The role of physical activity, cardiorespiratory fitness and exercise on the autonomic and arterial systems of healthy adolescents

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

Signature: ……..

Abstract

Cardiovascular diseases (CVD) are the leading cause of death worldwide and the atherosclerotic process that precedes CVD starts during childhood. Physical activity (PA), cardiorespiratory fitness (CRF) and exercise are well known as preventive strategies for CVD. One possible mechanism for such prevention is the role of PA, CRF and exercise on the arterial and autonomic systems. The aim of this thesis was to investigate using observational and experimental studies the role of PA, CRF and exercise on the autonomic and arterial systems of healthy adolescents. Chapter 4 systematically reviewed observational cross-sectional studies and provided level one evidence for a significant and positive association between resting parasympathetic function and moderate-to-vigorous PA in youth. Chapter 4 also indicated that gaps exist in the literature such as the associations between PA intensities, CRF and heart rate variability (HRV). These findings were furthered in Chapter 5 which showed that vigorous PA (VPA) and moderate PA (MPA) were positively related with HRV at rest and cardiac autonomic recovery following exercise in adolescents. In Chapter 6 a high-fat meal was used aiming to increase CVD risk in the postprandial state, and it was demonstrated that PA levels and CRF are not significantly associated with postprandial HRV and arterial stiffness in adolescents. Aiming to investigate possible associations between the vascular and autonomic system, measures of baroreflex sensitivity (BRS) were introduced. Chapter 7 showed that BRS and its autonomic and vascular components present a between-day coefficient of variation lower than 20% whilst within day coefficient of variations were lower than 34% in adolescents. In Chapter 8 acutely following high- and moderate-intensity interval exercise a decrease

in blood pressure was observed concomitantly with decreases in BRS. This was mainly mediated by decreases in the autonomic modulation, and the duration of the decreases in blood pressure was higher following high-intensity interval exercise. Chapter 9 extended these findings by demonstrating that the changes in BRS following the ingestion of glucose was not altered by the high or moderate-intensity exercise performed before glucose ingestion. Chapter 10 showed that following four weeks of high-intensity exercise interval training no improvements were observed in BRS and its autonomic and vascular components at rest or acutely following exercise. Collectively, the present thesis contributes significantly to the literature by providing novel evidence in healthy adolescents on the role of PA intensities, CRF and exercise on the arterial and autonomic systems at rest, acutely following exercise and in the postprandial state. The results gathered in this thesis indicate potential of the autonomic and vascular function as targets of CVD risk reduction in youth.

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To express my gratitude, I would like to adapt a quote taken from the book the pale blue dot by Carl Sagan: *“In the vastness of space and the immensity of time, it is my joy to share a planet and an epoch with you all.*”

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Publications

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Oral presentation: “Diminishing cardiovascular disease risk in adolescents: The role of exercise on autonomic and arterial function” Cardiovascular Health Research Group, University of Exeter, 2015.

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Abbreviations

AC	Arterial compliance
AD	Arterial distensibility
BF	Body fat
BMI	Body mass index
BP	Blood pressure
BRS	Baroreflex sensitivity
CCA	Common carotid artery
CI	Confidence interval
cIMT	Carotid intra-media thickness
CON	Control
CRF	Cardiorespiratory fitness
CV	Coefficient of variation
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
ECG	Electrocardiography
ES	Effect size
FMD	Flow mediated dilation
GET	Gas exchange threshold
GLU	Glucose
HDL	High-density lipoprotein
HF	High-frequency
HFM	High-fat meal
HIIE	High-intensity interval exercise
HIIE-T	High-intensity interval training
HR	Heart rate
HRR	Heart rate recovery
HRV	Heart rate variability
ICC	Intra-class coefficient of correlation
IEM	Incremental elastic modules
IMT	Intra-media thickness

