of the 95th percentile for age and sex | 10.2-23.9 | OB: 35.0<br><br>Severely OB: 31.0 | E/A, E/e', e'/a' | USA | cross-sectional case-control, single centre | Yes/No | No | poor |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Sharpe, 2006 *311* | Total: 43<br><br>OB: 18<br>Con: 15 | OB: 33.3 ± 1.0<br>BMI equivalent to ≥ 30 at age 18 (IOTF)<br><br>Con: 20.5 ± 0.7<br>BMI equivalent to < 25 at age 18 | nr<br><br>OB: 12.4 ± 0.4<br>Con: 13.3 ± 0.5 | OB: 50.0<br>Con: 53.3 | E-wave, A-wave, E/A, DT, e', a', E/e', e'/a' | Australia | cross-sectional case-control, single centre | Yes/No | No | poor |
| Singh, 2013 *351* | Total: 44<br><br>OB: 15<br>Con: 14<br>Other: 15 | OB: 34.5 ± 2.9<br>BMI of ≥ 95th percentile for age and sex (cd)<br><br>Con: 19.9 ± 1.6<br>cd<br><br>Other: 37.4 ± 5.9<br>OB with NAFLD | nr<br><br>OB: 15 (14-17) [median (IQR)]<br>Con: 15 (14-17)<br>Other: 15 (13-16) | OB: 40.0<br>Con: 57.1<br>Other: 60.0 | Diastolic strain | USA | cross-sectional case-control, single centre | Yes/Yes | Yes<br><br>Unclear<br><br>Not reported | fair |
| Van Putte-Katier, 2008 *347* | Total: 94<br><br>OB: 49<br>Con: 45 | OB: 28.3 ± 7.1<br>cd<br><br>Con: 17.2 ± 2.9<br>cd | nr<br><br>OB: 11.2 ± 2.8<br>Con: 11.5 ± 3.2 | OB: 44.9<br>Con: 48.9 | A-wave, E/A, S wave, D wave, IVRT, e', E/e' | Belgium | cross-sectional case-control, single centre | Yes/Yes | No | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Whalley, 2009 *365* | Total: 39<br><br>OW/OB: 11<br>Con: 9<br>Type 1 Diab: 11<br>Type 2 Diab: 8 | OW/OB: 30.9 ± 5.3<br>BMI ≥ 85th percentile for age and sex (IOTF)<br><br>Con: 20.8 ± 2 cd<br><br>Type 1 Diab: 24.5 ± 3.6<br>Type 2 Diab: 38.3 ± 7.4 | 12-18<br><br>OW/OB: 15.30 ± 1.51<br>Con: 14.90 ± 1.182<br>Type 1 Diab: 15.5 ± 1.1<br>Type 2 Diab: 14.9 ± 1.1 | OW/OB: 0<br>Con: 0<br>Diabetes: 0 | E-wave, A-wave, E/A, IVRT, e', E/e' | New Zealand | cross-sectional case-control, single centre | Yes/Yes | No | fair |
| Xie, 2015 *360* | Total: 90<br><br>OB: 52<br>Con: 38 | OB: 31.5 ± 3.7<br>BMI of ≥ 95th percentile for age and sex (cd)<br><br>Con: 19.6 ± 2.1 cd | 14-20<br><br>OB: 17.8 ± 1.7<br>Con: 18.1 ± 2.1 | OB: 48.1<br>Con: 44.7 | E-wave, A-wave, E/A, DT, e', a', E/e', e'/a', diastolic strain | China | cross-sectional case-control, single centre | Yes/No | Yes<br><br>Overnight fast<br><br>[fasting glucose (mmol/l) x fasting insulin (mU/l)]/22.5 | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Yang, 2019 [372] | Total: 181<br>OB: 40<br>Con: 102<br>Other: 39 | OB: nr<br>BMI ≥ 95th percentile and < 95th percentile x 120% for age and sex<br>Con: nr<br>BMI < 95$^{th}$ percentile for age and sex<br>Other: ≥ 95th percentile x 120% (severe obesity) | 10-20<br>OB: 13.5 ± 2.7<br>Con: 13.8 ± 2.5<br>Other: 13.6 ± 2.5 | OB: 67.5<br>Con: 64.7<br>Other: 69.2 | E/A, DT, IVRT, e', E/e', e'/a' | Taiwan | cross-sectional case-control, single centre | No/No | Yes<br>Unclear<br>[1.5 + fasting glucose × fasting C-peptide]/2800 | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Yildirim, 2018 [348] | Total: 95<br><br>OB: 33<br>Con: 33<br>Other: 29 | OB: nr<br>BMI ≥ 95th percentile for age and sex (cd)<br><br>Con: nr<br>BMI 3rd - 85th percentiles for age and sex<br><br>Other: nr<br>OB with anaemia | 12-17<br><br>OB: 14.18 ± 1.28<br>Con: 14.40 ± 1.42<br>Other: 13.89 ± 1.39 | OB: 54.5<br>Con: 54.5<br>Other: 44.8 | IVRT, e', a', E/e', e'/a' | Turkey | cross-sectional case-control, single centre | Yes/No | No | poor |
| Yu, 2006 [356] | Total: 40<br><br>OW/OB: 22<br>Con: 18 | OW/OB: 28.4 ± 3.6<br>BMI ≥ 85th percentile for age and sex (country specific - South Korea)<br><br>Con: 17.9 ± 2.5<br>BMI < 85th percentile for age and sex | >10<br><br>OW/OB: 13.4 ± 1.22<br>Con: 13.4 ± 2.19 | OW/OB: 72.7<br>Con: 61.1 | E-wave, A-wave, E/A, S wave, D wave, S/D, e', a', e'/a' | South Korea | cross-sectional case-control, single centre | Yes/Yes | No | fair |

| Study (First author, year) | N | BMI (kg/m2 - unless specified) and group definitions | Age range and mean age (years - unless specified) | Sex (male %) | LVDF Measures Reported | Country | Study Type | Included meta analysis / systematic review (Yes/No) | HOMA-IR analysis (included, fasted state, equation used) | Quality score |
|---|---|---|---|---|---|---|---|---|---|---|
| Zeybek, 2010 [349] | Total: 58<br>OB: 34<br>Con: 24 | OB: 32.55 ± 2.96<br>BMI ≥ 95th percentile for age and sex (country specific - Turkey)<br>Con: 17.52 ± 2.58<br>BMI 5th-85th percentile for age and sex | nr<br>OB: 11.75 ± 2.23<br>Con: 11.25 ± 1.75 | OB: 50<br>Con: 50 | E-wave, A-wave, E/A, IVRT, e', a', E/e', e'/a' | Turkey | pre-post | Yes/No | Yes<br>Unclear<br>[fasting insulin (μU/ml) x fasting glucose (mmol/L)]/22.5 | fair |
| Zhang, 2018 [357] | Total:<br>OB:<br>Con: | OB: 25.67 ± 3.78<br>BMI > 95th percentile for age and sex (country specific – China)<br>Con: 17.33 ± 2.58<br>BMI < 85th percentile for age and sex | 4-18<br>OB: 10.76 ± 2.72<br>Con: 11.49 ± 3.49 | OB: 57.5<br>Con: 49.3 | E-wave, A-wave, E/A, e', a', E/e', e'/a' | China | cross-sectional case-control, single centre | Yes/No | No | poor |

A-wave indicates, peak late Doppler mitral inflow wave; a', peak late diastolic tissue velocity; BMI, body mass index; cd, cannot determine; CDC, Centre for Disease Control and Prevention; CI, confidence interval; Con, control; D wave, peak pulmonary vein diastolic velocity; Diab, diabetes; DT, E-wave deceleration time; E-wave, peak early Doppler mitral inflow velocity; e', peak early diastolic tissue velocity; E/A, E-wave/A-wave ratio; E/e', E-wave/e' ratio; e'/a', e'/a' ratio; HOMA-IR, homeostatic model

assessment for insulin resistance; IOTF, International Obesity Taskforce; IVRT, isovolumic relaxation time; IQR, interquartile range; LVMI, left ventricular mass index; MetS, metabolic syndrome; NAFLD, Non-alcoholic fatty liver disease; nr, not-reported; OB, obese; OW, overweight; RWT, relative wall thickness; S wave, peak pulmonary vein systolic velocity; SDS, standard deviation score; WHO, World Health Organization.

**Appendix 1, Table 1.2: Total number of studies and participants available for meta-analysis with BMI for each LVDF measure**

| Measure | Total Number of Studies | Number of Studies in Meta-Analysis | Total Number of Participants | Number of Participants in Meta-Analysis |
|---|---|---|---|---|
| **E-wave** | 38 | 33 | 4056 | 3660 |
| **A-wave** | 39 | 34 | 4200 | 3754 |
| **E/A** | 52 | 42 | 7795 | 5668 |
| **DT** | 19 | 14 | 2203 | 1383 |
| **IVRT** | 24 | 17 | 4190 | 1890 |
| **e'** | 41 | 33 | 4464 | 3491 |
| **a'** | 28 | 22 | 3308 | 2534 |
| **E/e'** | 38 | 30 | 5048 | 4075 |
| **e'/a'** | 23 | 18 | 3874 | 2782 |

A-wave indicates late mitral inflow peak velocity; a', late diastolic tissue peak velocity; BMI, body mass index; DT, E-wave deceleration time; E-wave, early mitral inflow peak velocity; e', early diastolic tissue peak velocity; E/A, E-wave/A-wave ratio; E/e', E-wave/e' ratio; e'/a', e'/a' ratio; IVRT, isovolumic relaxation time.

**Appendix 1, Table 1.3: Study specific positive (↑), negative (↓), or non-significant (↔) associations of measures left ventricular diastolic function with measures of adiposity**

| Study (first author, year) | E-wave | A-wave | E/A | DT | IVRT | e' | a' | E/e' | e'/a' | Diastolic strain or strain-rate | Pulmonary vein flow |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Corica, 2020 [322] | | | ↓[a] | | | ↓[b] | | | | | |
| Cozzolino, 2015 [323] | | | | | | | | | ↔[a] | | |
| Dias, 2017 [326] | | | | | | | | | | ↓ $SR_E$[c], ↔ $SR_A$[c] | |
| El Saiedi, 2018 [327] | | | | | | | | ↑[d] | | | |
| Hui, 2019 [331] | | | | | | | | ↑[d] | | | |
| Kibar, 2015 [334] | | | | | | | | | | ↓ $SR_E/SR_A$[d] | |
| Korkmaz, 2016 [335] | ↑[a,d] | | ↓[d] | | | | | | | | |
| Lorch, 2007 [337] | | | | | | | | | | ↓ long and rad $SR_A$[d] | |
| Mangner, 2014 [338] | | | | | | ↓[d] | | | | | |
| Marcovecchio, 2016 [339] | ↔[a,b] | | | | | | | | | | |
| Mehta, 2009 [215] | | | | | | ↓[b,d,e] | | ↑[b,d] | ↓[b,d,e] | | |
| Obert, 2012 [340] | | | | | | ↓[a] | | ↑[a] | | | |
| Pacifico, 2014 [343] | | | | | | | | ↔[a,c] | | | |
| Porcar-Almela, 2015 [344] | | | | | | | | ↑[a,b,c] | | | |
| Saltijeral, 2011 [309] | ↓[d] | ↔[d] | ↔[d] | | | ↔[d] | ↔[d] | ↔[d] | ↓[d] | | |
| Sanchez, 2015 [368] | | | | | | | | | | ↔ $SR_E$[a] | |
| Shah, 2011 [346] | | | ↓[b] | | | | | ↑[a,b,c] | ↓[a,b,c] | | |
| Singh, 2013 [351] | | | | | | | | | | ↔ $SR_E$[c,d] | |
| Van Putte-Katier, 2008 [347] | | | | | | ↓[a] | | ↑[a] | | | ↑ S wave[a] |

Arrows indicate studies that report either positive associations (↑), negative associations (↓), or no significant associations (↔) between a measure of adiposity (e.g. body mass index) and a measure of left ventricle diastolic dysfunction. A-wave, peak late Doppler mitral inflow velocity; a', peak late diastolic tissue velocity; DT, E-wave deceleration time; E-wave, peak early Doppler mitral inflow velocity; e', peak early diastolic tissue velocity; E/A, E-wave/A-wave ratio; E/e', E-wave/e' ratio; e'/a', e'/a' ratio; IVRT, isovolumic relaxation time; long, longitudinal; rad, radial; S wave, peak pulmonary vein systolic velocity; SR, diastolic strain rate; $SR_A$, SR in late diastole; $SR_A$, SR in early diastole. [a] Body mass index (BMI) standard deviation score (z score); [b] Waist circumference; [c] Body fat percentage/adipose tissue volume; [d] BMI; [e] Body surface area.

**Appendix 1, Table 1.4: Association of left ventricular diastolic function measures with BMI, age and sex**

| Measure | N | Ns | $r^2$ | b | r | SE | z | LCI | UCI | p |
|---|---|---|---|---|---|---|---|---|---|---|
| **E-wave (m/s)** | 66 | 33 | 0.143 | | | | | | | |
| *BMI (kg/m2)* | | | | 0.177 | 0.178 | 0.072 | 2.450 | 0.035 | 0.319 | 0.014 |
| *Age (years)* | | | | -1.311 | -0.177 | 0.563 | -2.329 | -2.414 | -0.208 | 0.020 |
| *Sex (male %)* | | | | 0.007 | 0.001 | 0.058 | 0.120 | -0.107 | 0.121 | 0.905 |
| **A-wave (m/s)** | 68 | 34 | 0.272 | | | | | | | |
| *BMI (kg/m2)* | | | | 0.264 | 0.216 | 0.050 | 5.294 | 0.166 | 0.361 | 0.000 |
| *Age (years)* | | | | -2.031 | -0.224 | 0.544 | -3.736 | -3.097 | -0.966 | 0.000 |
| *Sex (male %)* | | | | 0.056 | 0.006 | 0.056 | 0.990 | -0.055 | 0.167 | 0.322 |
| **E/A** | 85 | 42 | 0.551 | | | | | | | |
| *BMI (kg/m2)* | | | | -0.006 | -0.147 | 0.001 | -4.561 | -0.008 | -0.003 | 0.000 |
| *Age (years)* | | | | 0.059 | 0.209 | 0.010 | 5.750 | 0.039 | 0.079 | 0.000 |
| *Sex (male %)* | | | | -0.001 | -0.003 | 0.002 | -0.448 | -0.005 | 0.003 | 0.654 |
| **DT (ms)** [a] | 31 | 14 | 0.263 | | | | | | | |
| *BMI (kg/m2)* | | | | -0.022 | -0.005 | 0.447 | -0.049 | -0.899 | 0.855 | 0.961 |
| *Age (years)* | | | | -5.887 | -0.169 | 6.151 | -0.957 | -17.942 | 6.168 | 0.338 |
| *Sex (male %)* | | | | -1.585 | -0.046 | 0.916 | -1.731 | -3.381 | 0.210 | 0.083 |
| **IVRT (ms)** | 37 | 17 | 0.152 | | | | | | | |
| *BMI (kg/m2)* | | | | 0.286 | 0.222 | 0.097 | 2.951 | 0.096 | 0.476 | 0.003 |
| *Age (years)* | | | | -1.749 | -0.182 | 1.244 | -1.406 | -4.188 | 0.689 | 0.160 |
| *Sex (male %)* | | | | -0.126 | -0.013 | 0.142 | -0.887 | -0.404 | 0.152 | 0.375 |
| **e' (cm/s)** [a] | 39 | 20 | 0.235 | | | | | | | |
| *BMI (kg/m2)* | | | | -0.091 | -0.294 | 0.020 | -4.583 | -0.130 | -0.052 | 0.000 |
| *Age (years)* | | | | 0.012 | 0.005 | 0.297 | 0.040 | -0.571 | 0.595 | 0.968 |
| *Sex (male %)* | | | | -0.023 | -0.010 | 0.019 | -1.221 | -0.060 | 0.014 | 0.222 |
| **e' sep (cm/s)** | 37 | 19 | 0.257 | | | | | | | |

| Measure | N | Ns | $r^2$ | b | r | SE | z | LCI | UCI | p |
|---|---|---|---|---|---|---|---|---|---|---|
| *BMI (kg/m2)* | | | | -0.075 | -0.413 | 0.016 | -4.726 | -0.106 | -0.044 | 0.000 |
| *Age (years)* | | | | 0.386 | 0.287 | 0.164 | 2.356 | 0.065 | 0.708 | 0.018 |
| *Sex (male %)* | | | | -0.019 | -0.014 | 0.017 | -1.083 | -0.052 | 0.015 | 0.279 |
| **e' lat (cm/s)** | 40 | 20 | 0.169 | | | | | | | |
| *BMI (kg/m2)* | | | | -0.116 | -0.247 | 0.021 | -5.554 | -0.157 | -0.075 | 0.000 |
| *Age (years)* | | | | 0.290 | 0.083 | 0.327 | 0.887 | -0.351 | 0.930 | 0.375 |
| *Sex (male %)* | | | | -0.011 | -0.003 | 0.038 | -0.283 | -0.086 | 0.064 | 0.777 |
| **a' (cm/s)** [a] | 27 | 14 | 0.295 | | | | | | | |
| *BMI (kg/m2)* | | | | 0.059 | 0.343 | 0.017 | 3.449 | 0.025 | 0.092 | 0.001 |
| *Age (years)* | | | | -0.609 | -0.476 | 0.177 | -3.433 | -0.957 | -0.261 | 0.001 |
| *Sex (male %)* | | | | -0.002 | -0.001 | 0.013 | -0.138 | -0.027 | 0.024 | 0.890 |
| **a' sep (cm/s)** | 31 | 16 | 0.421 | | | | | | | |
| *BMI (kg/m2)* | | | | 0.074 | 0.621 | 0.011 | 6.579 | 0.052 | 0.096 | 0.000 |
| *Age (years)* | | | | -0.470 | -0.528 | 0.115 | -4.104 | -0.695 | -0.246 | 0.000 |
| *Sex (male %)* | | | | 0.017 | 0.019 | 0.012 | 1.427 | -0.006 | 0.040 | 0.154 |
| **a' lat (cm/s)** | 28 | 14 | 0.179 | | | | | | | |
| *BMI (kg/m2)* | | | | 0.088 | 0.432 | 0.016 | 5.394 | 0.056 | 0.120 | 0.000 |
| *Age (years)* | | | | -0.067 | -0.044 | 0.187 | -0.359 | -0.433 | 0.299 | 0.720 |
| *Sex (male %)* | | | | -0.010 | -0.007 | 0.027 | -0.373 | -0.063 | 0.043 | 0.709 |
| **E/e' (cm/s)** | 29 | 16 | 0.646 | | | | | | | |
| *BMI (kg/m2)* | | | | 0.067 | 0.387 | 0.006 | 11.419 | 0.055 | 0.078 | 0.000 |
| *Age (years)* | | | | 0.166 | 0.130 | 0.068 | 2.460 | 0.034 | 0.299 | 0.014 |
| *Sex (male %)* | | | | 0.012 | 0.009 | 0.007 | 1.804 | -0.001 | 0.025 | 0.071 |
| **E/e' sep (cm/s)** [a] | 30 | 16 | 0.151 | | | | | | | |
| *BMI (kg/m2)* | | | | 0.081 | 0.431 | 0.011 | 7.220 | 0.059 | 0.104 | 0.000 |
| *Age (years)* | | | | -0.064 | -0.045 | 0.131 | -0.484 | -0.321 | 0.194 | 0.628 |
| *Sex (male %)* | | | | 0.022 | 0.015 | 0.014 | 1.588 | -0.005 | 0.048 | 0.112 |

| Measure | N | Ns | $r^2$ | b | r | SE | z | LCI | UCI | p |
|---|---|---|---|---|---|---|---|---|---|---|
| **E/e' lat (cm/s)** | 35 | 18 | 0.415 | | | | | | | |
| *BMI (kg/m2)* | | | | 0.046 | 0.237 | 0.006 | 7.174 | 0.034 | 0.059 | 0.000 |
| *Age (years)* | | | | 0.159 | 0.110 | 0.087 | 1.827 | -0.012 | 0.331 | 0.068 |
| *Sex (male %)* | | | | 0.003 | 0.002 | 0.008 | 0.391 | -0.013 | 0.019 | 0.696 |
| **e'/a'** | 21 | 11 | 0.570 | | | | | | | |
| *BMI (kg/m2)* | | | | -0.016 | -0.306 | 0.005 | -2.834 | -0.026 | -0.005 | 0.005 |
| *Age (years)* | | | | 0.106 | 0.282 | 0.040 | 2.678 | 0.029 | 0.184 | 0.007 |
| *Sex (male %)* | | | | 0.011 | 0.029 | 0.007 | 1.564 | -0.003 | 0.025 | 0.118 |
| **e'/a' sep** [a] | 25 | 13 | 0.544 | | | | | | | |
| *BMI (kg/m2)* | | | | -0.024 | -0.689 | 0.003 | -7.909 | -0.030 | -0.018 | 0.000 |
| *Age (years)* | | | | 0.096 | 0.370 | 0.033 | 2.887 | 0.031 | 0.161 | 0.004 |
| *Sex (male %)* | | | | -0.007 | -0.028 | 0.004 | -1.941 | -0.014 | 0.000 | 0.052 |
| **e'/a' lat** | 24 | 12 | 0.400 | | | | | | | |
| *BMI (kg/m2)* | | | | -0.037 | -0.593 | 0.008 | -4.525 | -0.052 | -0.021 | 0.000 |
| *Age (years)* | | | | 0.102 | 0.223 | 0.059 | 1.727 | -0.014 | 0.218 | 0.084 |
| *Sex (male %)* | | | | -0.006 | -0.012 | 0.014 | -0.400 | -0.032 | 0.021 | 0.689 |

A-wave indicates peak late Doppler mitral inflow velocity; a', peak late diastolic tissue velocity; Adj r2, adjusted r2; b, unstandardized regression coefficient; BMI, body mass index; CI, confidence interval (LCI, lower CI; UCI, upper CI); DT, E-wave deceleration time; E-wave, peak early Doppler mitral inflow velocity; e', peak early diastolic tissue velocity; E/A, E-wave/A-wave ratio; E/e', E-wave/e' ratio; e'/a', e'/a' ratio; IVRT, isovolumic relaxation time; N, number of observations; Ns, number of studies; r, standardized correlation coefficient; SE, standard error.

[a], Non-normal distributions.