LDL	Low-density lipoprotein
LDL	Lumen diastolic diameter
LF	Low-frequency
LPA	Light physical activity
LSD	Lumen systolic diameter
MAP	Mean arterial pressure
MAS	Maximum aerobic speed
MIIE	Moderate-intensity interval exercise
MPA	Moderate physical activity
MVPA	Moderate to vigorous physical activity
NO	Nitric oxide
NOS	Newcastle-Ottawascale
OGTT	Oral glucose tolerance test
PA	Physical activity
PHV	Peak height velocity
PP	Pulse pressure
PWV	Pulse wave velocity
$\dot{Q}$	Cardiac output
RMSSD	Square root of the mean of the sum of the squares of differences between adjacent heart beats
SBP	Systolic blood pressure
SD	Standard deviation
ST	Sedentary time
SV	Stroke volume
TAG	Triacylglycerol
TC	Total cholesterol
TE	Typical error
TPR	Total peripheral resistance
TRIMP	Training impulse
VLF	Very low-frequency
VPA	Vigorous physical activity
WC	Waist circumference
[]	Denotes concentration

To mum and dad, Fatima and Sidney Oliveira. Without your unconditional love I would be someone else, but not me.

Chapter 1: Introduction

Cardiovascular diseases (CVDs) are the leading cause of death by non-communicable diseases in the world. Around 17.8 million of deaths were attributed to CVD in 2012 (WHO, 2014), and it has been previously estimated that over 23.3 million people are expected to die from CVDs in 2030 (Mathers and Loncar, 2006). In the UK, CVDs are responsible for 29% of all cause of mortality, and the treatment and management of CVDs within the National Health Service in 2012 – 2013 costed more than £6.8 billion (Bhatnagar et al., 2015). Although these numbers stress the health and financial burden of CVDs in modern society, CVD prevention is key with an estimated ~ 90% lowered lifetime risk of CVD for adults without the presence of traditional CVD risk factors, such as low-density lipoprotein (LDL), total cholesterol (TC), body mass index (BMI), and blood pressure (BP) (Lloyd-Jones et al., 2006).

The underlying pathobiological cause of CVDs is the atherosclerotic process, which eventually culminates in the disease overtly appearing in late adulthood (Ross, 1993). Compelling evidence shows that the initial stages of atherosclerosis may have its origin during childhood (McGill et al., 2000), with atherosclerotic lesions present in nearly 70% of adolescents at the end of puberty (Stary, 2000). Furthermore, it is well established that the presence of four traditional CVD risk factors in children and adolescents (herein youth) increases the presence of arterial wall atherosclerotic lesions (Berenson et al., 1998). Traditional CVD risk factors have been shown to cluster in apparently health youth (Raitakari et al., 1994), and predict pre-clinical atherosclerosis later in adult life independently of adult CVD risk factor status (Li et al.,

2003). Therefore, interventions to decrease CVD risk would be most effective to prevent CVD risk factors early in life (Magnussen et al., 2013, McGill et al., 2008).

Regular physical activity (PA) and exercise training are well known as preventive strategies to lower CVD risk (Booth et al., 2012), with physical inactivity being considered the biggest health problem of the 21st century (Blair, 2009). A landmark investigation into the role of PA and CVD risk reduction was conducted by Morris et al. (1953), who demonstrated that physically active workers, such as bus conductors and postmen, had a lower incidence of coronary heart disease compared to less physically active workers, such as bus drivers, telephonists, and office workers. More recent epidemiological data shows that PA significantly lowers CVD risk in adults (Thompson et al., 2003), and the World Health Organisation recognises that physically active adults have a 30% lower chance of dying from all-cause mortality, with over three million deaths potentially being avoided by increasing current PA levels (WHO, 2014).

Similar to the overwhelming data in the adult literature, evidence exist showing that PA is inversely associated with traditional CVD risk factors in youth (Ekelund et al., 2012), and current guidelines suggest that children and adolescents should perform a minimum of 60 min·day^{-1} of moderate-to-vigorous physical activity (MVPA) (WHO, 2010). Despite this, over 80% of adolescents worldwide do not meet current guidelines for health (Hallal et al., 2012). Furthermore, strategies aimed at increasing PA levels in youth have led to a modest four min·day^{-1} overall increase in MVPA (Metcalf et al., 2012), which suggests that alternative approaches to decrease CVD risk should be considered.