**Appendix 1, Table 1.5: Associations of BMI with each left ventricular diastolic function measure, ranked by strength of association (r) – sensitivity analysis excluding >21 years of age**

| Measure (units per 10 point change in BMI) | Number of Studies | References | Correlation Coefficient (r) | b | 95% CI | Fisher's z-test |
|---|---|---|---|---|---|---|
| **e'/a' sep** ($1/kg/m^2$) | 13 | 215, 308, 311, 317, 323, 345, 349, 352-357 | -0.689 | -0.240 | **-0.299, -0.180** | 0.000 |
| **a' sep** ($cm.s^{-1}/kg/m^2$) | 16 | 215, 308, 311, 317, 323, 327, 339, 345, 349, 352-358 | 0.621 | 0.743 | **0.522, 0.965** | 0.239 |
| **e'/a' lat** ($1/kg/m^2$) | 12 | 215, 308, 315, 317, 345, 349, 352-355, 359, 360 | -0.593 | -0.366 | **-0.525, -0.208** | 0.315 |
| **E/e' sep** ($1/kg/m^2$) | 16 | 215, 308, 311, 323, 324, 327, 338, 339, 347, 353, 355, 357, 358, 361, 362 | 0.446 | 0.843 | **0.652, 1.035** | 0.831 |
| **a' lat** ($cm.s^{-1}/kg/m^2$) | 14 | 215, 308, 315, 317, 339, 345, 349, 352-355, 358-360 | 0.432 | 0.877 | **0.558, 1.195** | 0.879 |
| **e' sep** ($cm.s^{-1}/kg/m^2$) | 19 | 215, 308, 311, 317, 318, 323, 327, 338, 339, 345, 347, 349, 352-358 | -0.413 | -0.747 | **-1.057, -0.437** | 1.009 |
| **E/e' average** ($1/kg/m^2$) | 16 | 215, 308, 324, 326, 328, 333, 338, 339, 349, 353, 355, 358, 361, 364, 365 | 0.393 | 0.676 | **0.502, 0.850** | 0.982 |
| **a' average** ($cm.s^{-1}/kg/m^2$) | 14 | 215, 308, 310, 317, 326, 339, 345, 349, 352-355, 358, 364 | 0.343 | 0.589 | **0.255, 0.924** | 1.172 |
| **e' average** ($cm.s^{-1}/kg/m^2$) | 20 | 215, 308, 310, 317, 318, 326, 328, 333, 338, 339, 345, 349, 352-355, 358, 364-366 | -0.294 | -0.912 | **-1.302, -0.522** | 1.460 |
| **e' lat** ($cm.s^{-1}/kg/m^2$) | 20 | 215, 308, 315-318, 331, 336, 338, 339, 345, 349, 352-355, 358-360, 367 | -0.247 | -1.161 | **-1.571, -0.752** | 1.642 |
| **e'/a' average** ($1/kg/m^2$) | 11 | 215, 308, 317, 345, 349, 352-355, 366 | -0.223 | -0.113 | -0.239, 0.012 | 1.418 |
| **E/e' lat** ($1/kg/m^2$) | 18 | 133, 215, 308, 310, 317, 324, 331, 336, 338, 339, 349, 353-355, 358, 360, 361 | 0.222 | 0.432 | **0.303, 0.562** | 1.707 |
| **IVRT** ($ms/kg/m^2$) | 17 | 14,24,28,29,35,40, 43,46,48,52,53,57-59,61-63 | 0.222 | 2.861 | **0.961, 4.762** | 1.664 |
| **A-wave** | 34 | 12,14,15,18-48 | 0.184 | 2.237 | **1.096, 3.379** | 2.039 [a] |

| | | | | | | |
|---|---|---|---|---|---|---|
| ($cm.s^{-1}/kg/m^2$) | | | | | | |
| **E/A** ($1/kg/m^2$) | 42 | 12,14,15,18-30,32,34-38,40-60 | -0.154 | -0.058 | **-0.088, -0.029** | 2.216 [a] |
| **E-wave** ($cm.s^{-1}/kg/m^2$) | 33 | 12,14,15,18-47 | 0.088 | 0.872 | -0.607, 2.351 | 2.417 [a] |
| **DT** ($ms/kg/m^2$) | 14 | 14,15,21,24,26,29,34-36,40,44,53,58,61 | -0.005 | -0.220 | -8.987, 8.546 | 2.401 [a] |

Fisher z-test, which accounts for sample size, was used to compare the strength of the correlation coefficients (r) with the strongest association, septal (sep) e'/a', as a reference. Larger values of Fisher's z indicate that correlation coefficients are more likely to be statistically different (less strongly associated) with respect to the reference association. Those that were significantly different ($P < 0.05$) are marked with an [a]. Tissue Doppler imaging (TDI) measures are reported as an average of recordings from the septal and lateral wall (lat) of the left ventricle, and individually as sep and lat. A-wave indicates late mitral inflow peak velocity; a', late diastolic tissue peak velocity; Adj $r^2$, adjusted $r^2$; b, unstandardized regression coefficient; BMI, body mass index; CI, confidence interval; DT, E-wave deceleration time; E-wave, early mitral inflow peak velocity; e', early diastolic tissue peak velocity; E/A, E-wave/A-wave ratio; E/e', E-wave/e' ratio; e'/a', e'/a' ratio; IVRT, isovolumic relaxation time

**Appendix 1, Table 1.6: Increased (↑), decreased, (↓), or unchanged (↔) left Ventricular diastolic function in children and adolescents with overweight/obesity – results of studies included in the systematic review**

| Study (First Author, Year) | a' (+) | E/e' (+) | IVRT (+) | A-wave (+) | E-wave (+) | DT (=) | E/A (-) | e' (-) | e'/a' (-) |
|---|---|---|---|---|---|---|---|---|---|
| Ahmed, 2016 [315] | ↑[c] | | | | | | | ↓[c] | ↓[c] |
| Akcaboy, 2016 [133] | | ↔[c] | | | | | ↔ | | |
| Alkholy, 2016 [316] | | | | ↔ | ↔ | | ↔ | ↔[c] | |
| Alp, 2014 [317] | ↑[b] ↖[c] | ↑[c] | ↑[f] | ↑ | ↑ | | ↓ | ↔[b,c] | ↓[b,c] |
| Aslan, 2019 [318] | | | | ↔ | ↔ | | ↔ | ↔[b,c] | |
| Battal, 2011 [319] | | | | ↔ | ↓ | ↑ | ↓ | | |
| Bjornstad, 2016 [320] | ↑[b,c] | ↖[c] ↔[b] | | ↑ | ↔ | ↓ | ↓ | ↖[c] ↔[b] | |
| Chinali, 2008 [321] | | | ↔ | | | ↑ | ↓ | | |
| Corica, 2020 [322] | | | | | | | ↓ | | |
| Cozzolino, 2015 [323] | ↔[b] | ↔[b] | | ↔ | ↓ | | ↓ | ↔[b] | ↓[b] |
| Dahiya, 2015 [144] | | ↑[b] | | | | | ↔ | ↓[b] | |
| Dhuper, 2011 [324] | | ↑[b,c] | | | | | ↓ | | |
| Di Bonito, 2009 [325] | | | ↔ | | | | ↔ | | ↔[d] |
| Dias, 2017 [326] | ↓[a] | ↔[a] | | ↓ | ↔ | | ↑ | ↓[a] | |
| Dusan, 2015 [134] | | | ↔ | ↑ | ↔ | ↔ | ↓ | | |
| El Saiedi, 2018 [327] | ↑[b,d] | ↑[b] | | ↔ | ↓ | | ↔ | ↓[b] ↔[d] | |
| Franssen, 2019 [328] | | ↑[a] | | ↑ | ↔ | ↔ | ↔ | ↓[a] | |
| Ghanem, 2010 [329] | | | | | | | ↔ | | |
| Hirschler, 2006 [330] | | | | | | | ↔ | | |
| Hui, 2019 [331] | | ↑[c] | ↔ | ↑ | ↑ | | ↔ | ↓[c] | |
| Ingul, 2010 [333] | | ↑[a] | ↑ | ↔ | ↔ | ↑ | ↔ | ↓[a] | |
| Ingul, 2018 [332] | ↑[a] | ↑[a] | | | | | ↓ | ↓[a] | |
| Kibar, 2015 [334] | ↔[c] ↘[b] | | | | | | | ↔[b,c] | ↖[b] ↔[c] |
| Korkmaz, 2016 [335] | | | | | | | ↓[e] | | |

| Study (First Author, Year) | a' (+) | E/e' (+) | IVRT (+) | A-wave (+) | E-wave (+) | DT (=) | E/A (-) | e' (-) | e'/a' (-) |
|---|---|---|---|---|---|---|---|---|---|
| Labombarda, 2013 [336] | | ↖[c] | | ↔ | ↔ | ↔ | ↔ | ↘[c] | |
| Lorch, 2007 [337] | | | ↔ | ↔ | ↔ | ↔ | ↔ | | |
| Manger, 2014 [338] | | ↑[b,c] | ↖ | | | ↔ | ↔ | ↓[b,c] | |
| Marcovecchio, 2016 [339] | ↑[c] ↔[b] | ↔[b,c] | | ↔ | ↑ | ↑ | ↔ | ↔[b,c] | |
| Mehta, 2009 [215] | ↔[b,c] | ↑[b,c] | | ↑ | ↔ | | ↓ | ↓[b,c] | ↓[b,c] |
| Obert, 2012 [340] | ↔[d] | ↑[d] | ↔ | ↔ | ↔ | ↔ | ↔ | ↓[d] | |
| Obert, 2013 [341] | | | | | | | | | ↓[d] |
| Ozcetin, 2012 [308] | ↑[c] ↔[b] | ↑[c] ↖[b] | | ↔ | ↔ | | ↔ | ↘[c] ↔[b] | ↓[c] ↘[b] |
| Ozdemir, 2010 [342] | | | ↔ | | | | ↔ | | |
| Pacifico, 2014 [343] | ↔[a] | ↔[a] | ↑ | ↔ | ↔ | ↔ | ↔ | | ↘[a] |
| Porcar-Almela, 2015 [344] | | ↑[a] | ↔ | ↔ | ↖ | ↔ | ↔ | | |
| Saltijeral, 2011 [309] | ↔[c] | ↖[c] | | ↔ | ↔ | | ↔ | ↔[c] | ↔[c] |
| Schuster, 2009 [310] | ↔[a] | ↔[c] | ↑ | ↔ | ↔ | ↔ | ↔ | | |
| Schusterova, 2013 [350] | | | ↑ | ↑ | ↔ | ↑ | ↓ | | |
| Sert, 2013 [345] | ↑[b,c] | | | ↔ | ↓ | | ↓ | ↓[c] ↔[b] | ↓[b,c] |
| Shah, 2011 [346] | | ↑[a] | | ↑ | ↑ | | ↔ | | ↓[a] |
| Van Putte-Katier, 2008 [347] | | ↑[a] | ↔ | ↔ | | | ↔ | ↓[b] | |
| Yildirim, 2018 [348] | ↔[d] | ↖[d] | ↔ | | | | | ↔[d] | ↘[d] |
| Zeybek, 2010 [349] | ↔[b,c] | ↑[c,f] | ↔ | ↔ | ↔ | | ↔ | ↓[c] ↔[b] | ↓[c] ↔[b] |

Arrows indicate increased (↑), a statistical trend ($p < 0.1$) towards increased values (↖), unchanged (↔), a statistical trend towards decreased values (↘), or decreased (↓) left ventricular (LV) diastolic function (DF) measures in overweight/obese children and adolescents compared to control subjects. LV DF measures are listed from the left in order of the strongest positive (+), no association (=), to the strongest negative (-) association with body mass index on the right as identified in our quantitative analysis.

[a] Average of septal and lateral tissue Doppler imaging (TDI) measures; [b] Septal TDI measures; [c] Lateral TDI measures; [d] Cannot determine site of TDI; [e] In children aged 12-14 years only; [f] IVRT assessed by TDI.

**Appendix 1, Table 1.7: Association of left ventricular diastolic function measures with HOMA-IR, age and sex**

| Measure | N | Ns | $r^2$ | b | r | SE | z | LCI | UCI | p |
|---|---|---|---|---|---|---|---|---|---|---|
| **E-wave (m/s)** | 30 | 16 | 0.088 | | | | | | | |
| *HOMA-IR* | | | | 0.091 | 0.012 | 0.558 | 0.162 | -1.004 | 1.185 | 0.871 |
| *Age (years)* | | | | -0.544 | -0.055 | 1.109 | -0.491 | -2.718 | 1.630 | 0.624 |
| *Sex (male %)* | | | | -0.101 | -0.010 | 0.130 | -0.783 | -0.355 | 0.152 | 0.434 |
| **A-wave (m/s)** | 30 | 16 | 0.259 | | | | | | | |
| *HOMA-IR* | | | | 1.169 | 0.157 | 0.288 | 4.064 | 0.605 | 1.733 | 0.000 |
| *Age (years)* | | | | -3.684 | -0.375 | 0.932 | -3.953 | -5.510 | -1.857 | 0.000 |
| *Sex (male %)* | | | | 0.261 | 0.027 | 0.076 | 3.447 | 0.112 | 0.409 | 0.001 |
| **E/A** | 47 | 24 | 0.289 | | | | | | | |
| *HOMA-IR* | | | | -0.035 | -0.159 | 0.012 | -2.864 | -0.059 | -0.011 | 0.004 |
| *Age (years)* | | | | 0.103 | 0.354 | 0.027 | 3.826 | 0.050 | 0.156 | 0.000 |
| *Sex (male %)* | | | | 0.001 | 0.004 | 0.004 | 0.326 | -0.006 | 0.008 | 0.744 |
| **DT (ms)** | 14 | 27 | 0.321 | | | | | | | |
| *HOMA-IR* | | | | -2.654 | -0.103 | 2.221 | -1.195 | -7.007 | 1.699 | 0.232 |
| *Age (years)* | | | | -12.606 | -0.374 | 5.741 | -2.196 | -23.858 | -1.354 | 0.028 |
| *Sex (male %)* | | | | -1.979 | -0.059 | 1.028 | -1.925 | -3.995 | 0.036 | 0.054 |
| **IVRT (ms)** | 27 | 13 | 0.443 | | | | | | | |
| *HOMA-IR* | | | | 3.560 | 0.463 | 1.148 | 3.102 | 1.310 | 5.810 | 0.002 |
| *Age (years)* | | | | -4.219 | -0.418 | 1.257 | -3.356 | -6.683 | -1.755 | 0.001 |
| *Sex (male %)* | | | | -0.456 | -0.045 | 0.256 | -1.780 | -0.959 | 0.046 | 0.075 |
| **e' (cm/s)** | 23 | 12 | 0.403 | | | | | | | |
| *HOMA-IR* | | | | -0.673 | -0.332 | 0.231 | -2.915 | -1.125 | -0.220 | 0.004 |
| *Age (years)* | | | | -0.023 | -0.009 | 0.368 | -0.063 | -0.744 | 0.698 | 0.949 |
| *Sex (male %)* | | | | -0.043 | -0.016 | 0.054 | -0.792 | -0.148 | 0.063 | 0.428 |
| **e' sep (cm/s)** | 15 | 8 | 0.300 | | | | | | | |
| *HOMA-IR* | | | | -0.132 | -0.098 | 0.136 | -0.966 | -0.399 | 0.136 | 0.334 |
| *Age (years)* | | | | 0.608 | 0.346 | 0.448 | 1.358 | -0.270 | 1.486 | 0.174 |
| *Sex (male %)* | | | | -0.007 | -0.004 | 0.043 | -0.166 | -0.091 | 0.077 | 0.868 |
| **e' lat (cm/s)** | 23 | 12 | 0.526 | | | | | | | |

| Measure | N | Ns | $r^2$ | b | r | SE | z | LCI | UCI | p |
|---|---|---|---|---|---|---|---|---|---|---|
| *HOMA-IR* | | | | -0.730 | -0.247 | 0.212 | -3.436 | -1.146 | -0.313 | 0.001 |
| *Age (years)* | | | | -0.723 | -0.186 | 0.267 | -2.703 | -1.247 | -0.199 | 0.007 |
| *Sex (male %)* | | | | -0.098 | -0.025 | 0.058 | -1.695 | -0.212 | 0.015 | 0.090 |
| **a' (cm/s)** | 14 | 8 | 0.352 | | | | | | | |
| *HOMA-IR* | | | | 0.295 | 0.247 | 0.055 | 5.341 | 0.187 | 0.404 | 0.000 |
| *Age (years)* | | | | -1.092 | -0.695 | 0.381 | -2.866 | -1.839 | -0.345 | 0.004 |
| *Sex (male %)* | | | | 0.037 | 0.024 | 0.012 | 2.989 | 0.013 | 0.062 | 0.003 |
| **a' sep (cm/s)** | 11 | 6 | 0.663 | | | | | | | |
| *HOMA-IR* | | | | 0.387 | 0.402 | 0.075 | 5.180 | 0.241 | 0.534 | 0.000 |
| *Age (years)* | | | | -0.202 | -0.159 | 0.340 | -0.594 | -0.867 | 0.464 | 0.553 |
| *Sex (male %)* | | | | -0.011 | -0.009 | 0.032 | -0.339 | -0.074 | 0.052 | 0.734 |
| **a' lat (cm/s)** | 13 | 7 | 0.801 | | | | | | | |
| *HOMA-IR* | | | | 0.190 | 0.137 | 0.119 | 1.589 | -0.044 | 0.423 | 0.112 |
| *Age (years)* | | | | -0.708 | -0.390 | 0.121 | -5.843 | -0.945 | -0.471 | 0.000 |
| *Sex (male %)* | | | | -0.081 | -0.045 | 0.064 | -1.268 | -0.206 | 0.044 | 0.205 |
| **E/e' (cm/s)** | 14 | 7 | 0.282 | | | | | | | |
| *HOMA-IR* | | | | 0.509 | 0.600 | 0.109 | 4.678 | 0.296 | 0.723 | 0.000 |
| *Age (years)* | | | | -0.207 | -0.185 | 0.261 | -0.791 | -0.718 | 0.305 | 0.429 |
| *Sex (male %)* | | | | 0.018 | 0.016 | 0.013 | 1.363 | -0.008 | 0.043 | 0.173 |
| **E/e' sep (cm/s)** [a] | | | | | | | | | | |
| *HOMA-IR* | | | | | | | | | | |
| *Age (years)* | | | | | | | | | | |
| *Sex (male %)* | | | | | | | | | | |
| **E/e' lat (cm/s)** | 16 | 9 | 0.283 | | | | | | | |
| *HOMA-IR* | | | | 0.161 | 0.156 | 0.074 | 2.195 | 0.017 | 0.306 | 0.028 |
| *Age (years)* | | | | 0.184 | 0.135 | 0.229 | 0.805 | -0.264 | 0.632 | 0.421 |
| *Sex (male %)* | | | | 0.008 | 0.006 | 0.023 | 0.343 | -0.038 | 0.054 | 0.731 |
| **e'/a'** | 9 | 5 | 0.529 | | | | | | | |
| *HOMA-IR* | | | | -0.056 | -0.174 | 0.024 | -2.314 | -0.103 | -0.009 | 0.021 |
| *Age (years)* | | | | 0.093 | 0.221 | 0.116 | 0.805 | -0.134 | 0.321 | 0.421 |

| Measure | N | Ns | $r^2$ | b | r | SE | z | LCI | UCI | p |
|---|---|---|---|---|---|---|---|---|---|---|
| *Sex (male %)* | | | | 0.016 | 0.038 | 0.031 | 0.526 | -0.044 | 0.076 | 0.599 |
| **e'/a' sep** | 9 | 5 | 0.000 | | | | | | | |
| *HOMA-IR* | | | | -0.098 | -0.412 | 0.033 | -2.953 | -0.163 | -0.033 | 0.003 |
| *Age (years)* | | | | 0.002 | 0.006 | 0.077 | 0.026 | -0.148 | 0.152 | 0.980 |
| *Sex (male %)* | | | | -0.020 | -0.065 | 0.018 | -1.146 | -0.055 | 0.014 | 0.252 |
| **e'/a' lat** | 14 | 7 | 0.147 | | | | | | | |
| *HOMA-IR* | | | | -0.094 | -0.291 | 0.019 | -4.839 | -0.132 | -0.056 | 0.000 |
| *Age (years)* | | | | -0.073 | -0.173 | 0.129 | -0.568 | -0.325 | 0.179 | 0.570 |
| *Sex (male %)* | | | | -0.113 | -0.267 | 0.027 | -4.197 | -0.166 | -0.060 | 0.000 |

A-wave indicates peak late Doppler mitral inflow velocity; a', peak late diastolic tissue velocity; Adj r2, adjusted r2; b, unstandardized regression coefficient; CI, confidence interval (LCI, lower CI; UCI, upper CI); DT, E-wave deceleration time; E-wave, peak early Doppler mitral inflow velocity; e', peak early diastolic tissue velocity; E/A, E-wave/A-wave ratio; E/e', E-wave/e' ratio; e'/a', e'/a' ratio; HOMA-IR, homeostatic model assessment of insulin resistance; IVRT, isovolumic relaxation time; N, number of observations; Ns, number of studies; r, standardized correlation coefficient; SE, standard error.

[a], Insufficient number of studies to be included.

## Study 1: Quality assessment tools

These tools are modified from the Study Quality Assessment Tools by the National Heart, Lung, and Blood Institute (NHLBI) to assess study quality and risk of bias.[312] These tools score papers as *good*, *fair* or *poor* based on questions that focus on important key concepts for critical appraisal without providing a numeric score which have been described as misleading and unhelpful.

### Case Control and Cross-Sectional Studies

| | **Reference** | 1. Was the research question or objective in this paper clearly stated and appropriate? | 2. Was the study population clearly specified and defined? | 3. Did the authors include a sample size justification? | 4. Were controls selected or recruited from the same or similar population that gave rise to the cases (including the same timeframe)? | 5. Were the definitions, inclusion and exclusion criteria, algorithms or processes used to identify or select cases and controls valid, reliable, and implemented consistently across all study participants? | 6. Were the cases clearly defined and differentiated from controls? | 7. If less than 100 percent of eligible cases and/or controls were selected for the study, were the cases and/or controls randomly selected from those eligible? | 8. Was there use of concurrent controls? | 9. Were the investigators able to confirm that the exposure/risk occurred prior to the development of the condition or event that defined a participant as a case? | 10. For exposures that can vary in amount or level (obesity), did the study examine different levels of the exposure as related to the outcome [e.g., categories of obesity, or correlation statistics (r, r2 and/or β)]? | 11. Were the measures of diastolic function defined, valid, reliable, and implemented consistently (including the same time period) across all study participants? | 12. Were the assessors of diastolic function blinded to the case or control status of participants? | 13. Was the statistical analysis clear and appropriate? | 14. Were key potential confounding variables measured and adjusted statistically in the analyses? If matching was used, did the investigators account for matching during study analysis? | 15. Were the results internally consistent? | **Quality Rating (Good, Fair or Poor)** (see guidance) | Raters Initials | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | |

| | | |
|---|---|---|
| | | **Reference** |
| | | 1. Was the study question or objective clearly stated? |
| | | 2. Were eligibilitYes/selection criteria for the study population prespecified and clearly described? |
| | | 3. Were the participants in the study representative of those who would be eligible for the test/service/intervention in the general or clinical population of interest? |
| | | 4. Were all eligible participants that met the prespecified entry criteria enrolled? |
| | | 5. Was the sample size sufficiently large to provide confidence in the findings? |
| | | 6. Was the test/service/intervention clearly described and delivered consistently across the study population? |
| | | 7. Were the outcome measures prespecified, clearly defined, valid, reliable, and assessed consistently across all study participants? |
| | | 8. Were the people assessing the outcomes blinded to the participants' exposures/interventions? |
| | | 9. Was the loss to follow-up after baseline 20% or less? Were those lost to follow-up accounted for in the analysis? |
| | | 10. Did the statistical methods clear and appropriate and examine changes in outcome measures from before to after the intervention? Were statistical tests done that provided p values for the pre-to-post changes? |
| | | 11. Were outcome measures of interest taken multiple times before the intervention and multiple times after the intervention (i.e., did they use an interrupted time-series design)? |
| | | 12. If the intervention was conducted at a group level (e.g., a whole hospital, a community, etc.) did the statistical analysis take into account the use of individual-level data to determine effects at the group level? |
| | | **Quality Rating (Good, Fair or Poor) (see guidance)** |
| | | **Raters Initials** |
| | | **Comments** |

| | | |
|---|---|---|
| | | **Reference** |
| | | 1. Was the study described as randomized, a randomized trial, a randomized clinical trial, or an RCT? |
| | | 2. Was the method of randomization adequate (i.e., use of randomly generated assignment)? |
| | | 3. Was the treatment allocation concealed (so that assignments could not be predicted)? |
| | | 4. Were study participants and providers blinded to treatment group assignment? |
| | | 5. Were the people assessing the outcomes blinded to the participants' group assignments? |
| | | 6. Were the groups similar at baseline on important characteristics that could affect outcomes (e.g., demographics, risk factors, co-morbid conditions)? |
| | | 7. Was the overall drop-out rate from the study at endpoint 20% or lower of the number allocated to treatment? |
| | | 8. Was the differential drop-out rate (between treatment groups) at endpoint 15 percentage points or lower? |
| | | 9. Was there high adherence to the intervention protocols for each treatment group? |
| | | Were other interventions avoided or similar in the groups (e.g., similar background treatments)? |
| | | Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants? |
| | | Did the authors report that the sample size was sufficiently large to be able to detect a difference in the main outcome between groups with at least 80% |
| | | Were outcomes reported or subgroups analysed prespecified (i.e., identified before analyses were conducted)? |
| | | Were all randomized participants analysed in the group to which they were originally assigned, i.e., did they use an intention-to-treat analysis? |
| | | **Quality Rating (Good, Fair or Poor) (see guidance)** |
| | | **Raters Initials** |
| | | **Comments** |

# Appendix 2

## Study 2: Insulin resistance

A discussion on the different methods used to assess IR and IS is provided here, beginning with the gold-standard method, the hyperinsulinaemic-euglycaemic clamp technique.[558]

During the hyperinsulinaemic-euglycaemic clamp technique, insulin is continuously intravenously infused to maintain a high insulin concentration (~100 µU/ml), whilst plasma glucose is also infused to maintain euglycaemia (~90-100 mg/dL).[558, 559] This high level of circulating insulin stimulates the body to store the circulating glucose. Therefore, the rate of glucose infusion is directly related to the cellular uptake of glucose and subsequently reflects exogenous IS.[558] The main limitation of this technique is that it is time consuming, requiring a number of hours to be completed.

Alternatives to the hyperinsulinaemic-euglycaemic clamp technique include dynamic measures of glucose and/or insulin concentrations during a perturbation and steady-state (typically fasting) measures of glucose and/or insulin.[559-562] Dynamic methods use an exogenous bolus of either insulin (insulin tolerance test), glucose (hyperglycaemic clamp technique and oral glucose tolerance tests [OGTT]), or both (the insulin-modified frequently sampled intravenous glucose tolerance test) to understand how either glucose or insulin concentrations change to a known concentration of insulin/glucose bolus, subsequently acting as a marker of IR.[559, 562] Although these tests correlate well with the hyperinsulinaemic-euglycaemic clamp technique,[559, 562] these methods are time-consuming, requiring time for the bolus to take effect. Therefore, steady-state surrogates, which require only a single blood sample, are most commonly used to assess IR, as highlighted by the large number of studies that used HOMA-IR in Study 1.

A feedback loop between the liver and pancreatic β-cells maintains basal glucose and insulin levels.[563] Thus, fasting surrogates reflect hepatic IS and β-cell function. When in a fasting state, measures of fasting-insulin and fasting-glucose are recorded with the understanding that to maintain euglycaemia, higher levels of fasting-insulin indicate IR, whilst lower levels of fasting-insulin indicate IS.[559, 562] There are different methods used to estimate IR in children and adolescents using fasting surrogates, including: the fasting-insulin resistance index; fasting-glucose to fasting-insulin ratio; HOMA-IR; and the quantitative insulin sensitivity check index.[559, 562] These metrics typically account for fasting-glucose levels to adjust for the variable levels of insulin secretion because of differing inter-euglycaemic ranges between individuals. Although these metrics are slightly different in how they are calculated, they are, in essence, the same, as they can be mathematically derived from one-another.[560] Correlations of fasting surrogates with IS determined by the hyperinsulinaemic-euglycaemic clamp technique in children and adolescents have ranged between r=0.25-0.92.[422, 559, 564, 565] Differences in these associations likely reflect the methodological heterogeneity between studies.

The principle limitation of assessing IR is the possible inaccurate measurement of insulin and glucose. As discussed in the review by Brown and Yanovski,[559] there is inter-laboratory variation in insulin and glucose assays, as well as inter-assay variation, and differences in the calibrations used to quantify insulin and glucose. Therefore, the choice and number of assays can influence results,[566, 567] meaning results between laboratories and assays cannot necessarily be compared. Thus, numerical cut-points for the classification of IR are not available for clinical diagnosis and are limited to research only. Another limitation is that haemolysed blood cells can result in lower insulin concentrations in blood samples due to the release of insulin degrading enzymes.[568]

## Study 2: Supporting methods

### Glucose and insulin assays

Glucose was measured on an Abbott Architect c16000 Chemistry analyser (Abbott Laboratories, Abbott Park, Illinois, USA) using an enzymatic (hexokinase) method.

The principle of the hexokinase method, as described by Abbott is that "*glucose is phosphorylated by hexokinase in the presence of adenosine triphosphate and magnesium ions to produce glucose-6-phosphate and adenosine diphosphate. Glucose-6-phosphate dehydrogenase specifically oxidizes glucose-6-phosphate to 6-phosphogluconate with the concurrent reduction of nicotinamide adenine dinucleotide to nicotinamide adenine dinucleotide reduced. One micromole of nicotinamide adenine dinucleotide reduced is produced for each micromole of glucose consumed. The produced nicotinamide adenine dinucleotide reduced absorbs light at 340 nm and can be detected spectrophotometrically as an increased absorbance.*"

Insulin was measured on an Abbott Architect i2000 Immunoassay analyser using the Chemiluminescence microparticle immunoassay. The principle of this method, described by Abbot, is provided in Appendix 2.

The principle of the Chemiluminescence microparticle immunoassay, as described by Abbott is that "*sample, anti-insulin coated paramagnetic microparticles and anti-insulin acridinium-labeled conjugate are combined to create a reaction mixture. The Insulin present in the sample binds to the anti-insulin coated microparticles and anti-insulin acridinium-labeled conjugate. After washing, pre-trigger and trigger solutions are then added to the reaction mixture [and] the resulting chemiluminescent reaction is measured as relative light units. There is a direct relationship between the amount of insulin in the sample and the relative light units detected*."

The results included a haemolysis index, which was used in a sensitivity analysis to ensure that any degradation of insulin did not influence results. Similarly, in a second sensitivity analysis, any samples

that had a haemolysis index of ≥ 0.3 were excluded as recommended by Professor Timothy James, as this was identified to be a level at which constitutes a haemolysis level likely to compromise the results. Results were unchanged in both sensitivity analyses (data not shown).

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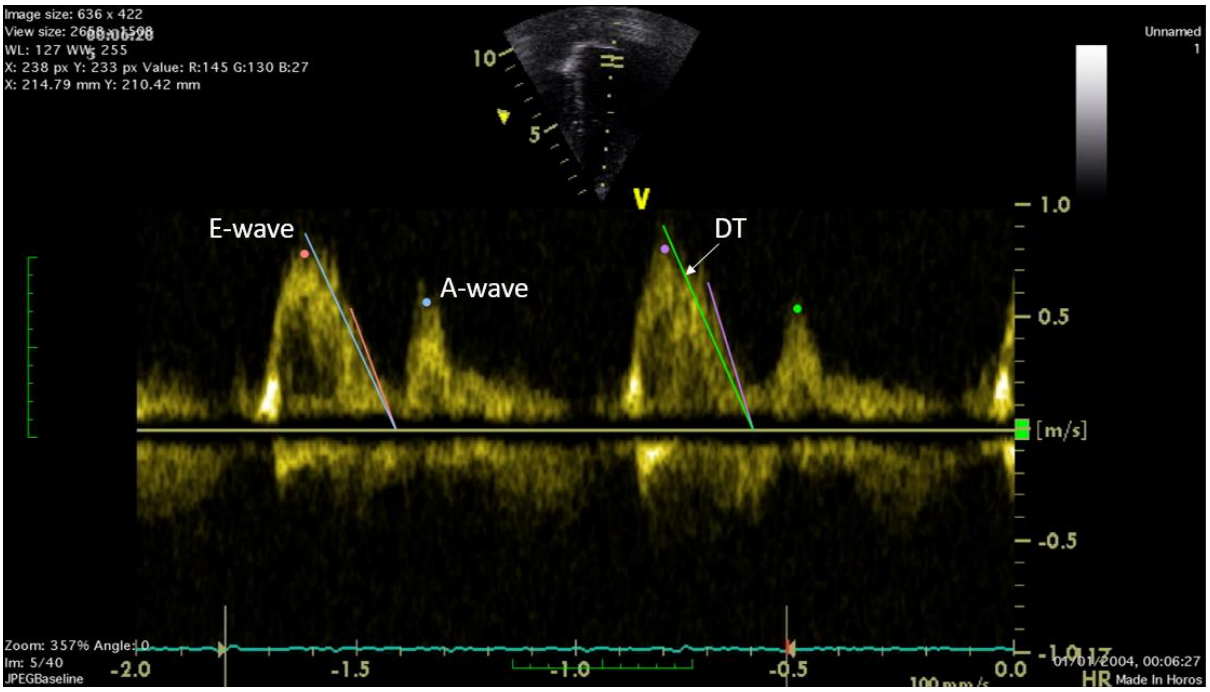
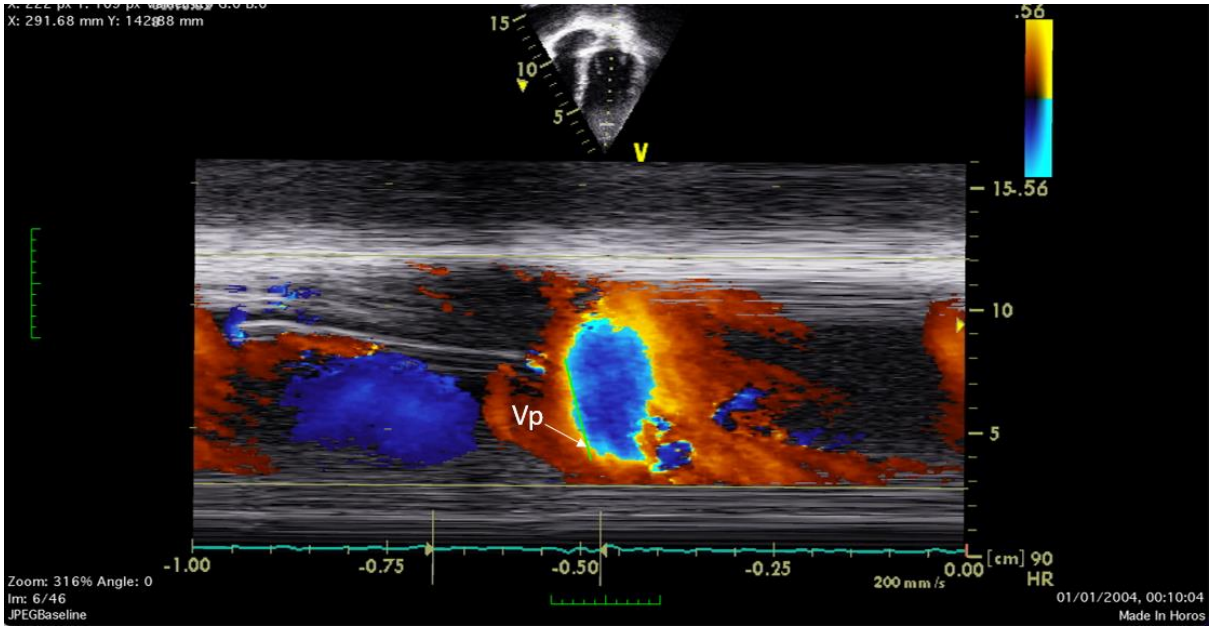
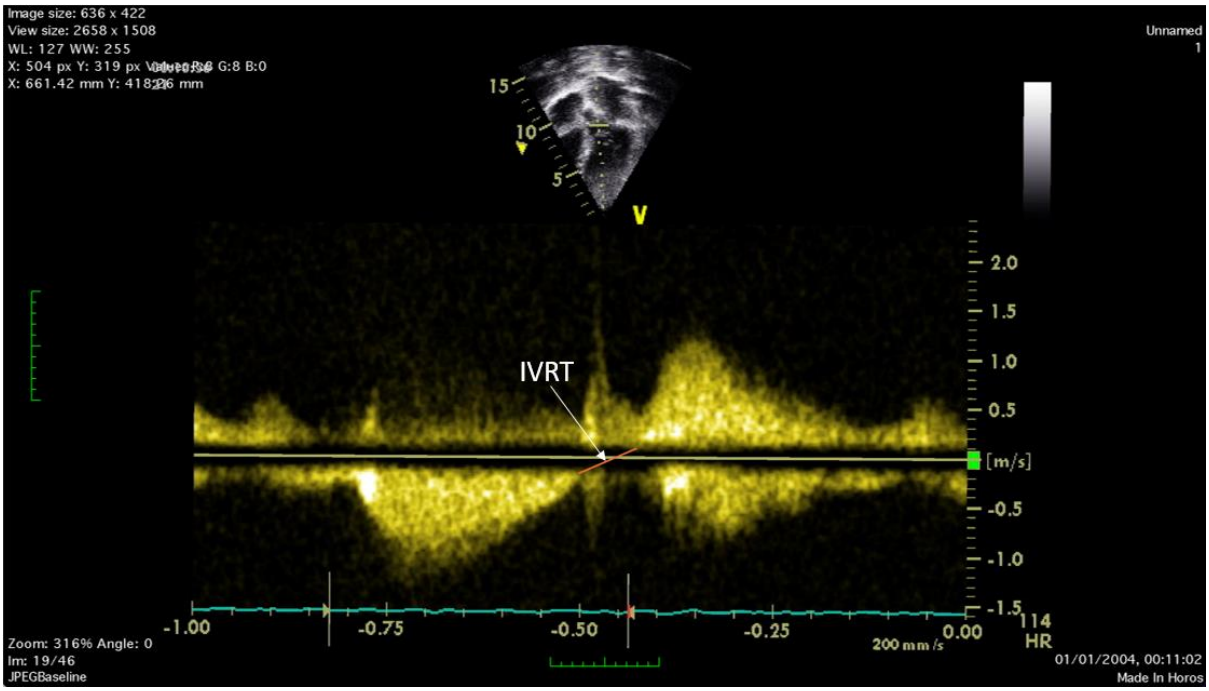


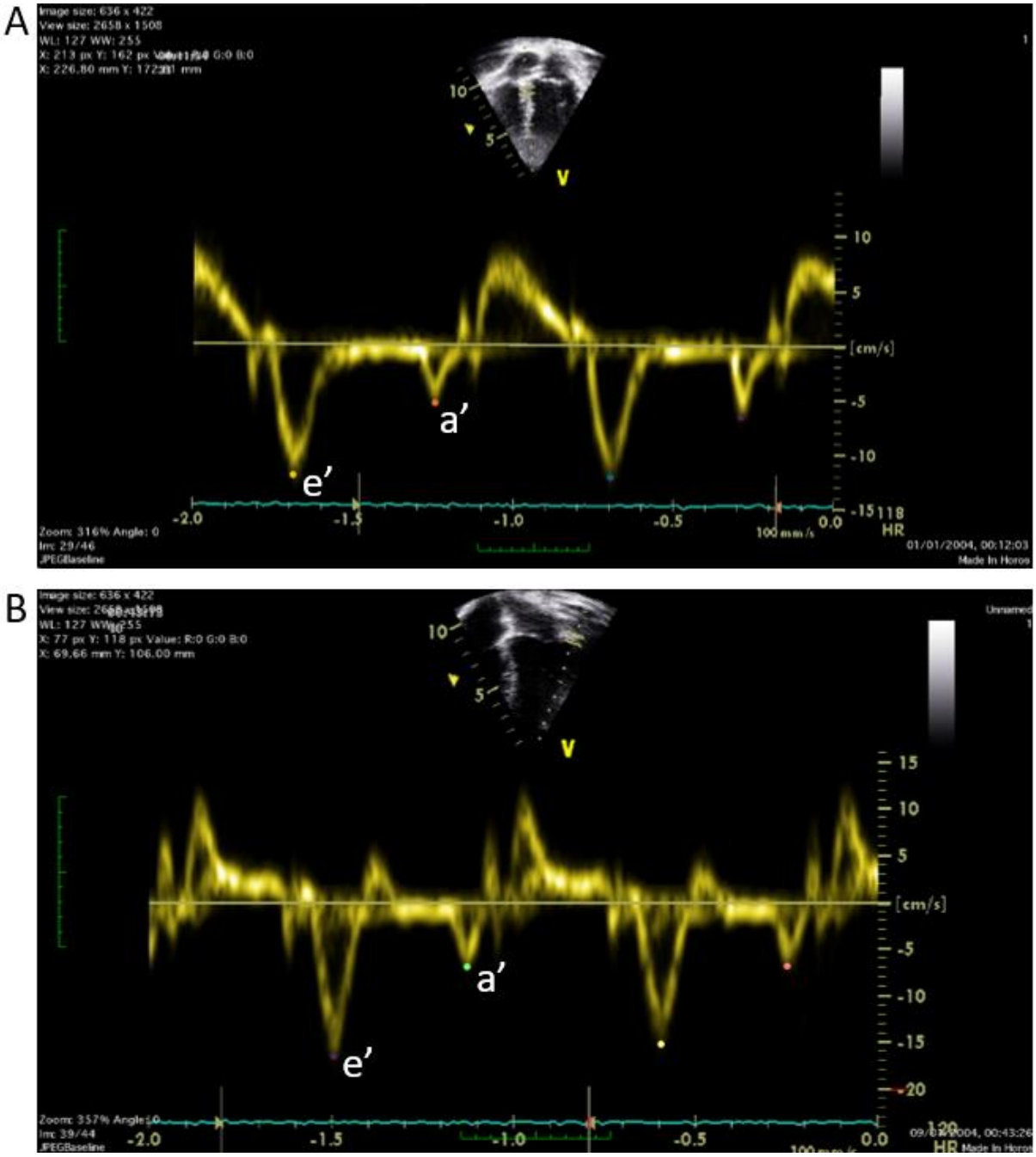

**Appendix 2, Figure 2.1: Mitral inflow analysis.** Early (E-wave) and late (A-wave) peak mitral inflow velocities were recorded. E-wave deceleration time was assessed as the interval between the peak E-wave velocity and the intersection of the deceleration of flow with the baseline. When performing the analyses, dialog boxes appeared with the respective values for each measure (not shown).




**Appendix 2, Figure 2.2: Propagation velocity analysis.** Propagation velocity (Vp) is measured as the slope of the first aliasing velocity during early filling. When performing the analyses, dialogue boxes appear with values for the change in velocity (*y*) and the change in time (*x*) (not shown). Vp can be calculated as the change in *y* divided by the change in *x*.




**Appendix 2, Figure 2.3: Isovolumic relaxation time analysis.** Measured as the time between aortic valve closure (end of aortic ejection) and mitral valve opening (beginning of mitral inflow). When performing the analyses, dialog boxes appeared with the isovolumic relaxation time (IVRT, not shown).




**Appendix 2, Figure 2.4: Myocardial tissue velocity analysis.** Early (e') and late (a') peak myocardial tissue velocities are measured. When performing the analyses, dialog boxes appear with the respective values for each measure (not shown). A, septal myocardial velocities – notice the placement of the sample volume at the basal region of the left ventricular (LV) septum; B, lateral myocardial velocities – notice the placement of the sample volume at the basal region of the LV lateral wall.

## LVDF and interbeat intervals

Heart rate has been shown to alter LVDF measures in adults.[436] As heart rate was not assessed by ECG during the echocardiography assessment, interbeat intervals were determined by measuring the time between the onset of two adjacent E-waves. Where possible, 5-10 measurements were taken and averaged. Two participants had missing heart rate as the echocardiography sweep-speed was set too high to determine successive R-R intervals.

## Study 2: Supporting results

### Tables

**Appendix 2, Table 2.1: Mean Z-score differences in tissue Doppler imaging LVDF measures between normal-weight and overweight/obesity groups**

| Measure | Mean z-score difference [a] |
|---|---|
| **E-wave (cm/s)** | 0.01 (-0.38, 0.40) |
| **A-wave (cm/s)** | 0.28 (-0.12, 0.68) |
| **E/A** | -0.24 (-0.62, 0.14) |
| **DT (ms)** | -0.20 (-0.59, 0.19) |
| **IVRT (ms)** | -0.003 (-0.38, 0.37) |
| **Vp (cm/s)** | 0.15 (-0.48, 0.77) |
| **e' (cm/s)** | |
| *Septal* | **-1.01 (-1.43, -0.60)** |
| *Lateral* | -0.24 (-0.71, 0.23) |
| *Averaged* | **-0.62 (-1.08, -0.16)** |
| **a' (cm/s)** | |
| *Septal* | **0.44 (0.01, 0.88)** |
| *Lateral* | 0.18 (-0.27, 0.63) |
| *Averaged* | 0.34 (-0.08, 0.76) |
| **e'/a'** | |
| *Septal* | **-0.86 (-1.26, -0.47)** |
| *Lateral* | -0.24 (-0.66, 0.19) |
| *Averaged* | **-0.57 (-1.00, -0.15)** |
| **E/e'** | |
| *Septal* | **0.94 (0.52, 1.36)** |
| *Lateral* | 0.15 (-0.32, 0.61) |
| *Averaged* | **0.61 (0.18, 1.03)** |

Data are represented as mean difference (95% confidence intervals [CIs]). Bold values indicate significance (95% CIs do not span zero). A' indicates late diastolic tissue peak velocity; e', early diastolic tissue peak velocity; E/e', E-wave/e' ratio; e'/a', e'/a' ratio. [a], displayed as mean difference of the overweight/obesity group from the normal-weight group.