Recently, studies have suggested that the negative associations between PA and CVD risk in youth reflect the time spent performing vigorous PA (VPA) but not moderate PA (MPA) (Barker et al., 2018, Fussenich et al., 2016). For example, only ~ seven min·day^{-1} of VPA but not ~ 46 min·day^{-1} of MPA was associated with 57 and 64% reduced risk of overweight and hypertension in children (Fussenich et al., 2016). Additionally, VPA is positively associated with cardiorespiratory fitness (CRF) (Aires et al., 2010) and VPA delivered as high-intensity interval exercise (HIIE) is a key component to increase cardiorespiratory fitness (CRF) (Costigan et al., 2015). This is important as CRF has recently been demonstrated to have negative associations with CVD risk in a sample of European adolescents independent of PA levels (Barker et al., 2018). However, guidelines for VPA frequency and amount are currently unclear in terms of CVD risk modification, with the only statement suggesting that children and adolescents should perform VPA at least three times per week for bone and muscular strength development (WHO, 2010). Given the above, there is a strong rationale to further investigate PA intensities and CVD risk in youth.

In youth the likely mechanism of CVD risk reduction associated with PA and CRF is via modification of traditional CVD risk factors, which are typically expressed as a clustered score. However, improvements in traditional CVD risk factors accrued by PA in adults account for ~ 60% of CVD risk reduction, leaving a ~ 40% risk factor gap in current understanding (Mora et al., 2007). Recently, the improvements attributed to PA on the arterial and autonomic systems are suggested to, at least in part, explain the 40% risk factor gap in adults (Joyner and Green, 2009). Research involving adolescents has demonstrated increases in autonomic and vascular function independently of concomitant increases in traditional CVD risk factors (Bond et al., 2015a), which suggests the autonomic and vascular systems may represent

components of the risk factor gap in youth. Furthermore, studies suggest the existence of a positive association between VPA levels and autonomic and arterial function in children and adolescents (Gutin et al., 2005, Hopkins et al., 2009). However, it remains to be investigated the role of PA intensities, exercise and CRF on a mechanism linking autonomic and arterial systems.

One attractive approach to investigate a mechanistic association between the arterial and autonomic systems is the baroreflex assessed as the baroreflex sensitivity (BRS). The arterial BRS can be divided into a vascular and an autonomic component (Taylor et al., 2014) with the first obtained as vascular compliance, and the later as the autonomic responses to arterial stretching. Although in adults evidence shows an important role of regular exercise on BRS and its vascular and autonomic components (Monahan et al., 2001b, Hunt et al., 2001a, Komine et al., 2009), currently there is a dearth of investigations exploring the effects of exercise on the BRS in youth.

Historically, the atherosclerotic process has been associated with traditional CVD risk factors such as blood concentration (herein concentration will be expressed as between []) of triacylglycerol (TAG) and glucose (GLU), and BP measured in the fasted state (Berenson et al., 1998). However, in adults the postprandial state has been suggested to increase CVD risk independently of fasted measurements (Ansar et al., 2011, Uetani et al., 2012, Pirillo et al., 2014). In youth, longitudinal investigations have also shown that non-fasting [TAG] and postprandial GLU intolerance predicts CVD events during adulthood even after adjustment for adult CVD risk factors (Morrison et al., 2009, Franks et al., 2010). This suggests that the postprandial state may better reflect CVD risk due the fact that humans spent most of the day in the postprandial state (Nakamura et al., 2016).

In a similar manner to fasting CVD risk factors, exercise has been shown to modulate postprandial CVD risk in youth. For example, moderate and high-intensity exercise performed before the ingestion of a high-fat meal (HFM) or GLU load have been shown to decrease postprandial [TAG] and [GLU] respectively(Cockcroft et al., 2017b, Cockcroft et al., 2015, Tolfrey et al., 2014), suggesting a decreased CVD risk. Similarly, while an impairment in vascular function have been described in adolescents following a HFM (Bond et al., 2015b), performing moderate-intensity exercise before the meal maintains vascular function, whereas high-intensity interval exercise (HIIE) augments vascular function in the postprandial state (Bond et al., 2015b). However, it remains to be shown the possible effect of the postprandial state on the interactions between autonomic and vascular functions, as well as to investigate the effects of habitual PA and CRF on the postprandial responses.