**Appendix 2, Table 2.2: Associations of unadjusted physical activity intensities and residualised physical activity intensities with LVDF**

| Measure | VPA [a] | | MPA | | LPA [a] | | ST [a] | | $r^2$ |
|---|---|---|---|---|---|---|---|---|---|
| | b (95% CI) | r | b (95% CI) | r | b (95% CI) | r | b (95% CI) | r | |
| **e' sep** | | | | | | | | | |
| *Model 1* | **1.70 (0.71, 2.69)** | **0.35** | **0.01 (0.001, 0.02)** | **0.23** | **3.80 (0.61, 7.00)** | **0.24** | -0.61 (-13.84, 1.62) | -0.16 | - |
| *Model 2* | **1.77 (0.46, 3.07)** | **0.36** | -0.01 (-0.03, 0.01) | -0.20 | 3.80 (-0.91, 8.51) | 0.24 | -1.47 (-9.81, 6.87) | -0.04 | 0.24 |
| *Model 3* | **1.71 (0.72, 2.70)** | **0.35** | -0.001 (-0.01, 0.01) | -0.02 | 3.70 (-1.01, 8.41) | 0.16 | -1.47 (-9.81, 6.87) | -0.03 | 0.24 |
| **e' lat** | | | | | | | | | |
| *Model 1* | **2.53 (0.71, 4.35)** | **0.31** | 0.01 (-0.01, 0.03) | 0.15 | 3.51 (-2.36, 9.38) | 0.13 | -7.18 (-21.21, 6.85) | -0.11 | - |
| *Model 2* | **2.91 (0.52, 5.29)** | **0.35** | -0.01 (-0.05, 0.02) | -0.16 | 3.19 (-5.40, 11.78) | 0.12 | -1.11 (-16.40, 14.17) | -0.02 | 0.11 |
| *Model 3* | **2.56 (0.71, 4.40)** | **0.31** | -0.01 (-0.03, 0.02) | -0.06 | 3.11 (-5.48, 11.70) | 0.08 | -1.12 (-16.40, 14.17) | -0.02 | 0.11 |
| **e' avg** | | | | | | | | | |
| *Model 1* | **2.08 (0.86, 3.30)** | **0.36** | 0.01 (-0.001, 0.02) | 0.20 | 3.51 (-0.48, 7.50) | 0.19 | -6.21 (-15.81, 3.38) | -0.14 | - |
| *Model 2* | **2.32 (0.73, 3.92)** | **0.40** | -0.01 (-0.03, 0.01) | -0.20 | 3.46 (-2.29, 9.20) | 0.18 | -1.14 (-11.37, 9.09) | -0.03 | 0.18 |
| *Model 3* | **2.10 (0.87, 3.34)** | **0.36** | -0.004 (-0.02, 0.01) | -0.05 | 3.38 (-2.37, 9.13) | 0.12 | -1.14 (-11.37, 9.08) | -0.02 | 0.18 |
| **a' sep** | | | | | | | | | |
| *Model 1* | **-0.86 (-1.44, -0.28)** | **-0.31** | -0.002 (-0.01, 0.004) | -0.07 | -0.30 (-2.21, 1.61) | -0.04 | 3.22 (-1.27, 7.71) | 0.15 | - |
| *Model 2* | **-1.18 (-1.95, -0.41)** | **-0.43** | 0.01 (-0.003, 0.02) | 0.28 | -0.48 (-3.25, 2.29) | -0.05 | 1.85 (-3.06, 6.75) | 0.09 | 0.17 |
| *Model 3* | **-0.88 (-1.46, -0.29)** | **-0.32** | 0.006 (-0.002, 0.01) | 0.18 | -0.35 (-3.13, 2.42) | -0.03 | 1.85 (-3.06, 6.75) | 0.08 | 0.17 |
| **a' lat** | | | | | | | | | |
| *Model 1* | -0.17 (-0.85, 0.50) | -0.06 | $4.4x10^{-5}$ (-0.01, 0.01) | 0.001 | 0.17 (-1.93, 2.26) | 0.02 | 1.12 (-3.87, 6.11) | 0.05 | - |
| *Model 2* | -0.24 (-1.12, 0.65) | -0.08 | 0.002 (-0.01, 0.01) | 0.06 | 0.15 (-3.05, 3.34) | 0.02 | 1.05 (-4.61, 6.72) | 0.05 | 0.07 |
| *Model 3* | -0.17 (-.086, 0.51) | -0.06 | 0.002 (-0.01, 0.01) | 0.05 | 0.22 (-2.98, 3.42) | 0.02 | 1.05 (-4.61, 6.72) | 0.04 | 0.07 |
| **a' avg** | | | | | | | | | |
| *Model 1* | **-0.52 (-1.04, -0.01)** | **-0.22** | -0.001 (-0.01, 0.004) | -0.04 | -0.17 (-1.80, 1.46) | -0.02 | 2.38 (-1.47, 6.24) | 0.13 | - |
| *Model 2* | **-0.69 (-1.36, -0.03)** | **-0.29** | 0.005 (-0.004, 0.01) | 0.22 | -0.38 (-2.79, 2.02) | -0.05 | 1.75 (-2.51, 6.02) | 0.10 | 0.15 |
| *Model 3* | **-0.53 (-1.05, -0.01)** | **-0.22** | 0.004 (-0.003, 0.01) | 0.13 | -0.26 (-2.67, 2.14) | -0.02 | 1.75 (2.51, 6.01) | 0.09 | 0.15 |
| **e'/a' sep** | | | | | | | | | |
| *Model 1* | **0.51 (0.27, 0.75)** | **0.44** | 0.002 (-0.0003, 0.005) | 0.20 | 0.74 (-0.06, 1.54) | 0.19 | -1.76 (-3.67, 0.14) | -0.19 | - |
| *Model 2* | **0.62 (0.31, 0.93)** | **0.53** | -0.004 (-0.01, 0.0004) | -0.33 | 0.85 (-0.27, 1.98) | 0.22 | -0.63 (-2.61, 1.35) | -0.07 | 0.26 |
| *Model 3* | **0.52 (0.28, 0.75)** | **0.44** | -0.002 (-0.01, 0.001) | -0.13 | 0.81 (-0.31, 1.93) | 0.14 | -0.63 (-2.61, 1.35) | -0.06 | 0.26 |
| **e'/a' lat** | | | | | | | | | |

| Measure | VPA [a] | | MPA | | LPA [a] | | ST [a] | | $r^2$ |
|---|---|---|---|---|---|---|---|---|---|
| | b (95% CI) | r | b (95% CI) | r | b (95% CI) | r | b (95% CI) | r | |
| *Model 1* | **0.46 (0.06, 0.87)** | **0.26** | 0.002 (-0.002, 0.01) | 0.11 | 0.40 (-0.87, 1.68) | 0.07 | -2.51 (-5.51, 0.49) | -0.18 | - |
| *Model 2* | **0.50 (-0.02, 1.02)** | **0.28** | -0.003 (-0.01, 0.005) | -0.14 | 0.25 (-1.63, 2.13) | 0.04 | -1.73 (-5.06, 1.61) | -0.13 | 0.11 |
| *Model 3* | **0.47 (0.06, 0.87)** | **0.26** | -0.002 (-0.01, 0.004) | -0.07 | 0.13 (-1.75, 2.01) | 0.02 | -1.73 (-5.06, 1.61) | -0.11 | 0.11 |
| **e'/a' avg** | | | | | | | | | |
| *Model 1* | **0.49 (0.21, 0.76)** | **0.37** | 0.002 (-0.001, 0.01) | 0.17 | 0.58 (-0.32, 1.49) | 0.14 | **-2.15 (-4.28, -0.01)** | **-0.22** | - |
| *Model 2* | **0.55 (0.20, 0.91)** | **0.42** | -0.003 (-0.01, 0.002) | -0.26 | 0.61 (-0.68, 1.89) | 0.14 | -1.25 (-3.53, 1.03) | -0.13 | 0.19 |
| *Model 3* | **0.49 (0.21, 0.77)** | **0.38** | -0.001 (-0.01, 0.002) | -0.11 | 0.52 (-0.77, 1.81) | 0.08 | -1.25 (-3.53, 1.03) | -0.11 | 0.19 |
| **E/e' sep** | | | | | | | | | |
| *Model 1* | **-0.84 (-1.48, -0.20)** | **-0.27** | -0.005 (-0.01, 0.002) | -0.16 | -1.52 (-3.56, 0.52) | -0.15 | **5.09 (0.29, 9.88)** | **0.21** | - |
| *Model 2* | -0.83 (-1.67, 0.02) | -0.27 | 0.006 (-0.006, 0.02) | 0.18 | -1.46 (-4.50, 1.58) | -0.15 | 3.49 (-1.89, 8.87) | 0.15 | 0.21 |
| *Model 3* | **-0.84 (-1.49, -0.20)** | **-0.27** | 0.002 (-0.01, 0.01) | 0.04 | -1.22 (-4.26, 1.82) | -0.08 | 3.49 (-1.89, 8.87) | 0.13 | 0.21 |
| **E/e' lat** | | | | | | | | | |
| *Model 1* | **-0.86 (-1.55, -0.17)** | **-0.27** | -0.004 (-0.01, 0.003) | -0.14 | -0.85 (-3.08, 1.37) | -0.08 | 5.08 (-0.14, 10.30) | 0.21 | - |
| *Model 2* | -0.86 (-1.77, 0.04) | -0.27 | 0.004 (-0.01, 0.02) | 0.12 | -0.43 (-3.68, 2.83) | -0.04 | 3.58 (-2.21, 9.37) | 0.15 | 0.12 |
| *Model 3* | **-0.87 (-1.57, -0.17)** | **-0.27** | 0.002 (-0.01, 0.01) | 0.05 | -0.18 (-3.43, 3.07) | -0.01 | 3.58 (-2.21, 9.37) | 0.13 | 0.12 |
| **E/e' avg** | | | | | | | | | |
| *Model 1* | **-0.82 (-1.38, -0.25)** | **-0.30** | -0.004 (-0.01, 0.002) | -0.16 | -1.07 (-2.90, 0.76) | -0.12 | **4.81 (0.54, 9.08)** | **0.23** | - |
| *Model 2* | **-0.83 (-1.57, -0.10)** | **-0.31** | 0.005 (-0.005, 0.02) | 0.18 | -0.92 (-3.55, 1.73) | -0.10 | 3.40 (-1.29, 8.10) | 0.16 | 0.20 |
| *Model 3* | **-0.83 (-1.39, -0.26)** | **-0.31** | 0.002 (-0.01, 0.01) | 0.06 | -0.68 (-3.32, 1.96) | -0.05 | 3.40 (-1.29, 8.10) | 0.15 | 0.20 |

All analyses were adjusted for age, sex, and Tanner score. Model 1, univariate associations of unadjusted physical activity (PA) intensities with LVDF measures; Model 2, fully-adjusted model containing all unadjusted PA intensities; Model 3, Model 2 but with the replacement of all PA intensities with residualised PA (rPA) intensities. a' indicates late diastolic tissue peak velocity; BMI, body mass index; e', early diastolic tissue peak velocity; E/e', E-wave/e' ratio; e'/a', e'/a' ratio; FMI, fat mass index; lat, lateral; LPA, light physical activity; MPA, moderate physical activity; sep, septal; ST, sedentary time; 95% CI, 95% confidence interval. [a], zero-skewed, log adjusted.

**Appendix 2, Table 2.3: Associations of physical activity intensities with tissue Doppler measures of LVDF, adjusted for adiposity**

| Measure | VPA [a] | | MPA | | LPA [a] | | ST [a] | | Adiposity | | $r^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | b (95% CI) | r | b (95% CI) | r | b (95% CI) | r | b (95% CI) | r | b (95% CI) | r | |
| **e' sep** | | | | | | | | | | | |
| *Model 4* | 0.94 (-0.36, 2.24) | 0.19 | -0.01 (-0.03, 0.01) | -0.17 | 3.52 (-0.87, 7.91) | 0.22 | 0.25 (-7.58, 8.07) | 0.01 | **-0.65 (-1.00, -0.29)** | **-0.37** | 0.35 |
| *Model 5* | 0.85 (-0.19, 1.89) | 0.17 | -0.001 (-0.01, 0.01) | -0.01 | 3.54 (-0.86, 7.93) | 0.15 | 0.25 (7.58, 8.07) | 0.01 | **-0.65 (-1.00, -0.29)** | **-0.37** | 0.35 |
| *Model 6* | 0.73 (-0.75, 2.20) | 0.15 | -0.01 (-0.03, 0.01) | -0.17 | 3.41 (-1.68, 8.50) | 0.20 | -2.89 (-12.64, 6.87) | -0.07 | **-1.23 (-1.93, -0.53)** | **-0.40** | 0.36 |
| *Model 7* | 0.77 (-0.37, 1.91) | 0.16 | -0.0002 (-0.01, 0.01) | -0.01 | 3.21 (-2.01, 8.43) | 0.13 | -2.89 (-12.65, 6.87) | 0.13 | **-1.23 (-1.93, -0.53)** | **-0.40** | 0.36 |
| **e' lat** | | | | | | | | | | | |
| *Model 4* | **2.88 (0.33, 5.44)** | **0.35** | -0.01 (-0.05, 0.02) | -0.16 | 3.18 (-5.47, 11.83) | 0.12 | -1.07 (-16.55, 14.41) | -0.02 | -0.02 (-0.71, 0.68) | -0.01 | 0.11 |
| *Model 5* | **2.53 (0.46, 4.60)** | **0.31** | -0.01 (-0.03, 0.02) | -0.06 | 3.12 (-5.54, 11.76) | 0.08 | -1.07 (-16.55, 14.41) | -0.02 | -0.02 (-0.71, 0.68) | -0.01 | 0.11 |
| *Model 6* | 2.77 (-0.13, 5.66) | 0.34 | -0.02 (-0.05, 0.02) | -0.19 | 5.10 (-4.90, 15.09) | 0.17 | -2.23 (-21.58, 17.12) | -0.03 | -0.15 (-1.53, 1.24) | -0.03 | 0.11 |
| *Model 7* | **2.49 (0.23, 4.74)** | **0.30** | -0.005 (-0.03, 0.02) | -0.05 | 4.94 (-5.32, 15.20) | 0.12 | -2.24 (-21.59, 17.11) | -0.03 | -0.15 (-1.53, 1,24) | -0.03 | 0.11 |
| **e' avg** | | | | | | | | | | | |
| *Model 4* | **1.91 (0.22, 3.60)** | **0.33** | -0.01 (-0.03, 0.01) | -0.19 | 3.32 (-2.39, 9.04) | 0.18 | -0.31 (-10.53, 9.92) | -0.01 | -0.33 (-0.79, 0.13) | -0.16 | 0.20 |
| *Model 5* | **1.67 (0.30, 3.04)** | **0.29** | -0.003 (-0.02, 0.01) | -0.05 | 3.30 (-2.41, 9.02) | 0.12 | -0.31 (-10.53, 9.92) | -0.01 | -0.33 (-0.79, 0.13) | -0.16 | 0.20 |
| *Model 6* | 1.75 (-0.17, 3.66) | 0.30 | -0.01 (-0.04, 0.01) | -0.21 | 4.24 (-2.39, 10.87) | 0.21 | -2.44 (-15.27, 10.39) | -0.05 | -0.68 (-1.60, 0.23) | -0.19 | 0.21 |
| *Model 7* | **1.61 (0.12, 3.11)** | **0.28** | -0.003 (-0.02, 0.01) | -0.04 | 4.07 (-2.73, 10.87) | 0.14 | -2.44 (-15.27, 10.39) | -0.04 | -0.68 (-1.60, 0.23) | -0.19 | 0.21 |
| **a' sep** | | | | | | | | | | | |
| *Model 4* | **-1.01 (-1.83, -0.19)** | **-0.37** | 0.01 (-0.003, 0.02) | 0.27 | -0.42 (-3.19, 2.34) | -0.05 | 1.50 (-3.43, 6.43) | 0.07 | 0.13 (-0.09, 0.35) | 0.13 | 0.19 |
| *Model 5* | **-0.70 (-1.36, -0.05)** | **-0.26** | 0.006 (-0.002, 0.01) | 0.18 | -0.32 (-3.09, 2.45) | -0.02 | 1.50 (-3.43, 6.43) | 0.06 | 0.13 (-0.09, 0.35) | 0.13 | 0.19 |
| *Model 6* | **-1.02 (-1.92, -0.12)** | **-0.39** | 0.04 (-0.01, 0.02) | 0.13 | -0.14 (-3.25, 2.96) | -0.02 | 0.81 (-5.15, 6.76) | 0.04 | 0.03 (-0.40, 0.46) | 0.02 | 0.16 |
| *Model 7* | **-0.87 (-1.57, -0.18)** | **-0.34** | 0.003 (-0.01, 0.01) | 0.09 | -0.09 (-3.28, 3.10) | -0.01 | 0.81 (-5.15, 6.77) | 0.03 | 0.03 (-0.04, 0.01) | 0.02 | 0.16 |
| **a' lat** | | | | | | | | | | | |
| *Model 4* | -0.03 (-0.97, 0.91) | -0.01 | 0.001 (-0.01, 0.01) | 0.04 | 0.23 (-2.95, 3.42) | 0.02 | 0.62 (-5.06, 6.30) | 0.03 | 0.17 (-0.09, 0.42) | 0.16 | 0.09 |
| *Model 5* | 0.04 (-0.72, 0.80) | 0.01 | 0.002 (-0.01, 0.01) | 0.04 | 0.28 (-2.91, 3.46) | 0.02 | 0.62 (-5.06, 6.30) | 0.02 | 0.17 (-0.09, 0.42) | 0.16 | 0.09 |
| *Model 6* | 0.13 (-0.89, 1.16) | -0.05 | -0.004 (-0.02, 0.01) | -0.13 | 0.71 (-2.86, 4.28) | 0.07 | -2.06 (-8.93, 4.82) | -0.09 | 0.40 (-0.09, 0.90) | 0.22 | 0.09 |
| *Model 7* | 0.09 (-0.71, 0.89) | 0.03 | -0.002 (-0.01, 0.01) | -0.05 | 0.57 (-3.09, 4.23) | 0.04 | -2.06 (-8.93, 4.82) | -0.07 | 0.40 (-0.09, 0.90) | 0.22 | 0.09 |
| **a' avg** | | | | | | | | | | | |
| *Model 4* | -0.52 (-1.23, 0.18) | -0.22 | 0.005 (-0.005, 0.01) | 0.20 | -0.31 (2.70, 2.08) | -0.04 | 1.39 (-2.88, 5.65) | 0.08 | 0.14 (-0.05, 0.33) | 0.17 | 0.17 |
| *Model 5* | -0.35 (-0.92, 0.22) | -0.15 | 0.004 (-0.003, 0.01) | 0.13 | -0.21 (-2.62, 2.18) | 0.02 | 1.39 (-2.88, 5.65) | 0.07 | 0.14 (-.05, 0.33) | 0.17 | 0.17 |
| *Model 6* | -0.44 (-1.21, 0.32) | -0.20 | 0.0003 (-0.01, 0.01) | 0.01 | 0.06 (-2.61, 2.72) | 0.01 | -0.18 (-5.31, 4.96) | -0.01 | 0.20 (-0.17, 0.57) | 0.14 | 0.14 |
| *Model 7* | -0.41 (-1.01, 0.19) | -0.18 | 0.0005 (-0.01, 0.01) | 0.02 | 0.04 (-2.69, 2.78) | 0.004 | -0.18 (-5.31, 4.96) | -0.01 | 0.20 (-0.17, 0.57) | 0.14 | 0.14 |

| Measure | VPA [a] | | MPA | | LPA [a] | | ST [a] | | Adiposity | | $r^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | b (95% CI) | r | b (95% CI) | r | b (95% CI) | r | b (95% CI) | r | b (95% CI) | r | |
| **e'/a' sep** | | | | | | | | | | | |
| *Model 4* | **0.45 (0.13, 0.76)** | **0.38** | -0.004 (-0.008, 0.001) | -0.31 | 0.80 (-0.27, 1.86) | 0.21 | -0.28 (-2.18, 1.62) | -0.03 | **-0.13 (-0.22, -0.05)** | **-0.32** | 0.34 |
| *Model 5* | **0.34 (0.09, 0.59)** | **0.29** | -0.002 (-0.005, 0.001) | -0.13 | 0.78 (-0.29, 1.85) | 0.14 | -0.28 (-2.18, 1.62) | -0.03 | **-0.13 (-0.22, -0.05)** | **-0.32** | 0.34 |
| *Model 6* | **0.40 (0.05, 0.75)** | **0.36** | -0.002 (-0.01, 0.002) | -0.19 | 0.63 (-0.57, 1.84) | 0.16 | -0.49 (-2.81, 1.82) | -0.05 | **-0.19 (-0.35, -0.02)** | **-0.26** | 0.31 |
| *Model 7* | **0.37 (0.10, 0.64)** | **0.33** | -0.001 (-0.63, 1.84) | -0.05 | 0.60 (-0.64, 1.84) | 0.10 | -0.49 (-2.81, 1.82) | -0.05 | **-0.19 (-0.35, -0.02)** | **-0.26** | 0.31 |
| **e'/a' lat** | | | | | | | | | | | |
| *Model 4* | 0.43 (-0.12, 0.99) | 0.24 | -0.002 (-0.01, 0.01) | -0.13 | 0.22 (-1.67, 2.11) | 0.04 | -1.59 (-4.96, 1.78) | -0.11 | -0.05 (-0.21, 0.10) | -0.08 | 0.11 |
| *Model 5* | 0.40 (-0.05, 0.85) | 0.22 | -0.001 (-0.01, 0.004) | -0.07 | 0.11 (-1.78, 2.00) | 0.01 | -1.59 (-4.96, 1.78) | -0.10 | -0.05 (-0.21, 0.10) | -0.08 | 0.11 |
| *Model 6* | 0.38 (-0.23, 0.99) | 0.21 | -0.001 (-0.01, 0.01) | -0.05 | 0.49 (-1.64, 2.62) | 0.08 | -0.16 (-4.26, 3.94) | -0.01 | -0.14 (-0.43, 0.16) | -0.12 | 0.11 |
| *Model 7* | 0.40 (-0.09, 0.87) | 0.23 | 0.0003 (-0.01, 0.01) | 0.01 | 0.48 (-1.71, 2.66) | 0.05 | -0.16 (-4.27, 3.94) | -0.01 | -0.14 (-0.43, 0.16) | -0.12 | 0.11 |
| **e'/a' avg** | | | | | | | | | | | |
| *Model 4* | **0.44 (0.07, 0.81)** | **0.34** | -0.003 (-0.01, 0.002) | -0.24 | 0.56 (-0.72, 1.83) | 0.13 | -1.01 (-3.28, 1.26) | -0.10 | -0.09 (-0.19, 0.01) | -0.20 | 0.22 |
| *Model 5* | **0.37 (0.07, 0.68)** | **0.29** | -0.002 (-0.01, 0.001) | -0.10 | 0.49 (-0.78, 1.76) | 0.08 | -1.01 (-3.28, 1.26) | -0.09 | -0.09 (-0.19, 0.01) | -0.20 | 0.22 |
| *Model 6* | 0.39 (-0.03, 0.81) | 0.31 | -0.003 (-0.01, 0.004) | -0.12 | 0.61 (-0.84, 2.06) | 0.14 | -0.44 (-3.23, 2.36) | -0.04 | -0.16 (-0.36, 0.04) | -0.19 | 0.22 |
| *Model 7* | **0.39 (0.06, 0.72)** | **0.31** | -0.0002 (-0.004, 0.004) | -0.01 | 0.58 (-0.91, 2.07) | 0.09 | -0.44, (-3.23, 2.36) | -0.04 | -0.16 (-0.36, 0.04) | -0.19 | 0.22 |
| **E/e' sep** | | | | | | | | | | | |
| *Model 4* | -0.37 (-1.23, 0.49) | -0.12 | 0.005 (-0.01, 0.02) | 0.06 | -1.30 (-4.20, 1.59) | -0.13 | 2.54 (-2.61, 7.69) | 0.11 | **0.36 (0.13, 0.59)** | **0.33** | 0.30 |
| *Model 5* | -0.37 (-1.05, 0.32) | -0.12 | 0.001 (-0.01, 0.01) | 0.04 | -1.13 (-4.02, 1.77) | -0.08 | 2.54 (-2.62, 7.69) | 0.09 | **0.36 (0.13, 0.59)** | **0.33** | 0.30 |
| *Model 6* | -0.21 (-1.20, 0.79) | -0.07 | 0.003 (-0.01, 0.02) | 0.10 | -0.68 (-4.12, 2.76) | -0.06 | 5.04 (-1.56, 11.64) | 0.20 | **0.62 (0.14, 1.09)** | **0.31** | 0.28 |
| *Model 7* | -0.35 (-1.12, 0.42) | -0.11 | 0.001 (-0.01, 0.01) | 0.01 | -0.33 (-3.87, 3.20) | -0.02 | 5.04 (-1.56, 11.64) | 0.17 | **0.62 (0.14, 1.09)** | **0.31** | 0.28 |
| **E/e' lat** | | | | | | | | | | | |
| *Model 4* | **-0.98 (-1.95, -0.02)** | **-0.31** | 0.004 (-0.01, 0.02) | 0.13 | -0.47 (-3.73, 2.80) | -0.05 | 3.83 (-2.02, 9.67) | 0.16 | -0.10 (-0.36, 0.17) | -0.09 | 0.13 |
| *Model 5* | **-0.99 (-1.77, -0.21)** | **-0.31** | 0.002 (-0.01, 0.01) | 0.05 | -0.20 (-3.47, 3.06) | -0.01 | 3.83 (-2.02, 9.67) | 0.14 | -0.10 (-0.36, 0.16) | -0.09 | 0.13 |
| *Model 6* | -0.89 (-1.98, 0.20) | -0.28 | 0.004 (-0.01, 0.02) | 0.12 | -0.67 (-4.44, 3.09) | -0.06 | 4.72 (-2.56, 12.01) | 0.18 | -0.17 (-0.69, 0.35) | -0.09 | 0.13 |
| *Model 7* | **-0.99 (-1.84, -0.14)** | **-0.32** | 0.001 (-0.01, 0.01) | 0.03 | -0.35 (-4.21, 3.51) | -0.02 | 4.72 (-2.56, 12.01) | 0.16 | -0.17 (-0.69, 0.35) | -0.09 | 0.13 |
| **E/e' avg** | | | | | | | | | | | |
| *Model 4* | -0.67 (-1.45, 0.11) | -0.25 | 0.005 (-0.006, 0.02) | 0.17 | -0.86 (-3.49, 1.77) | -0.10 | 3.08 (-1.63, 7.79) | 0.15 | 0.13 (-0.04, 0.005) | 0.14 | 0.22 |
| *Model 5* | **-0.66 (-1.29, -0.03)** | **-0.25** | 0.002 (-0.01, 0.01) | 0.06 | -0.65 (-3.28, 1.99) | -0.05 | 3.08 (-1.63, 7.79) | 0.13 | 0.13 (-0.04, 0.005) | 0.14 | 0.22 |
| *Model 6* | -0.55 (-1.44, 0.34) | -0.20 | 0.004 (-0.01, 0.02) | 0.13 | -0.66 (-3.75, 2.43) | -0.07 | 4.74 (-1.24, 10.71) | 0.21 | 0.22 (-0.21, 0.65) | 0.13 | 0.22 |
| *Model 7* | -0.66 (-1.35, 0.04) | -0.24 | 0.001 (-0.01, 0.01) | 0.03 | -0.33 (-3.50, 2.83) | -0.02 | 4.74 (-1.24, 10.71) | 0.18 | 0.22 (-0.21, 0.65) | 0.13 | 0.22 |