Given the above, the overall aim of this thesis is to address the role of PA intensities, CRF, and exercise on the autonomic and vascular systems. For this purpose, a series of original investigations were conducted aiming to address the following broad questions:

1) Is there a role of the autonomic and arterial systems to the risk factor gap in adolescents?

2) How different PA intensities, CRF and exercise modulates autonomic and vascular functions?

3) Is there a dependence between the autonomic and vascular systems measured as the BRS?

4) Does PA, CRF and exercise modulate the postprandial changes in autonomic and vascular function?

Addressing these questions is important given the position of the American Heart Association which stresses the need of identifying novel CVD risk factors in youth, to help the management of the initial process of atherosclerosis in this population (Balagopal et al., 2011).

Chapter 2: Literature Review

The present literature review provides a comprehensive and critical justification for the work undertaken in this thesis. Where necessary, the reader is directed to contemporary reviews which offer a deeper discussion of the relevant themes. The literature review starts with a brief introduction of CVD and its childhood origins (sections 2.1 and 2.2) followed by discussion about the effects of PA, PA intensities, exercise and CRF on CVD risk reduction (section 2.3). The subsequent sections will discuss the risk factor gap concept and how PA intensities, exercise and CRF are associated with CVD risk reduction in adolescents by modifying the autonomic and vascular systems (sections 2.4 – 2.6). Finally, an overview of CVD risk and the postprandial state (section 2.7) will be introduced, with focus on the possible effects of PA intensities, exercise and CRF. When possible, paediatric literature will be critically scrutinised and gaps in the current evidence base highlighted. The aim of the literature review is to provide a context and rationale for each experimental chapter within this thesis (section 2.9).

2.1 Cardiovascular diseases

Cardiovascular diseases, including heart disease, cerebrovascular disease and peripheral vascular disease, share the underlying pathophysiological process of atherosclerosis. Atherosclerosis is a long lasting condition involving changes in the function and structure of the artery wall (Figure 2.1), culminating in CVD events such as myocardial infarction and stroke (Ross, 1993). Although the clinical overt characteristics of atherosclerosis occur during adulthood, there is compelling evidence

indicating that the initial stages of the atherosclerotic process may have its origins during childhood.