All analyses were adjusted for age, sex, and Tanner score. Model 4, multiple regression with all unadjusted physical activity (PA) intensities, body mass index (BMI) z-score and LVDF measures; Model 5, Model 4 but with the replacement of unadjusted PA intensities with residualised PA (rPA) intensities; Model 6, Model 4 but with the replacement of BMI z-score with fat-mass index (FMI); Model 7, Model 5 but with the replacement of BMI z-score with FMI. FMI was zero-skewed, log transformed. a' indicates late diastolic tissue peak velocity; e', early diastolic tissue peak velocity; E/e', E-wave/e' ratio; e'/a', e'/a' ratio; lat, lateral; LPA, light physical activity; MPA, moderate physical activity; sep, septal; ST, sedentary time; 95% CI, 95% confidence interval. [a], zero-skewed, log adjusted.

**Appendix 2, Table 2.4: Association of septal early-to-late ratios with physical activity intensities, adjusted for adiposity**

| PA method | VPA | | MPA | | LPA | | ST | | Adiposity | | $r^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | b (95% CI) | r | b (95% CI) | r | b (95% CI) | r | b (95% CI) | r | b (95% CI) | r | |
| **ENMOz** [a] | | | | | | | | | | | |
| *Model 4* | **0.32 (0.04, 0.59)** | **0.36** | -0.35 (-1.22, 0.53) | -0.17 | 0.002 (-0.01, 0.01) | 0.08 | -0.02 (-0.31, 0.27) | -0.02 | **-0.14 (-0.23, -0.05)** | **-0.34** | 0.32 |
| *Model 5* | 0.19 (-0.11, 0.50) | 0.24 | -0.01 (-0.92, 0.91) | -0.003 | 0.002 (-0.01, 0.01) | 0.07 | -0.07 (-0.41, 0.28) | -0.04 | **-0.23 (-0.40, -0.06)** | **-0.32** | 0.30 |
| *Model 6* | **0.24 (0.05, 0.43)** | **0.28** | -0.17 (-0.75, 0.40) | -0.06 | 0.002 (-0.01, 0.01) | 0.05 | -0.02 (-0.31, 0.27) | -0.01 | **-0.14 (-0.23, -0.05)** | **-0.34** | 0.32 |
| *Model 7* | **0.23 (0.02, 0.43)** | **0.28** | 0.14 (-0.48, 0.75) | 0.05 | 0.001 (-0.01, 0.01) | 0.03 | -0.07 (-0.41, 0.28) | -0.04 | **-0.23 (-0.40, -0.06)** | **-0.32** | 0.30 |
| **HFEN** [b] | | | | | | | | | | | |
| *Model 4* | **0.46 (0.12, 0.80)** | **0.37** | -0.004 (-0.01, 0.001) | -0.30 | 0.77 (-0.36, 1.90) | 0.19 | -0.0001 (-0.001, 0.001) | -0.02 | **-0.14 (-0.23, -0.06)** | **-0.34** | 0.34 |
| *Model 5* | **0.41 (0.03, 0.79)** | **0.35** | -0.002 (-0.01, 0.002) | -0.18 | 0.58 (-0.71, 1.88) | 0.13 | -0.0003 (-0.002, 0.001) | -0.05 | **-0.21 (-0.28, -0.05)** | **-0.30** | 0.32 |
| *Model 6* | **0.33 (0.07, 0.60)** | **0.27** | -0.002 (-0.01, 0.001) | -0.14 | 0.76 (-0.38, 1.89) | 0.13 | -0.0001 (-0.001, 0.001) | -0.02 | **-0.14 (-0.23, -0.06)** | **-0.34** | 0.34 |
| *Model 7* | **0.37 (0.08, 0.65)** | **0.31** | -0.001 (-0.004, 0.002) | -0.06 | 0.55 (-0.79, 1.88) | 0.09 | -0.0003 (-0.002, 0.001) | -0.04 | **-0.21 (-0.38, -0.05)** | **-0.30** | 0.32 |
| **HFEN+** [c] | | | | | | | | | | | |
| *Model 4* | **0.36 (0.09, 0.62)** | **0.36** | -0.004 (-0.01, 0.001) | -0.24 | 0.53 (-0.33, 1.39) | 0.17 | -0.0002 (-0.002, 0.001) | -0.03 | **-0.15 (-0.23, -0.06)** | **-0.35** | 0.34 |
| *Model 5* | **0.31 (0.02, 0.60)** | **0.33** | -0.002 (-0.01, 0.80) | -0.14 | 0.41 (-0.56, 1.39) | 0.12 | -0.0005 (-0.002, 0.001) | -0.07 | **-0.22 (-0.38, -0.05)** | **-0.31** | 0.32 |
| *Model 6* | **0.28 (0.06, 0.49)** | **0.28** | -0.002 (-0.01, 0.002) | -0.09 | 0.52 (-0.34, 1.38) | 0.11 | -0.0002 (-0.002, 0.001) | -0.03 | **-0.15 (-0.23, -0.06)** | **-0.35** | 0.34 |
| *Model 7* | **0.29 (0.06, 0.52)** | **0.31** | -0.002 (-0.004, 0.004) | -0.01 | 0.39 (-0.61, 1.38) | 0.08 | -0.0005 (-0.002, 0.001) | -0.06 | **-0.22 (-0.38, -0.05)** | **-0.31** | 0.32 |
| **GT3Y5** [d] | | | | | | | | | | | |
| *Model 4* | **0.34 (0.11, 0.58)** | **0.40** | -38.30 (-85.49, 8.89) | -0.28 | 0.69 (-0.41, 1.79) | 0.17 | 0.00003 (-0.001, 0.001) | 0.005 | **-0.14 (-0.23, -0.06)** | **-0.34** | 0.34 |
| *Model 5* | **0.27 (0.01, 0.53)** | **0.33** | -16.83 (-69.56, 35.90) | -0.12 | 0.30 (-0.89, 1.50) | 0.07 | -0.0003 (-0.002, 0.001) | -0.05 | **-0.22 (-0.39, -0.06)** | **-0.32** | 0.31 |
| *Model 6* | **0.23 (0.05, 0.41)** | **0.27** | -20.21 (-54.01, 13.58) | -0.12 | 0.69 (-0.41, 1.80) | 0.12 | 0.00003 (-0.001, 0.001) | 0.004 | **-0.14 (-0.23, -0.06)** | **-0.34** | 0.34 |
| *Model 7* | **0.24 (0.05, 0.42)** | **0.29** | -7.00 (-44.59, 30.58) | -0.04 | 0.28 (-0.94, 1.50) | 0.05 | -0.0003 (-0.002, 0.001) | -0.04 | **-0.22 (-0.39, -0.06)** | **-0.32** | 0.31 |
| **GT3VM5** [e] | | | | | | | | | | | |
| *Model 4* | **0.38 (0.11, 0.65)** | **0.37** | -14.37 (-30.39, 1.66) | -0.28 | 0.79 (-0.26, 1.84) | 0.18 | -0.0002 (-0.002, 0.001) | -0.03 | **-0.14 (-0.22, -0.05)** | **-0.33** | 0.34 |
| *Model 5* | **0.32 (0.03, 0.61)** | **0.32** | -8.32 (-25.72, 9.08) | -0.16 | 0.51 (-0.68, 1.69) | 0.11 | -0.0004 (-0.002, 0.001) | -0.07 | **-0.22 (-0.39, -0.06)** | **-0.31** | 0.32 |
| *Model 6* | **0.28 (0.06, 0.49)** | **0.27** | -8.75 (-21.38, 3.89) | -0.14 | 0.77 (-0.28, 1.83) | 0.14 | -0.0002 (-0.002, 0.001) | -0.02 | **-0.14 (-0.22, -0.05)** | **-0.33** | 0.34 |
| *Model 7* | **0.28 (0.06, 0.51)** | **0.29** | -3.80 (-17, 53, 9.93) | -0.06 | 0.47 (-0.75, 1.68) | 0.08 | -0.0004 (-0.002, 0.001) | -0.06 | **-0.22 (-0.39, -0.06)** | **-0.31** | 0.32 |

Model 4, multiple regression with all unadjusted physical activity (PA) intensities, body mass index (BMI) z-score and LVDF measures; Model 5, Model 4 but with the replacement of unadjusted PA intensities with residualised PA (rPA) intensities; Model 6, Model 4 but with the replacement of BMI z-score with fat-mass index (FMI); Model 7, Model 5 but with the replacement of BMI z-score with FMI. CI indicates confidence interval; ENMOz, Euclidean norm minus one with negative values rounded to zero;

GT3VM5, ActiGraph counts calculated using the vector magnitude of the three raw axes; GT3Y5, ActiGraph counts calculated using the vertical axis; HFEN, high-pass filtered Euclidean norm; HFEN+, HFEN plus low-pass filtered Euclidean norm; LPA, light physical activity (PA); MPA, moderate PA; ST, sedentary time; VPA, vigorous PA.

[a], ENMOz VPA, MPA, and ST were zero-skewed, log transformed; [b], HFEN VPA and LPA were zero-skewed, log transformed; [c], HFEN+ VPA and LPA were zero-skewed, log transformed; [d], GT3Y5 VPA, MPA and LPA were zero-skewed, log transformed; [e], VPA, MPA and LPA were zero-skewed, log transformed.

**Appendix 2, Table 2.5: Associations of cardiorespiratory fitness with LVDF**

| Measure | $VO_2$ $peak_{kg}$ b (95% CI) | r | $VO_2$ $peak_{FFMkg}$ b (95% CI) | r |
|---|---|---|---|---|
| **E-wave (cm/s)** | 0.01 (-0.27, 0.30) | 0.01 | -0.04 (-0.36, 0.28) | -0.03 |
| **A-wave (cm/s)** | **-0.34 (-0.63, -0.06)** | **-0.27** | **-0.43 (-0.76, -0.11)** | **-0.30** |
| **E/A** | **0.01 (0.001, 0.01)** | **0.24** | **0.01 (0.001, 0.02)** | **0.24** |
| **DT (ms)** | 0.55 (-0.11, 1.20) | 0.19 | **0.80 (0.06, 1.55)** | **0.24** |
| **IVRT (ms)** | 0.03 (-0.18, 0.25) | 0.04 | 0.04 (-0.21, 0.28) | 0.03 |
| **VP (cm/s)** | 0.08 (-0.43, 0.59) | 0.04 | 0.16 (-0.42, 0.75) | 0.06 |
| **e' (cm/s)** | | | | |
| *Septal* | **0.09 (0.05, 0.13)** | **0.45** | **0.09 (0.04, 0.13)** | **0.38** |
| *Lateral* | **0.10 (0.02, 0.17)** | **0.30** | **0.12 (0.03, 0.20)** | **0.31** |
| *Averaged* | **0.09 (0.05, 0.14)** | **0.41** | **0.10 (0.05, 0.16)** | **0.39** |
| **a' (cm/s)** | | | | |
| *Septal* | **-0.04 (-0.06, -0.02)** | **-0.35** | **-0.05 (-0.08, -0.02)** | **-0.39** |
| *Lateral* | -0.01 (-0.04, 0.02) | -0.07 | -0.003 (-0.03, 0.03) | -0.02 |
| *Averaged* | **-0.02 (-0.04, -0.002)** | **-0.24** | -0.02 (-0.05, 0.0003) | -0.22 |
| **e'/a'** | | | | |
| *Septal* | **0.02 (0.01, 0.03)** | **0.49** | **0.03 (0.01, 0.04)** | **0.47** |
| *Lateral* | **0.02 (0.001, 0.03)** | **0.24** | 0.02 (-0.002, 0.03) | 0.21 |
| *Averaged* | **0.02 (0.01, 0.03)** | **0.38** | **0.02 (0.01, 0.03)** | **0.35** |
| **E/e'** | | | | |
| *Septal* | **-0.05 (-0.08, -0.03)** | **-0.40** | **-0.06 (-0.09, -0.03)** | **-0.38** |
| *Lateral* | **-0.03 (-0.06, -0.004)** | **-0.25** | **-0.04 (-0.08, -0.01)** | **-0.30** |
| *Averaged* | **-0.04 (-0.06, -0.02)** | **-0.38** | **-0.05 (-0.08, -0.03)** | **-0.40** |

All analyses were adjusted for age, sex, and Tanner score. a' indicates late diastolic tissue peak velocity; e', early diastolic tissue peak velocity; E/e', E-wave/e' ratio; e'/a', e'/a' ratio; FFM, fat-free mass; $VO_2$ $peak_{kg}$, peak oxygen consumption adjusted for body mass; $VO_2$ $peak_{FFMkg}$, peak oxygen consumption adjusted for fat-free mass; 95% CI, 95% confidence interval.

**Appendix 2, Table 2.6: Associations of cardiorespiratory fitness with tissue Doppler measures of LVDF, adjusted for adiposity**

| Measure | CRF | | Adiposity | | $r^2$ |
|---|---|---|---|---|---|
| | b (95% CI) | r | b (95% CI) | r | |
| **e' sep** | | | | | |
| *Model 8* | 0.04 (-0.03, 0.10) | 0.19 | **-0.55 (-1.10, -0.005)** | **-0.32** | 0.32 |
| *Model 9* | 0.04 (-0.03, 0.11) | 0.21 | -0.84 (-1.91, 0.23) | -0.28 | 0.30 |
| *Model 10* | 0.03 (-0.02, 0.09) | 0.14 | **-0.66 (-1.06, -0.25)** | **-0.38** | 0.32 |
| *Model 11* | 0.04 (-0.02, 0.09) | 0.15 | **-1.08 (-1.81, -0.35)** | **-0.37** | 0.30 |
| **e' lat** | | | | | |
| *Model 8* | **0.14 (0.01, 0.26)** | **0.42** | 0.41 (-0.66, 1.49) | 0.14 | 0.10 |
| *Model 9* | **0.15 (0.01, 0.29)** | **0.46** | 0.93 (-1.15, 3.01) | 0.19 | 0.10 |
| *Model 10* | **0.12 (0.01, 0.22)** | **0.31** | 0.01 (-0.79, 0.80) | 0.003 | 0.10 |
| *Model 11* | **0.12 (0.01, 0.22)** | **0.31** | -0.03 (-1.44, 1.39) | -0.01 | 0.10 |
| **e' avg** | | | | | |
| *Model 8* | **0.09 (0.01, 0.17)** | **0.39** | -0.04 (-0.75, 0.67) | -0.02 | 0.20 |
| *Model 9* | **0.10 (0.01, 0.19)** | **0.43** | 0.10 (-1.27, 1.48) | 0.03 | 0.20 |
| *Model 10* | 0.08 (-0.83, 0.22) | 0.29 | -0.31 (-0.83, 0.22) | -0.15 | 0.20 |
| *Model 11* | **0.08 (0.01, 0.15)** | **0.29** | -0.52 (-1.45, 0.41) | -0.15 | 0.20 |
| **a' sep** | | | | | |
| *Model 8* | -0.04 (-0.08, 0.005) | -0.32 | 0.04 (-0.31, 0.38) | 0.04 | 0.18 |
| *Model 9* | **-0.06 (-0.10, -0.01)** | **-0.51** | -0.33 (-0.99, 0.33) | -0.20 | 0.19 |
| *Model 10* | **-0.04 (-0.08, -0.01)** | **-0.34** | 0.08 (-0.17, 0.33) | 0.08 | 0.21 |
| *Model 11* | **-0.05 (-0.09, -0.02)** | **-0.40** | -0.02 (-0.47, 0.42) | -0.01 | 0.21 |
| **a' lat** | | | | | |
| *Model 8* | 0.02 (-0.03, 0.06) | 0.14 | 0.26 (-0.12, 0.65) | 0.26 | 0.06 |
| *Model 9* | 0.03 (-0.02, 0.08) | 0.28 | 0.72 (-0.02, 1.45) | 0.41 | 0.09 |
| *Model 10* | 0.02 (-0.01, 0.06) | 0.18 | 0.26 (-0.02, 0.54) | 0.26 | 0.08 |
| *Model 11* | 0.03 (-0.01, 0.07) | 0.22 | **0.55 (0.05, 1.04)** | **0.31** | 0.09 |
| **a' avg** | | | | | |
| *Model 8* | -0.01 (-0.04, 0.03) | -0.08 | 0.16 (-0.13, 0.45) | 0.20 | 0.14 |
| *Model 9* | -0.01 (-0.05, 0.03) | -0.12 | 0.19 (-0.37, 0.76) | 0.14 | 0.13 |
| *Model 10* | -0.01 (-0.04, 0.02) | -0.09 | 0.17 (-0.05, 0.38) | 0.21 | 0.14 |
| *Model 11* | -0.01 (-0.04, 0.02) | -0.10 | 0.25 (-0.14, 0.63) | 0.18 | 0.13 |
| **e'/a' sep** | | | | | |
| *Model 8* | 0.01 (-0.002, 0.03) | 0.28 | -0.10 (-0.23, 0.02) | -0.26 | 0.32 |
| *Model 9* | **0.02 (0.002, 0.04)** | **0.42** | -0.06 (-0.31, 0.19) | -0.09 | 0.29 |
| *Model 10* | **0.01 (0.002, 0.03)** | **0.27** | **-0.13 (-0.22, -0.03)** | **-0.31** | 0.33 |
| *Model 11* | **0.02 (0.004, 0.03)** | **0.32** | -0.17 (-0.34, 0.004) | -0.24 | 0.31 |
| **e'/a' lat** | | | | | |
| *Model 8* | 0.02 (-0.01, 0.04) | 0.24 | -0.0001 (-0.23, 0.23) | -0.0002 | 0.07 |
| *Model 9* | 0.01 (-0.02, 0.04) | 0.18 | -0.08 (-0.53, 0.38) | -0.07 | 0.07 |
| *Model 10* | 0.01 (-0.01, 0.03) | 0.14 | -0.07 (-0.24, 0.11) | -0.11 | 0.06 |
| *Model 11* | 0.01 (-0.01, 0.03) | 0.11 | -0.17 (-0.48, 0.14) | -0.16 | 0.07 |
| **e'/a' avg** | | | | | |
| *Model 8* | 0.01 (-0.004, 0.03) | 0.28 | -0.05 (-0.21, 0.11) | -0.12 | 0.18 |
| *Model 9* | 0.02 (-0.005, 0.04) | 0.31 | -0.07 (-0.38, 0.24) | -0.08 | 0.18 |
| *Model 10* | 0.01 (-0.003, 0.03) | 0.21 | -0.10 (-0.22, 0.02) | -0.22 | 0.18 |
| *Model 11* | 0.01 (-0.003, 0.03) | 0.22 | -0.16 (-0.38, 0.05) | -0.21 | 0.18 |
| **E/e' sep** | | | | | |
| *Model 8* | -0.02 (-0.07, 0.02) | -0.18 | 0.30 (-0.06, 0.66) | 0.27 | 0.28 |

| Measure | CRF | | Adiposity | | $r^2$ |
|---|---|---|---|---|---|
| | b (95% CI) | r | b (95% CI) | r | |
| *Model 9* | -0.04 (-0.09, 0.01) | -0.34 | 0.15 (-0.56, 0.86) | 0.08 | 0.26 |
| *Model 10* | -0.03 (-0.06, 0.01) | -0.20 | **0.33 (0.06, 0.59)** | **0.29** | 0.30 |
| *Model 11* | -0.04 (-0.07, 0.0003) | -0.25 | 0.39 (-0.09, 0.87) | 0.21 | 0.27 |
| **E/e' lat** | | | | | |
| *Model 8* | **-0.05 (-0.10, -0.01)** | **-0.42** | -0.23 (-0.64, 0.18) | -0.20 | 0.11 |
| *Model 9* | **-0.07 (-0.12, -0.02)** | **-0.56** | -0.69 (-1.48, 0.09) | -0.37 | 0.14 |
| *Model 10* | **-0.05 (-0.09, -0.01)** | **-0.36** | -0.10 (-0.40, 0.20) | -0.09 | 0.13 |
| *Model 11* | **-0.06 (-0.10, -0.02)** | **-0.39** | -0.26 (-0.79, 0.27) | -0.14 | 0.14 |
| **E/e' avg** | | | | | |
| *Model 8* | **-0.04 (-0.08, -0.001)** | **-0.37** | 0.02 (-0.31, 0.35) | 0.02 | 0.23 |
| *Model 9* | **-0.06 (-0.10, -0.02)** | **-0.55** | -0.32 (-0.96, 0.31) | -0.20 | 0.24 |
| *Model 10* | **-0.04 (-0.07, -0.01)** | **-0.34** | 0.10 (-0.14, 0.34) | 0.10 | 0.25 |
| *Model 11* | **-0.05 (-0.08, -0.02)** | **-0.39** | 0.04 (-0.39, 0.47) | 0.02 | 0.24 |

All analyses were adjusted for age, sex, and Tanner score. Model 8, multiple regression containing body mass index (BMI) z-score, peak oxygen consumption adjusted for body mass ($VO_2$ $peak_{kg}$) and LVDF measures; Model 9, Model 8 but with the replacement of BMI z-score with fat mass index (FMI); Model 10, Model 8 but with the replacement of $VO_2$ $peak_{kg}$ with peak oxygen consumption adjusted for fat-free mass ($VO_2$ $peak_{FFMkg}$); Model 11, Model 10 but with the replacement of BMI z-score with FMI. FMI was zero-skewed, log transformed. a' indicates late diastolic tissue peak velocity; CRF, cardiorespiratory fitness; e', early diastolic tissue peak velocity; E/e', E-wave/e' ratio; e'/a', e'/a' ratio; lat, lateral; sep, septal; 95% CI, 95% confidence interval.

**Appendix 2, Table 2.7: Associations of insulin resistance with measures of LVDF, adjusted for adiposity**

| Measure | HOMA2-IR | | Adiposity (HOMA2-IR) | | $r^2$ *HOMA2-IR* | Matsuda index | | Adiposity (Matsuda index) | | $r^2$ *Matsuda* |
|---|---|---|---|---|---|---|---|---|---|---|
| | b (95% CI) | r | b (95% CI) | r | | b (95% CI) | r | b (95% CI) | r | |
| **E-wave** | | | | | | | | | | |
| *Model 12* | 2.46 (-3.84, 8.67) | 0.11 | - | - | - | -4.49 (-13.19, 4.21) | 0.02 | - | - | - |
| *Model 13* | 4.74 (-2.72, 12.19) | 0.21 | -1.75 (-4.82, 1.32) | -0.18 | 0.07 | -8.79 (-19.27, 1.70) | -0.29 | -2.37 (-5.67, 0.93) | -0.24 | 0.09 |
| *Model 14* | 5.61 (-2.30, 13.53) | 0.24 | -3.95 (-9.75, 1.85) | -0.24 | 0.10 | -10.68 (-21.86, 0.51) | -0.34 | -5.70 (-12.17, 0.77) | -0.33 | 0.14 |
| **A-wave** | | | | | | | | | | |
| *Model 12* | **7.15 (1.01, 13.28)** | **0.29** | - | - | - | **-9.97 (-18.44, -1.49)** | **-0.30** | - | - | - |
| *Model 13* | **8.12 (0.80, 15.43)** | **0.33** | -0.75 (-3.76, 2.27) | -0.07 | 0.23 | **-11.38 (-21.78, -0.99)** | **-0.34** | -0.78 (-4.06, 2.49) | -0.07 | 0.23 |
| *Model 14* | **9.13 (1.90, 16.36)** | **0.39** | -1.46 (-6.76, 3.84) | -0.09 | 0.24 | **-11.56 (-22.06, -1.06)** | **-0.38** | -1.28 (-7.35, 4.79) | -0.08 | 0.22 |
| **E/A** | | | | | | | | | | |
| *Model 12* | -0.11 (-2.67, 0.04) | -0.19 | - | - | - | 0.13 (-0.08, 0.34) | 0.16 | - | - | - |
| *Model 13* | -0.09 (-0.27, 0.09) | -0.15 | -0.02 (-0.09, 0.06) | -0.06 | 0.23 | 0.09 (-0.17, 0.34) | 0.11 | -0.03 (-0.11, 0.06) | -0.09 | 0.23 |
| *Model 14* | -0.11 (-0.27, 0.06) | -0.20 | -0.03 (-0.15, 0.10) | -0.07 | 0.23 | 0.06 (-0.18, 0.30) | 0.09 | -0.06 (-0.20, 0.08) | -0.16 | 0.22 |
| **DT** | | | | | | | | | | |
| *Model 12* | -9.54 (-24.49, 5.42) | -0.17 | - | - | - | 3.20 (-17.72, 24.11) | 0.04 | - | - | - |
| *Model 13* | -14.94 (-32.29, 2.42) | -0.27 | 4.28 (-2.78, 11.33) | 0.19 | 0.12 | 7.13 (-17.97, 32.23) | 0.10 | 2.27 (-5.62, 10.16) | 0.10 | 0.07 |
| *Model 14* | -17.23 (-35.62, 1.15) | -0.30 | 8.63 (-4.72, 21.99) | 0.22 | 0.14 | 9.96 (-17.06, 36.97) | 0.14 | 5.59 (-10.16, 21.33) | 0.14 | 0.08 |
| **IVRT** | | | | | | | | | | |
| *Model 12* | -2.86 (-7.14, 1.41) | -0.18 | - | - | - | **6.87 (1.27, 12.48)** | **0.33** | - | - | - |
| *Model 13* | -3.96 (-9.04, 1.13) | 0.12 | 0.84 (-1.25, 2.93) | 0.12 | 0.11 | **9.58 (2.82, 16.34)** | **0.45** | 1.49 (-0.64, 3.62) | 0.22 | 0.20 |
| *Model 14* | -4.61 (-9.85, 0.64) | -0.28 | 1.13 (-2.72, 4.97) | 0.10 | 0.16 | **10.85 (3.93, 17.76)** | **0.52** | 2.63 (-1.37, 6.63) | 0.23 | 0.26 |
| **Vp** | | | | | | | | | | |
| *Model 12* | -2.26 (-19.10, 14.59) | -0.04 | - | - | - | 3.23 (-19.88, 26.34) | 0.04 | - | - | - |
| *Model 13* | 0.49 (-19.74, 20.72) | 0.01 | -2.07 (-10.36, 6.22) | -0.08 | 0.04 | -1.39 (-29.92, 27.13) | -0.02 | -2.51 (-11.46, 6.44) | -0.10 | 0.05 |
| *Model 14* | 2.49 (-12.91, 17.90) | 0.06 | -2.53 (-13.82, 8.76) | -0.08 | 0.04 | -9.83 (-31.45, 11.80) | -0.17 | -5.85 (-18.36, 6.65) | -0.18 | 0.07 |
| **e' sep** | | | | | | | | | | |
| *Model 12* | **-1.01 (-1.86, -0.17)** | **-0.31** | - | - | - | 1.02 (-0.16, 2.19) | 0.24 | - | - | - |
| *Model 13* | -0.45 (-1.41, 0.52) | -0.14 | **-0.43 (-0.83, -0.04)** | **-0.32** | 0.22 | -0.01 (-1.36, 1.34) | -0.002 | **-0.57 (-0.99, -0.14)** | **-0.41** | 0.23 |
| *Model 14* | -0.52 (-1.52, 0.48) | -0.16 | **-0.84 (-1.57, -0.10)** | **-0.36** | 0.26 | -0.19 (-1.59, 1.22) | -0.04 | **-1.20 (-2.01, -0.39)** | **-0.51** | 0.27 |

| Measure | HOMA2-IR | | Adiposity (HOMA2-IR) | | $r^2$ *HOMA2-IR* | Matsuda index | | Adiposity (Matsuda index) | | $r^2$ *Matsuda* |
|---|---|---|---|---|---|---|---|---|---|---|
| | b (95% CI) | r | b (95% CI) | r | | b (95% CI) | r | b (95% CI) | r | |
| **e' lat** | | | | | | | | | | |
| *Model 12* | -0.42 (-1.79, 0.94) | -0.08 | - | - | - | -0.44 (-2.33, 1.44) | -0.07 | - | - | - |
| *Model 13* | -0.27 (-1.90, 1.37) | -0.05 | -0.12 (-0.79, 0.55) | -0.06 | 0.06 | -1.03 (-3.33, 1.27) | -0.15 | -0.32 (-1.05, 0.40) | -0.15 | 0.06 |
| *Model 14* | -0.39 (-2.11, 1.32) | -0.08 | -0.37 (-1.62, 0.89) | -0.10 | 0.07 | -1.04 (-3.48, 1.40) | -0.22 | -0.78 (-2.19, 0.63) | -0.22 | 0.06 |
| **e' avg** | | | | | | | | | | |
| *Model 12* | -0.72 (-1.66, 0.22) | -0.20 | - | - | - | 0.29 (-1.02, 1.59) | 0.06 | - | - | - |
| *Model 13* | -0.36 (-1.47, 0.75) | -0.10 | **-0.28 (-0.73, -0.18)** | **-0.19** | 0.13 | -0.52 (-2.08, 1.04) | -0.11 | -0.44 (-0.93, 0.05) | -0.29 | 0.13 |
| *Model 14* | -0.46 (-1.62, 0.70) | -0.13 | -0.60 (-1.45, 0.25) | -0.24 | 0.16 | -0.61 (-2.26, 1.03) | -0.13 | **-0.99 (-1.94, -0.04)** | **-0.39** | 0.16 |
| **a' sep** | | | | | | | | | | |
| *Model 12* | **0.93 (0.38, 1.47)** | **0.42** | - | - | - | **-1.25 (-2.01, -0.49)** | **-0.43** | - | - | - |
| *Model 13* | **0.68 (0.03, 1.32)** | **0.31** | 0.19 (-0.07, 0.46) | 0.21 | 0.23 | -0.81 (-0.04, 0.53) | -0.28 | 0.24 (-0.04, 0.53) | 0.25 | 0.25 |
| *Model 14* | **0.82 (0.16, 1.48)** | **0.38** | 0.22 (-0.27, 0.70) | 0.14 | 0.23 | -0.90 (-1.85, 0.06) | -0.32 | 0.33 (-0.22, 0.88) | 0.21 | 0.23 |
| **a' lat** | | | | | | | | | | |
| *Model 12* | 0.23 (-0.36, 0.82) | 0.10 | - | - | - | -0.52 (-1.34, 0.29) | -0.18 | - | - | - |
| *Model 13* | -0.03 (-0.72, 0.66) | -0.01 | 0.20 (-0.09, 0.49) | 0.21 | 0.15 | -0.15 (-1.12, 0.82) | -0.05 | 0.21 (-0.10, 0.52) | 0.22 | 0.16 |
| *Model 14* | 0.02 (-0.67, 0.72) | 0.01 | 0.37 (-0.15, 0.88) | 0.24 | 0.15 | -0.12 (-1.10, 0.86) | -0.04 | 0.44 (-0.13, 1.01) | 0.29 | 0.17 |
| **a' avg** | | | | | | | | | | |
| *Model 12* | **0.55 (0.10, 1.00)** | **0.32** | - | - | - | **-0.84 (-1.45, -0.22)** | **-0.36** | - | - | - |
| *Model 13* | 0.32 (-0.21, 0.85) | 0.18 | 0.18 (-0.04, 0.40) | 0.25 | 0.20 | -0.46 (-1.19, 0.28) | -0.20 | 0.21 (-0.02, 0.44) | 0.28 | 0.24 |
| *Model 14* | 0.42 (-0.09, 0.94) | 0.26 | 0.25 (-0.13, 0.64) | 0.22 | 0.20 | -0.49 (-1.21, 0.24) | -0.23 | 0.36 (-0.06, 0.78) | 0.30 | 0.24 |
| **e'/a' sep** | | | | | | | | | | |
| *Model 12* | **-0.39 (-0.62, -0.16)** | **-0.42** | - | - | - | **0.48 (0.15, 0.80)** | **0.38** | - | - | - |
| *Model 13* | -0.23 (-0.49, 0.04) | -0.24 | **-0.13 (-0.23, -0.02)** | **-0.32** | 0.29 | 0.20 (-0.18, 0.57) | 0.16 | **-0.15 (-0.27, -0.04)** | **-0.38** | 0.29 |
| *Model 14* | **-0.29 (-0.55, -0.01)** | **-0.31** | -0.19 (-0.39, 0.004) | -0.30 | 0.30 | 0.19 (-0.21, 0.58) | 0.16 | **-0.27 (-0.50, -0.05)** | **-0.41** | 0.28 |
| **e'/a' lat** | | | | | | | | | | |
| *Model 12* | -0.15 (-0.50, 0.19) | -0.12 | - | - | - | 0.06 (-0.43, 0.55) | 0.04 | - | - | - |
| *Model 13* | -0.04 (-0.45, 0.37) | -0.03 | -0.09 (-0.26, 0.08) | -0.17 | 0.06 | -0.19 (-0.77, 0.40) | -0.11 | -0.14 (-0.32, 0.05) | -0.25 | 0.07 |
| *Model 14* | -0.08 (-0.48, 0.33) | -0.06 | -0.17 (-0.47, 0.14) | -0.20 | 0.07 | -0.18 (-0.77, 0.41) | -0.11 | -0.27 (-0.61, 0.07) | -0.32 | 0.09 |
| **e'/a' avg** | | | | | | | | | | |
| *Model 12* | **-0.27 (-0.52, -0.02)** | **-0.28** | - | - | - | 0.26 (-0.10, 0.61) | 0.20 | - | - | - |

| Measure | HOMA2-IR | | Adiposity (HOMA2-IR) | | $r^2$ *HOMA2-IR* | Matsuda index | | Adiposity (Matsuda index) | | $r^2$ *Matsuda* |
|---|---|---|---|---|---|---|---|---|---|---|
| | b (95% CI) | r | b (95% CI) | r | | b (95% CI) | r | b (95% CI) | r | |
| *Model 13* | -0.13 (-0.42, 0.16) | -0.14 | -0.10 (-0.22, 0.02) | -0.26 | 0.17 | -0.0002 (-0.41, 0.41) | -0.0001 | **-0.14 (-0.27, -0.01)** | **-0.35** | 0.17 |
| *Model 14* | -0.18 (-0.47, 0.12) | -0.19 | -0.17 (-0.39, 0.04) | -0.27 | 0.18 | 0.0001 (-0.43, 0.43) | 0.0001 | -0.27 (-0.52, 0.02) | -0.40 | 0.18 |
| **E/e' sep** | | | | | | | | | | |
| *Model 12* | **0.92 (0.38, 1.46)** | **0.41** | - | - | - | **-1.11 (-1.88, -0.36)** | **-0.38** | - | - | - |
| *Model 13* | **0.74 (0.10, 1.39)** | **0.33** | 0.14 (-0.13, 0.40) | 0.15 | 0.25 | -0.82 (-1.74, 0.11) | -0.28 | 0.17 (-0.14, 0.46) | 0.17 | 0.23 |
| *Model 14* | **-0.87 (0.18, 1.56)** | **0.37** | 0.20 (-0.31, 0.71) | 0.12 | 0.27 | -0.88 (-1.89, 0.14) | -0.29 | 0.27 (-0.31, 0.86) | 0.17 | 0.23 |
| **E/e' lat** | | | | | | | | | | |
| *Model 12* | 0.30 (-0.18, 0.77) | 0.16 | - | - | - | -0.11 (-0.77, 0.55) | -0.05 | - | - | - |
| *Model 13* | 0.38 (-0.19, 0.94) | 0.21 | -0.06 (-0.29, 0.17) | -0.08 | 0.14 | -0.16 (-0.97, 0.65) | -0.07 | -0.03 (-0.28, 0.23) | -0.04 | 0.11 |
| *Model 14* | 0.47 (-0.10, 1.04) | 0.26 | -0.10 (-0.51, 0.32) | -0.08 | 0.16 | -0.26 (-1.10, 0.58) | -0.11 | -0.05 (-0.53, 0.43) | -0.04 | 0.10 |
| **E/e' avg** | | | | | | | | | | |
| *Model 12* | **0.61 (0.18, 1.03)** | **0.35** | - | - | - | **-0.61 (-1.22, -0.01)** | **-0.27** | - | - | - |
| *Model 13* | **0.56 (0.05, 1.07)** | **0.32** | 0.04 (-0.17, 0.25) | 0.05 | 0.24 | -0.49 (-1.23, 0.25) | -0.21 | 0.07 (-0.16, 0.30) | 0.09 | 0.19 |
| *Model 14* | **0.67 (0.13, 1.21)** | **0.37** | 0.05 (-0.34, 0.44) | 0.04 | 0.27 | -0.57 (-1.36, 0.29) | -0.25 | 0.11 (-0.35, 0.57) | 0.09 | 0.20 |

All analyses were adjusted for age, sex, and Tanner score. Model 12, univariate associations of insulin resistance measures with LVDF measures; Model 13; multiple linear regressions containing either the homeostatic model assessment of insulin resistance version two (HOMA2-IR) or the Matsuda index, body mass index (BMI) z-scores, and LVDF measures; Model 14, Model 13 but with the replacement of BMI z-scores with fat mass index (FMI). A-wave indicates late diastolic peak mitral inflow velocity; a', late diastolic tissue peak velocity; DT, E-wave deceleration time; E-wave, early diastolic peak mitral inflow velocity; e', early diastolic tissue peak velocity; E/e', E-wave/e' ratio; e'/a', e'/a' ratio; IVRT, isovolumetric relaxation time; lat, lateral; sep, septal; Vp, mitral propagation velocity; 95% CI, 95% confidence interval.

**Appendix 2, Table 2.8: Associations (r) of ambulatory blood pressure with measures of LVDF, adjusted for adiposity**

| Measure | **Awake systolic** | | **Awake diastolic** | | **Sleep systolic** | | **Sleep diastolic** | |
|---|---|---|---|---|---|---|---|---|
| | *Unadjusted* | *Adjusted* | *Unadjusted* | *Adjusted* | *Unadjusted* | *Adjusted* | *Unadjusted* | *Adjusted* |
| **e'** | | | | | | | | |
| *Septal* | **-0.28 (p=0.010)** | -0.16 (p=0.13) | **-0.26 (p=0.016)** | -0.15 (p=0.15) | -0.17 (p=0.15) | -0.04 (p=0.72) | **-0.23 (p=0.029)** | -0.11 (p=0.29) |
| *Lateral* | **-0.33 (p=0.004)** | **-0.32 (p=0.010)** | **-0.41 (p<0.001)** | **-0.43 (p<0.001)** | **-0.27 (p=0.032)** | -0.25 (p=0.077) | **-0.23 (p=0.040)** | **-0.26 (p=0.040)** |
| *Average* | **-0.35 (p=0.002)** | **-0.30 (p=0.013)** | **-0.40 (p<0.001)** | **-0.37 (p=0.001)** | **-0.26 (p=0.034)** | -0.20 (p=0.15) | **-0.26 (p=0.016)** | **-0.24 (p=0.049)** |
| **a'** | | | | | | | | |
| *Septal* | **0.25 (p=0.026)** | 0.15 (p=0.22) | 0.22 (p=0.050) | 0.12 (p=0.32) | **0.25 (p=0.041)** | 0.14 (p=0.31) | **0.28 (p=0.008)** | 0.22 (p=0.072) |
| *Lateral* | 0.09 (p=0.46) | 0.03 (p=0.79) | 0.10 (p=0.39) | 0.05 (p=0.71) | 0.14 (p=0.27) | 0.04 (p=0.79) | 0.12 (p=0.27) | 0.02 (p=0.85) |
| *Average* | 0.16 (p=0.16) | 0.07 (p=0.58) | 0.16 (p=0.16) | 0.07 (p=0.57) | 0.18 (p=0.16) | 0.04 (p=0.77) | 0.19 (p=0.088) | 0.08 (p=0.51) |
| **e'/a'** | | | | | | | | |
| *Septal* | **-0.34 (p=0.002)** | -0.19 (p=0.087) | **-0.30 (p=0.006)** | -0.17 (p=0.13) | **-0.27 (p=0.029)** | -0.12 (p=0.36) | **-0.31 (p=0.004)** | -0.19 (p=0.085) |
| *Lateral* | **-0.29 (p=0.013)** | -0.24 (p=0.060) | **-0.37 (p=0.001)** | **-0.36 (p=0.003)** | **-0.30 (p=0.021)** | -0.19 (p=0.18) | **-0.25 (p=0.024)** | -0.22 (p=0.083) |
| *Average* | **-0.34 (p=0.003)** | **-0.24 (p=0.044)** | **-0.38 (p=0.001)** | **-0.32 (p=0.006)** | **-0.30 (p=0.017)** | -0.16 (p=0.23) | **-0.30 (p=0.007)** | -0.22 (p=0.066) |
| **E/e'** | | | | | | | | |
| *Septal* | 0.17 (p=0.13) | 0.07 (p=0.52) | 0.04 (p=0.69) | -0.06 (p=0.57) | 0.03 (p=0.80) | -0.10 (p=0.40) | 0.03 (p=0.79) | -0.11 (p=0.32) |
| *Lateral* | 0.22 (p=0.051) | 0.22 (p=0.078) | **0.22 (p=0.049)** | **0.24 (p=0.046)** | 0.17 (p=0.19) | 0.14 (p=0.33) | 0.09 (p=0.44) | 0.11 (p=0.40) |
| *Average* | 0.22 (p=0.052) | 0.16 (p=0.18) | 0.14 (p=0.19) | 0.09 (p=0.42) | 0.11 (p=0.38) | 0.01 (p=0.93) | 0.07 (p=0.53) | -0.004 (p=0.97) |

Data are reported as standardised correlation coefficients (r). All analyses were adjusted for age, sex, and Tanner score, with the "adjusted" analyses also accounting for fat mass index. a' indicates late diastolic tissue peak velocity; e', early diastolic tissue peak velocity; E/e', E-wave/e' ratio; e'/a', e'/a' ratio

**Appendix 2, Table 2.9: Intra-observer reliability for LVDF measures**

| LVDF Measure | Intra-observer ICC (95% CI) |
|---|---|
| E-wave | 0.99 (0.97, 1.00) |
| A-wave | 0.99 (0.95, 1.00) |
| E/A | 0.99 (0.95, 1.00) |
| DT | 0.60 (0.00, 0.89) |
| IVRT | 0.91 (0.68, 0.98) |
| Vp | 0.68 (0.15, 0.92) |
| e' | |
| *Septal* | 0.98 (0.94, 1.00) |
| *Lateral* | 0.97 (0.89, 0.99) |
| *Averaged* | 0.98 (0.92, 0.99) |
| a' | |
| *Septal* | 0.89 (0.62, 0.97) |
| *Lateral* | 0.86 (0.55, 0.96) |
| *Averaged* | 0.85 (0.50, 0.96) |
| e'/a' | |
| *Septal* | 0.96 (0.85, 0.99) |
| *Lateral* | 0.98 (0.93, 1.00) |
| *Averaged* | 0.98 (0.91, 0.99) |
| E/e' | |
| *Septal* | 0.96 (0.86, 0.99) |
| *Lateral* | 0.92 (0.74, 0.98) |
| *Averaged* | 0.94 (0.79, 0.99) |

A-wave indicates late mitral inflow peak velocity; a', late diastolic tissue peak velocity; CI, confidence interval; DT, E-wave deceleration time; E-wave, early mitral inflow peak velocity; e', early diastolic tissue peak velocity; E/A, E-wave/A-wave ratio; E/e', E-wave/e' ratio; e'/a', e'/a' ratio; ICC, intraclass correlation coefficient; IVRT, isovolumic relaxation time; LVDF, left ventricular diastolic function; Vp, propagation velocity.

# Appendix 3

## Study 3: Supporting methods

### Partial multivariable linear regression model construction

Abbreviations for below:

- LPA – light physical activity
- MPA – moderate physical activity
- ST – sedentary time
- VPA – vigorous physical activity

Step 1: linear regression (dependent variable: MPA; independent variable: VPA)

Step 2: predict residuals from regression. Add these to the mean of MPA to generate residualised MPA.

Step 3: multivariable linear regression (dependent variable: LPA; independent variables: VPA and residualised MPA)

Step 4: predict residuals from regression. Add these to the mean of LPA which generates residualised LPA.

Step 5: multivariable linear regression (dependent variable: ST; independent variables: VPA, residualised MPA, and residualised LPA)

Step 6: predict residuals from regression. Add these to the mean of ST, which generates residualised ST.

This was repeated but with ST as the base variable:

Step 1: linear regression (dependent variable: LPA; independent variable: ST)

Step 2: predict residuals from regression. Add these to the mean of LPA to generate residualised LPA.

Step 3: multivariable linear regression (dependent variable: MPA; independent variables: ST and residualised LPA)

Step 4: predict residuals from regression. Add these to the mean of MPA which generates residualised MPA.

Step 5: multivariable linear regression (dependent variable: VPA; independent variables: ST, residualised LPA, and residualised MPA)

Step 6: predict residuals from regression. Add these to the mean of VPA, which generates residualised VPA.

## Study 3: Supporting results

**Socioeconomic status and ethnicity**

The Office for Standards in Education, Children's Services and Skills (Ofsted) reported in 2015 for one of the schools that "less than half [of students were] White British and just over one in 10 being Pakistani. Nearly a third of students speak English as an additional language." The other school reported in 2013 that "Most students are of White British heritage." The Index of Multiple Deprivation (IMD), a marker of socioeconomic status, ranged between the 2nd to the 10th decile for one school and 5th to the 10th decile for the other school in 2019.

### Tables

**Appendix 3, Table 3.1:** Correlation coefficient matrix of the association between intensities of PA

| Intensities of PA | VPA | MPA | LPA | ST |
|---|---|---|---|---|
| VPA | 1 | - | - | - |
| MPA | 0.59* | 1 | - | - |
| LPA | -0.37* | -0.72* | 1 | - |
| ST | 0.13† | -0.10‡ | 0.29* | 1 |

LPA, light physical activity (PA); MPA, moderate PA; ST, sedentary time; VPA, vigorous PA; *, $P < 0.001$; †, $P < 0.01$; ‡, $P < 0.05$.

**Appendix 3, Table 3.2:** Correlation coefficient matrix of the association between bands of residualised PA

| Intensities of PA | VPA | rMPA | rLPA | rST |
|---|---|---|---|---|
| VPA | 1 | - | - | - |
| rMPA | 0.0* | 1 | - | - |
| rLPA | 0.0* | 0.0* | 1 | - |
| rST | 0.0* | 0.0* | 0.0* | 1 |

rLPA; light physical activity; rMPA, residualised moderate physical activity; rST, residualised sedentary time; VPA, vigorous physical activity; *, $P > 0.05$.

**Appendix 3, Table 3.3:** Physical activity (PA) and sedentary time (ST) using different metrics of PA processing

| PA / ST | BFEN | ENMOz | HFEN | HFEN+ | GT3VM5 | GT3Y5 | *p* |
|---|---|---|---|---|---|---|---|
| VPA | 10.6 (7.2, 15.7) | 6.9 ± 5.0* | 11.9 (8.2, 17.1)* | 9.0 (5.5, 14.2)* | 9.0 (5.9, 14.5)* | 6.8 (4.0, 11.1)* | < 0.001 |
| MPA | 153.2 ± 42.9 | 44.9 ± 17.3* | 157.2 ± 43.6 | 119.1 ± 34.4* | 155.6 ± 48.9 | 106.8 ± 35.2* | < 0.001 |
| LPA | 202.5 (166.3, 228.8) | 72.9 ± 19.1* | 204.1 (168.6, 232.4) | 244.1 (196.9, 276.2)* | 256.1 (210.6, 284.0)* | 256.2 (213.3, 287.8)* | < 0.001 |
| ST | 470.1 ± 99.5 | 747.8 (627.4, 801.9)* | 462.2 ± 98.6 | 467.0 ± 99.6 | 418.3 ± 93.5* | 466.0 ± 100.1 | < 0.001 |

Data are reported as mean ± standard deviation or median (interquartile range) for normal and non-normally distributed data, respectively. BFEN indicates bandpass-filtered followed by Euclidean norm; ENMOz, Euclidean norm minus one with negative values rounded to zero; GT3VM5, ActiGraph counts calculated using the vector magnitude of the three raw axes; GT3Y5, ActiGraph counts calculated using the vertical axis; HFEN, high-pass filtered Euclidean norm; HFEN+, HFEN plus low-pass filtered Euclidean norm; LPA, light physical activity (PA); MPA, moderate PA; ST, sedentary time; VPA, vigorous PA; *, $P < 0.05$ between BFEN.

**Appendix 3, Table 3.4:** Participant characteristics in adolescents with and without valid physical activity wear time

| Measure | Valid PA wear time | Invalid PA wear time | *p* |
|---|---|---|---|
| N (male/female) | 339 (169/170) | 123 (71/52) | 0.28 |
| Height (cm)[a] | 165.2 ± 8.7 | 164.5 ± 8.8 | 0.48 |
| Weight (kg)[a] | 56.0 ± 12.3 | 57.3 ± 14.9 | 0.37 |
| BMI (kg/m²)[a] | 20.4 ± 3.8 | 21.1 ± 4.7 | 0.11 |
| BMI z-score (WHO)[a] | 0.3 ± 1.2 | 0.4 ± 1.3 | 0.45 |
| 20mSRT (total laps)[b, c] | 44 (32, 62) | 41 (28, 61) | 0.09 |
| 20mSRT (z-score total laps)[b, c] | -0.4 (-1.0, 0.5) | -0.6 (-1.1, $1.3x10^{-2}$) | 0.02 |

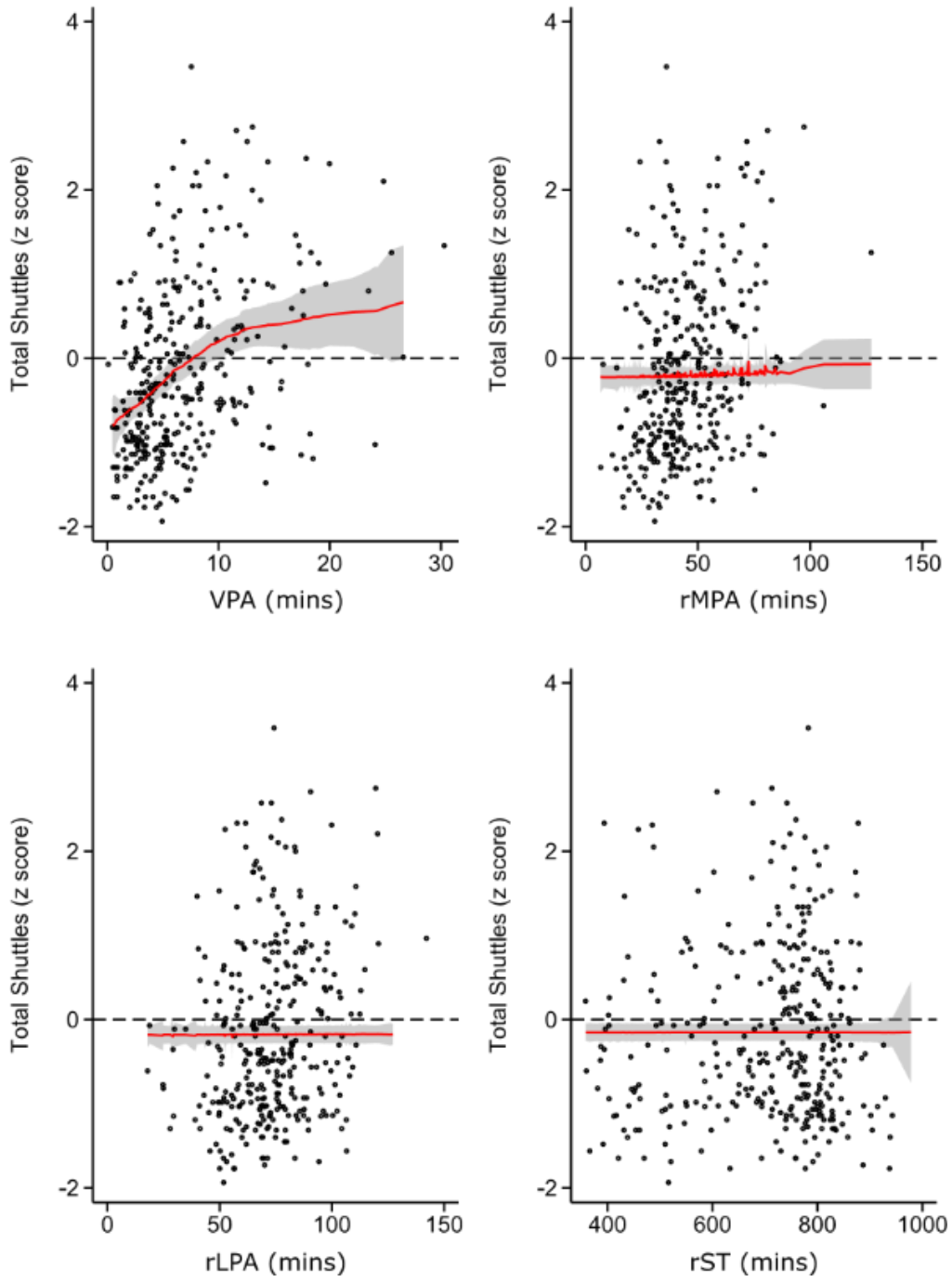
BMI indicates body mass index; WHO, World Health Organization; [a], mean ± standard deviation; [b], median (interquartile range); [c], variable with non-normal distribution.

**Appendix 3, Table 3.5: Moving average model results for other physical activity metrics**

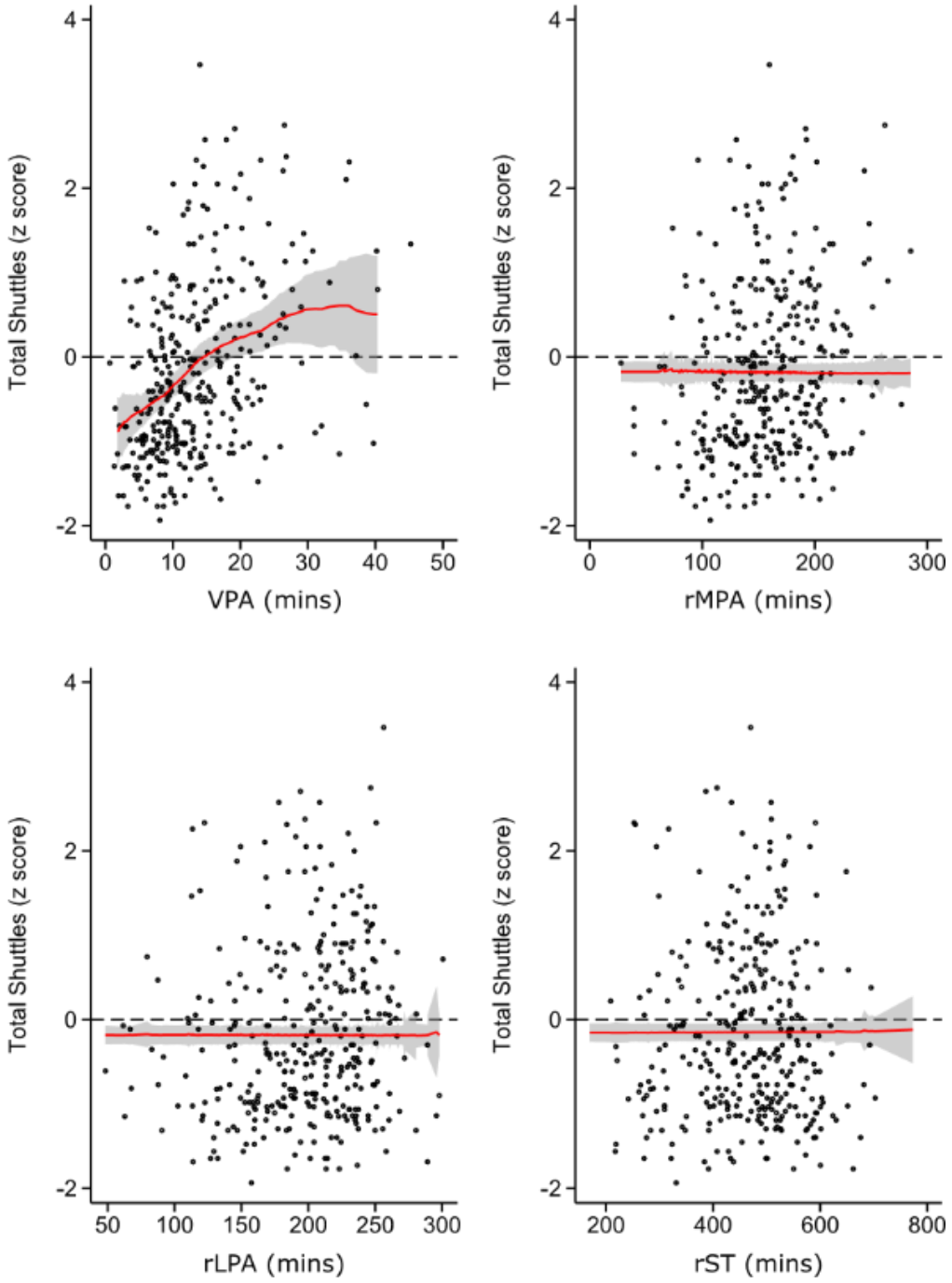
| PA Metric (mins) | Plateau | Median | Median Lower Limit | Median Upper Limit |
|---|---|---|---|---|
| **ENMOz** | 10.0 | 7.5 | 5.8 | 9.4 |
| **HFEN** | 17.4 | 14.4 | 12.2 | 18.1 |
| **HFENp** | 14.3 | 11.4 | 9.1 | 14.0 |
| **GT3Y5** | 12.9 | 8.7 | 7.5 | 10.5 |
| **GT3VM5** | 15.4 | 10.8 | 9.3 | 13.7 |

Median represents the point where the moving average model reached a cardiorespiratory fitness (CRF) z-score of zero. The lower and upper limits represent the range of vigorous physical activity (PA) values where the 95% confidence limits of the CRF z-score crossed the median. ENMOz represents Euclidean norm minus one with negative values rounded to zero; GT3VM5, ActiGraph counts calculated using the vector magnitude of the three raw axes; GT3Y5, ActiGraph counts calculated using the vertical axis; HFEN, high-pass filtered Euclidean norm; HFENp, high-pass filtered Euclidean norm HFEN plus Euclidean norm of the three signals after low-pass filtering minus 1g.

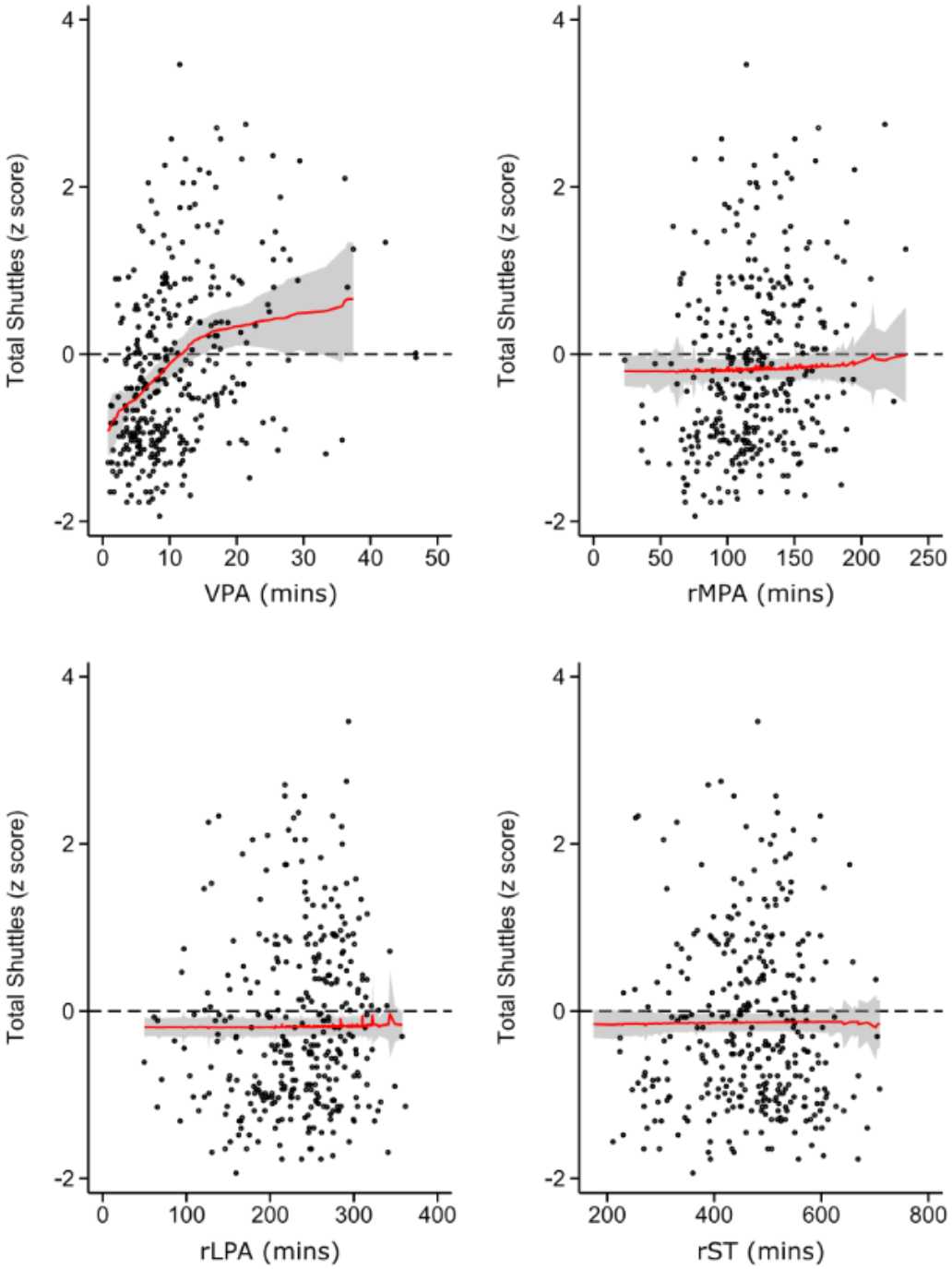
Figures



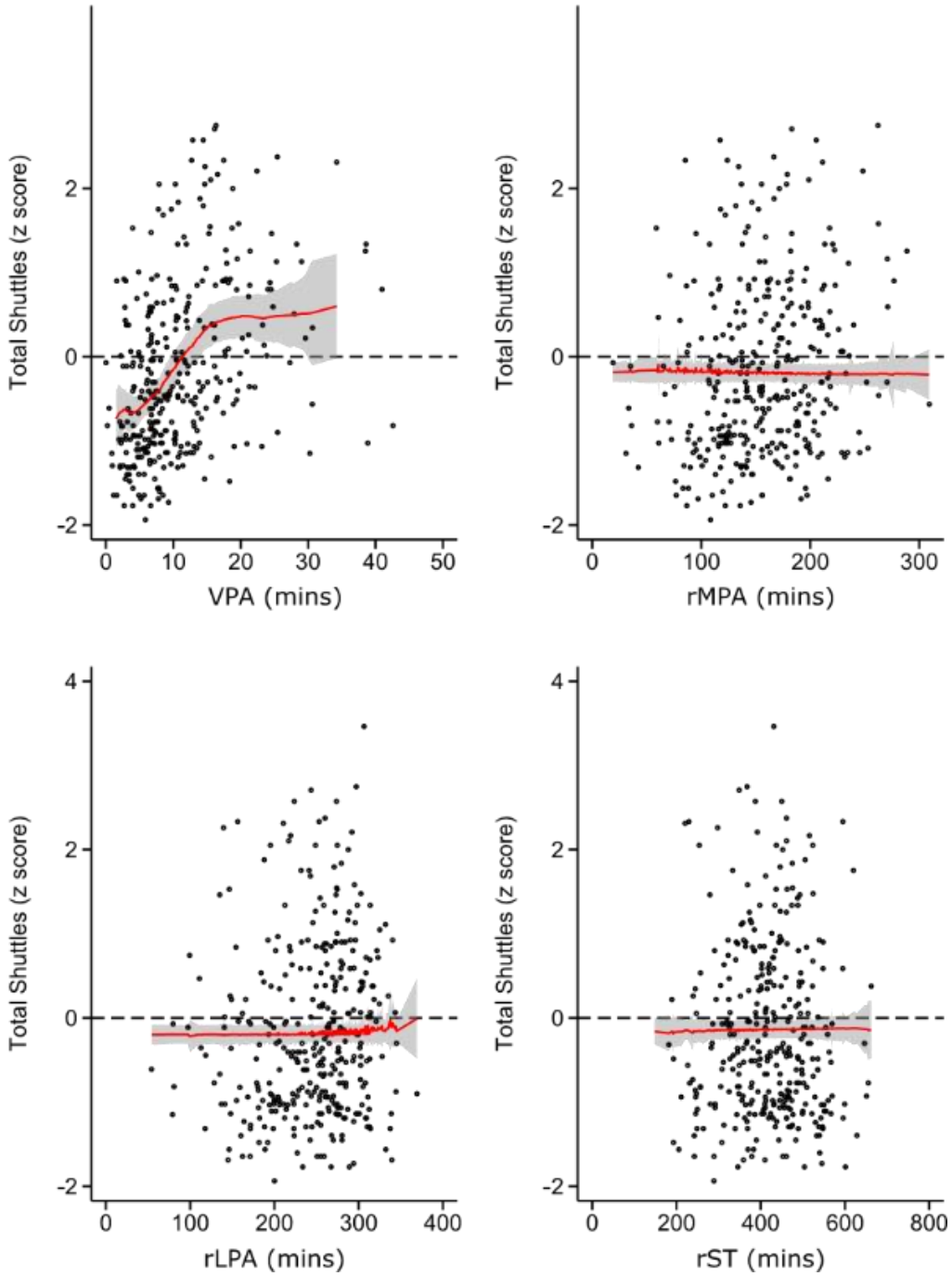

**Appendix 3, Figure 3.1: Moving average model of the association of Euclidean norm minus one with negative values rounded to zero (ENMOz) residualised physical activity (rPA) with the z-score of total number of shuttles run (cardiorespiratory fitness - CRF).** The red line indicates the best-fit moving-average non-linear relationship between PA and CRF with 95% confidence intervals (grey). These variables were adjusted by resdualised modelling for the confounding effect of correlations between activity levels. rLPA indicates residualised light physical activity; rMPA, residualised moderate physical activity; rST, residualised sedentary time; VPA, vigorous PA (base variable).



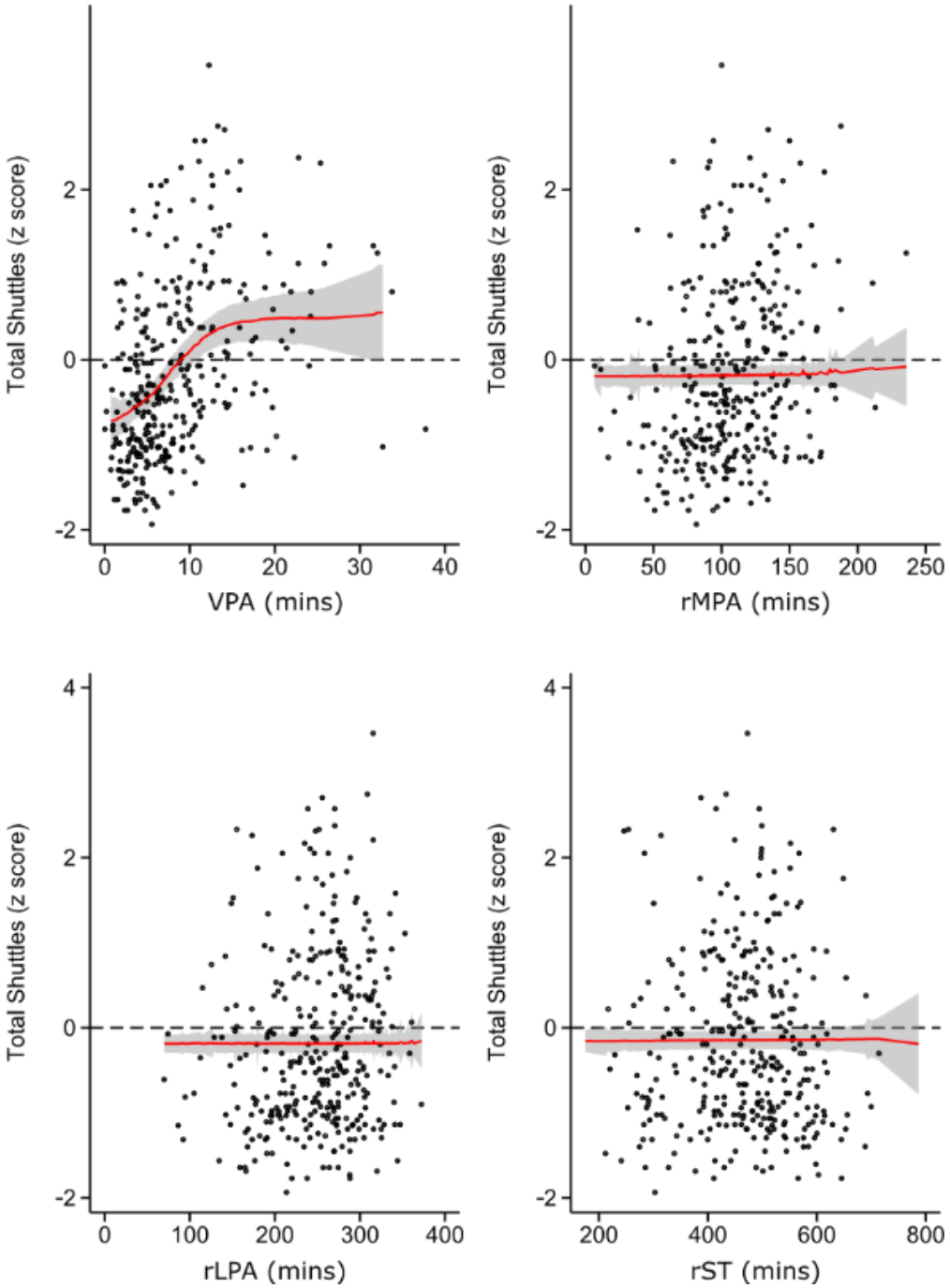

**Appendix 3, Figure 3.2: Moving average model of the association of high-pass filtered Euclidean norm (HFEN) residualised physical activity (rPA) with the z-score of total number of shuttles run (cardiorespiratory fitness - CRF).** The red line indicates the best-fit moving-average non-linear relationship between PA and CRF with 95% confidence intervals (grey). These variables were adjusted by resdualised modelling for the confounding effect of correlations between activity levels. rLPA indicates residualised light physical activity; rMPA, residualised moderate physical activity; rST, residualised sedentary time; VPA, vigorous physical activity (base variable).



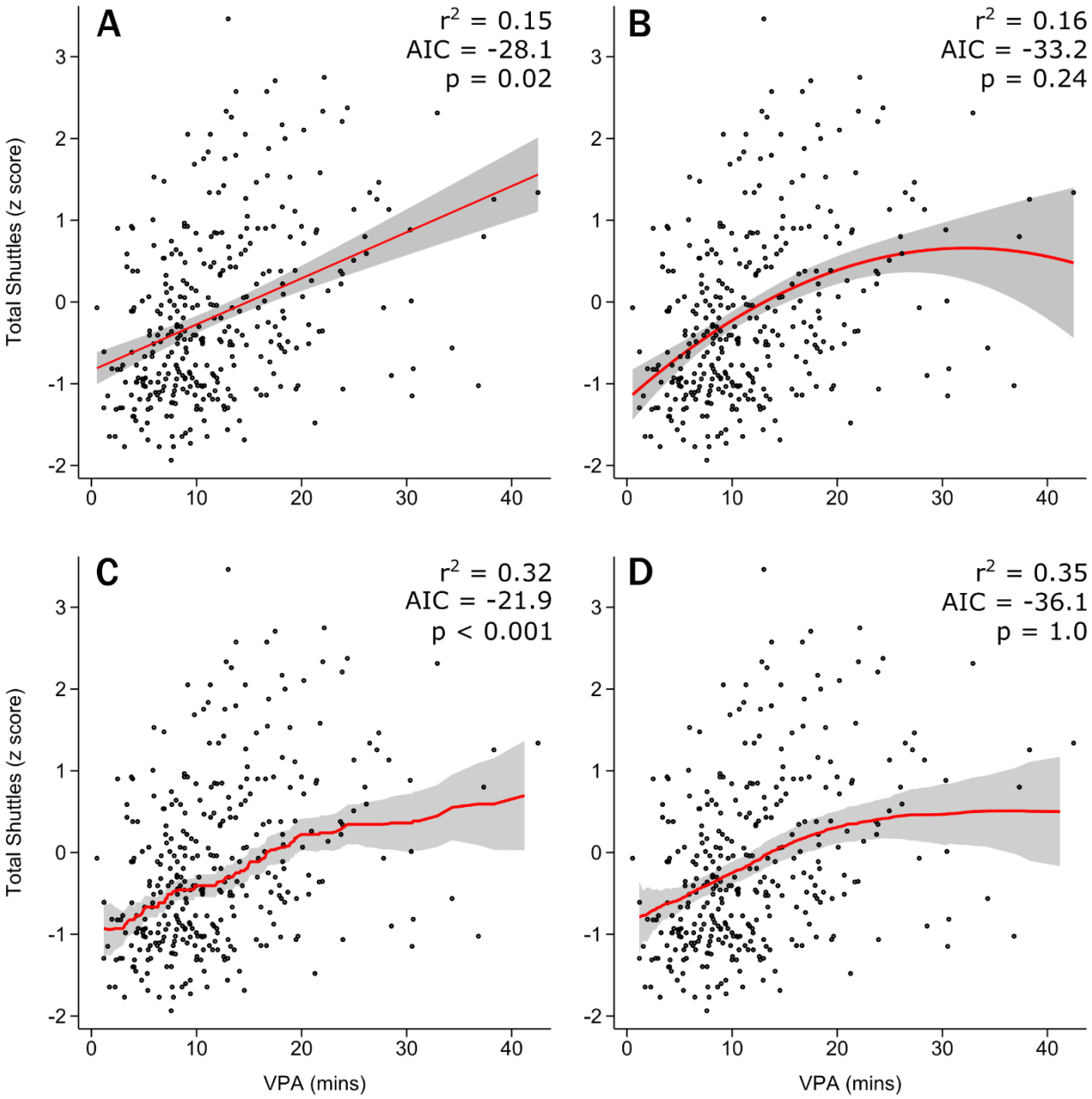

**Appendix 3, Figure 3.3: Moving average model of the association of high-pass filtered Euclidean norm HFEN plus EN of the three signals after low-pass filtering minus 1g (HFEN+) residualised physical activity (rPA) with the z-score of total number of shuttles run (cardiorespiratory fitness - CRF).** The red line indicates the best-fit moving-average non-linear relationship between PA and CRF with 95% confidence intervals (grey). These variables were adjusted by resdualised modelling for the confounding effect of correlations between activity levels. rLPA indicates residualised light physical activity; rMPA, residualised moderate physical activity; rST, residualised sedentary time; VPA, vigorous physical activity (base variable).



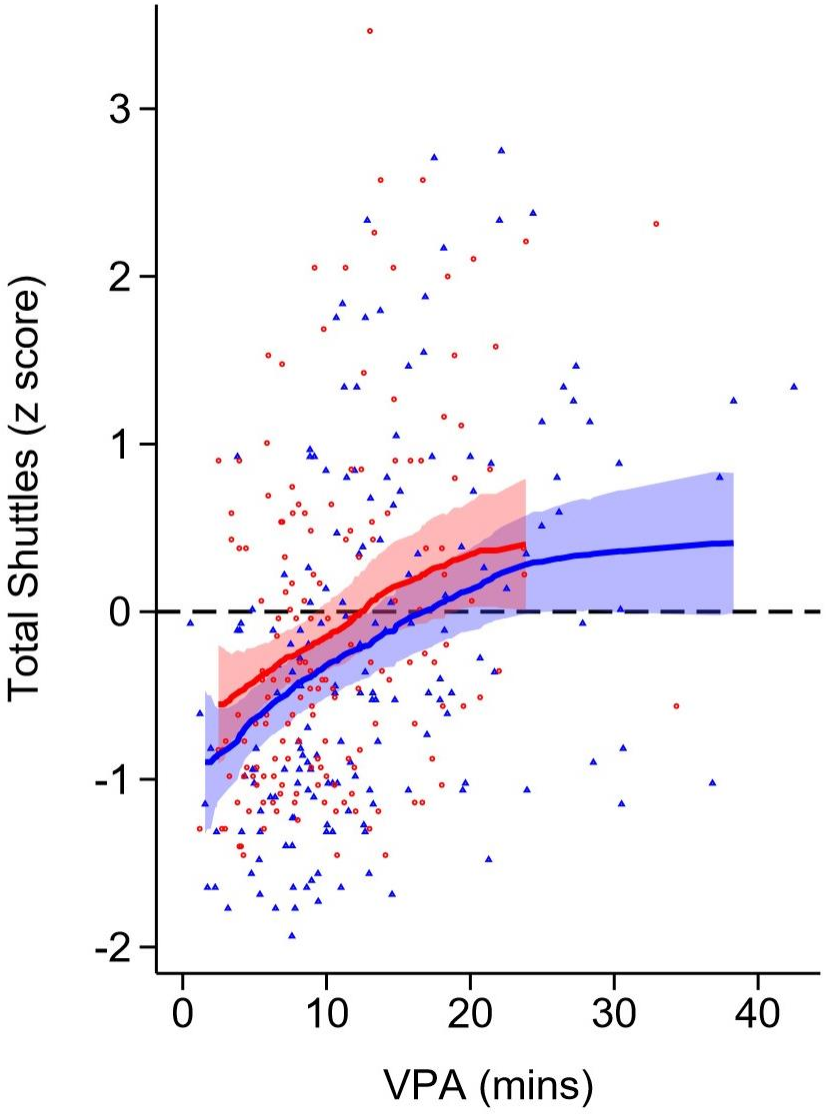

**Appendix 3, Figure 3.4: Moving average model of the association of ActiGraph counts calculated using the vector magnitude of the three raw axes (GT3VM5) residualised physical activity (rPA) with the z-score of total number of shuttles run (cardiorespiratory fitness - CRF).** The red line indicates the best-fit moving-average non-linear relationship between PA and CRF with 95% confidence intervals (grey). These variables were adjusted by resdualised modelling for the confounding effect of correlations between activity levels. rLPA indicates residualised light physical activity; rMPA, residualised moderate physical activity; rST, residualised sedentary time; VPA, vigorous physical activity (base variable).



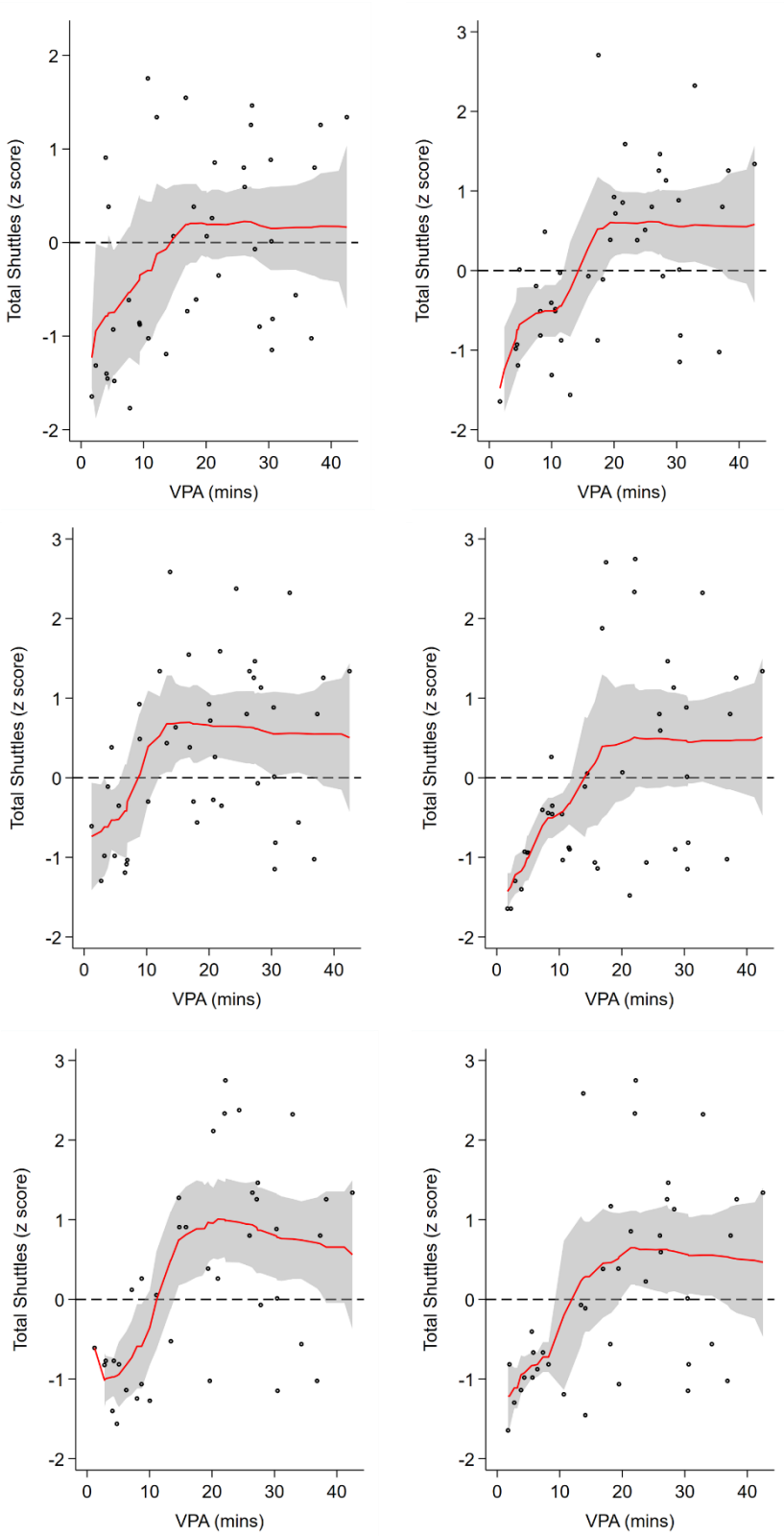

**Appendix 3, Figure 3.5: Moving average model of the association of ActiGraph counts calculated using the vertical axis (GT3Y5) residualised physical activity (rPA) with the z-score of total number of shuttles run (cardiorespiratory fitness - CRF).** The red line indicates the best-fit moving-average non-linear relationship between PA and CRF with 95% confidence intervals (grey). These variables were adjusted by resdualised modelling for the confounding effect of correlations between activity levels. rLPA indicates residualised light physical activity; rMPA, residualised moderate physical activity; rST, residualised sedentary time; VPA, vigorous physical activity (base variable).



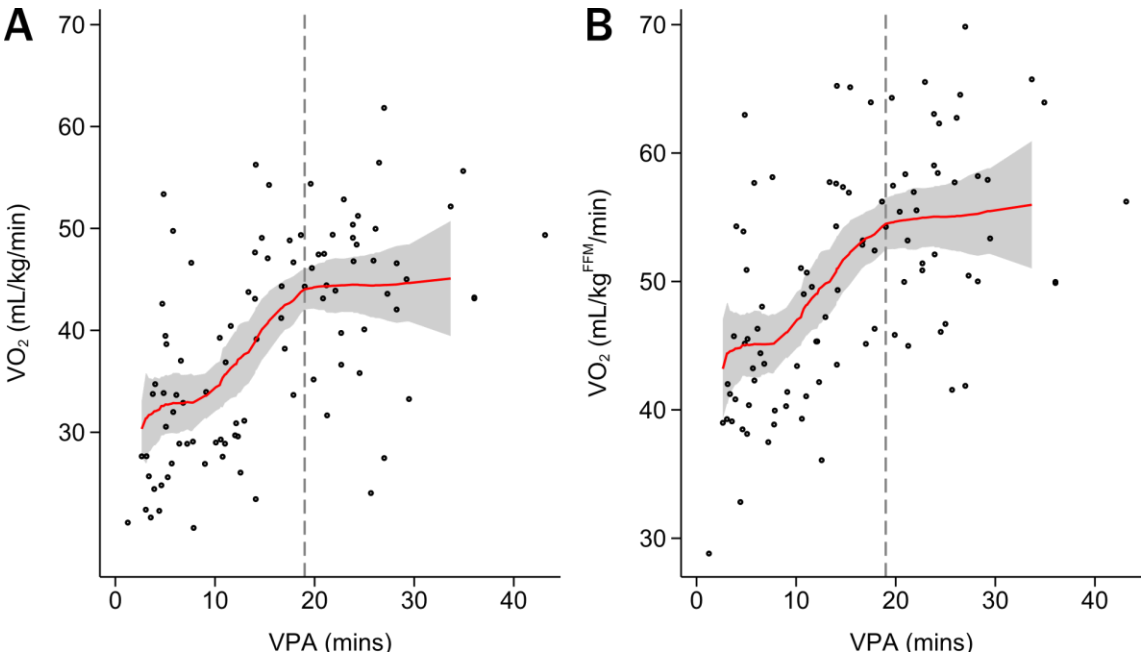

**Appendix 3, Figure 3.6: Different model fits of the association of daily vigorous physical activity (VPA) with the z-score of total number of shuttles run (cardiorespiratory fitness - CRF).** A represents a linear model; AIC, Akaike information criterion; B, 2nd order polynomial model; C, moving median model; D moving average model; p, p-value of the difference in AIC values compared to the moving average model (D).




**Appendix 3, Figure 3.7: Sex-specific moving average models of the association of vigorous physical activity (VPA) with the z-score of total number of shuttles run (cardiorespiratory fitness - CRF).** Boys are represented by blue and girls by red colouring. The blue and red lines indicate the best-fit moving-average non-linear relationships between VPA and CRF in boys and girls, respectively**.** VPA based on bandpass-filtered followed by Euclidean norm (BFEN).




**Appendix 3, Figure 3.8: Six randomly downsampled moving average models of the association of vigorous physical activity (VPA) with cardiorespiratory fitness.** The presence of a plateau in each of the six plots indicates that the plateau is not due to a reduced number of datapoints above 20 minutes of daily VPA.




**Appendix 3, Figure 3.9: Moving average model of the association of vigorous physical activity (VPA) with cardiorespiratory fitness, as determined by a cardiopulmonary exercise test in a sub-population of adolescents.** The vertical grey dashed line indicates the point at which the moving-average model plateaus at roughly 20 minutes. A) peak oxygen consumption per kilogram per minute [$VO_2$ (mL/kg/min)]; B) peak oxygen consumption per kilogram of fat free mass per minute [$VO_2$ (mL/kg$^{FFM}$/min)].

# Appendix 4

## STEM for Britain abstract

**THE CASE FOR VIGOROUS PHYSICAL ACTIVITY IN ADOLESCENTS – EVIDENCE FROM THE OXSOCRATES STUDY**

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Introduction: The World Health Organization currently recommends that adolescents perform an average of 60 min/day of moderate-to-vigorous physical activity (PA). However, some have suggested that vigorous PA (VPA) is most important for adolescent cardiometabolic and cardiovascular health. Therefore, we aimed to determine the strength of associations between each PA intensity and cardiometabolic and cardiovascular risk factors, independently of other intensities, and the PA duration at each intensity associated with the healthiest cardiometabolic and cardiovascular profile in adolescents.

Methods: Pre-screening and baseline data collected from the OxSOCRATES study (NCT04118543) were used. Wrist-worn accelerometery was assessed in 12–18-year-olds with and without overweight/obesity. Cardiorespiratory fitness (CRF) was assessed by bleep tests (n=339) and cardiopulmonary exercise tests (CPET [n=82]), adiposity by bioelectrical impedance analysis (n=84), fasting and dynamic insulin resistance/sensitivity by blood sampling (n=56), and left ventricular diastolic function (LVDF) by echocardiography (n=89). Multiple linear regression, moving average models, and one-way ANOVAs were used to compare the independent effects of PA intensities with cardiometabolic and cardiovascular risk factors, controlling for age, sex, Tanner score, and adiposity where possible.

Results: Greater VPA was associated with lower adiposity (r=-0.60, p<0.001), with participants in the upper quartile of VPA having 43% lower adiposity than those in the lowest quartile. Greater VPA was associated with better CRF until about 20 minutes of daily VPA, when the relationship plateaued. Participants in the upper quartile of VPA had 1.03 z-scores higher CRF (bleep test) than those in the lowest quartile. This was also evident when testing CRF by CPET (r=0.75, p<0.001), which remained when controlling for adiposity (r=0.51, p<0.001). Participants in the upper quartile of VPA had 23% (p<0.001) higher CRF, measured by CPET, than those in the lowest quartile. This was 17% higher (p<0.001) when controlling for adiposity. VPA was negatively and positively associated with fasting insulin resistance (r=-0.50, p=0.003) and dynamic insulin sensitivity (r=0.50, p=0.002), respectively, but this was removed when controlling for adiposity. VPA was associated with better LVDF. Tissue Doppler measures were better than other measures of LVDF at detecting this association, with septal early-to-late diastolic peak velocity ratios being most strongly associated (r=0.53, p<0.001), which remained when adjusting for adiposity (r=0.36, p=0.025). Participants in the upper quartile of VPA had 32% (p<0.001) higher septal early-to-late diastolic peak velocity ratios than those in the lowest quartile. This was 23% higher (p=0.013) when controlling for adiposity. Adjusted for adiposity and other confounders, those in the upper quartile of VPA performed a median of 19.4 min/day (interquartile range: 17.5, 24.2) of VPA. Other PA intensities were unrelated with cardiometabolic and cardiovascular risk factors, apart from moderate PA (MPA) being adversely related with some measures, but this was removed when controlling for adiposity.

Conclusions: VPA is associated with better cardiometabolic and cardiovascular health in adolescents, independent of lesser intensities. Critically, these associations persisted with CRF and LVDF when controlling for adiposity, suggesting that VPA may protect against the adverse cardiac effects of excess adiposity. Our data show that VPA is a critical component for maximising cardiometabolic and cardiovascular health in adolescents and that an average of 20 min/day of VPA may be best. As moderate-to-vigorous PA guidelines can be satisfied by only undertaking MPA, with no apparent

independent benefit on adiposity, CRF, insulin resistance, and LVDF, we provide evidence for specific VPA recommendations to improve these aspects of health in adolescents.