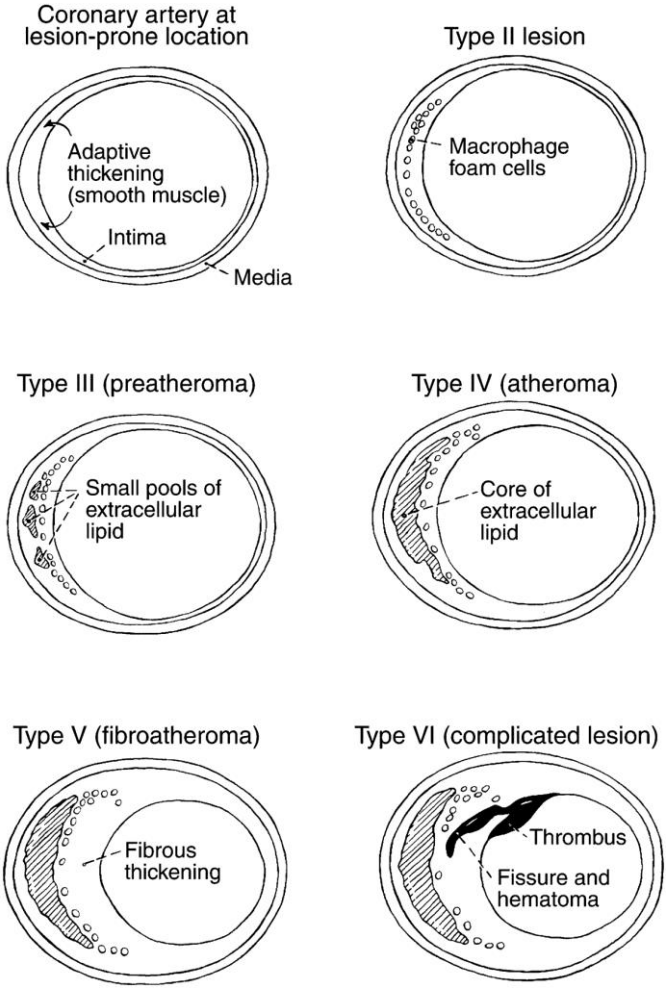

Figure 2.1: Stages of atherosclerosis. The initial stage is depicted in the top left which leads to a process of functional and structural changes to the vessel wall, resulting in a final stage of plaque rupture and thrombosis which is depicted in the bottom right. Adaptive thickening is the first observable structural change in the atherosclerotic process. Macrophage accumulation and lipid deposition with consequent formation of fatty streaks characterise type II lesions. Continued foam cell formation and macrophage necrosis can produce small extracellular pools of lipid in type III lesions. Areas of extracellular lipid represent the “core” of the atherosclerotic lesion in type IV. In this phase, a relatively thin tissue separation of the lipid core from the arterial lumen is observed. In type V lesions, a fibrous thickening of this structure forms and is also known as the lesion “cap”. Phase VI lesions exhibit architecture characterised by

calcified fibrous areas with visible ulceration. Reproduced from Stocker and Keaney (2004) with permission.

2.2 Childhood origins of cardiovascular diseases

A critical review of the evidence base for the childhood origins of atherosclerosis has been published by the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Youth (2011). Additionally, the initial atherosclerotic lesions in youth and the natural history of the disease have been reviewed (McGill et al., 2000). The evidence shows that the initial stages of atherosclerosis usually starts during childhood. Original data giving support to the prevalence of initial atherosclerotic lesions in the arterial wall measured post-mortem in young people have been collected in a series of epidemiological surveys by the Bogalusa Heart Study (Berenson et al., 1998, Berenson et al., 1992, Newman et al., 1991, Berenson, 2002). From these surveys, a seminal publication by Berenson et al. (1998), showed that there is a significant predisposition towards higher prevalence of fatty streaks in the coronary arteries of participants who died of traumatic diseases at the age of 15 – 19 years old (80% of prevalence) compared to participants who died at the age of 2 – 15 years old (50% of prevalence). Although the cross-sectional comparison between the different age groups impedes the conclusion of a possible longitudinal age effect, the data suggest an increase in the presence of type III arterial lesions during the first two decades of life.

Further support for the initial atherosclerotic progression in young people has been gathered in observational studies showing post-mortem histological adaptations of the arterial wall, which allowed classification of the lesion type according to the progression of atherosclerosis depicted in Figure 2.1. Phase III – IV lesions were not

present in children younger than 11 years old, and at the end of puberty these lesions were present in 69% of the participants (Stary, 2000). Although it is not possible to infer progression of lesions with ageing, the presence of type III – IV lesions in 95% of the 40 years old participants indicates that atherosclerosis lesions may follow the initial vessel adaptations observed at younger ages.

The studies by the Bogalusa Heart Study, and later by the Pathobiological Determinants of Atherosclerosis in Youth Study, also demonstrated that the atherosclerotic lesions were positively associated with the presence of traditional CVD risk factors in young people, highlighting the importance of measuring and tracking the progression of traditional CVD risk factors from a young age (Berenson et al., 1992, Newman et al., 1991).

2.2.1 Traditional cardiovascular disease risk factors

The term risk factor refers to a "measurable biological characteristics of an individual that precede a well-defined outcome of a disease" (Balagopal et al., 2011, p.2750). Cardiovascular disease risk factors were first identified by the Framingham Heart Study over half a century ago, and since then have been used in the investigation, risk stratification and progression of CVD (Bitton and Gaziano, 2010). According to an American Heart Association statement (Balagopal et al., 2011), traditional CVD risk factors in youth can be divided into four categories: constitutional (i.e. family history, age and sex); behavioural (i.e. diet, PA, tobacco exposure and perinatal exposures); physiological (i.e. BP, blood [lipids], obesity, and GLU metabolism); and medical diagnoses (i.e. diabetes and kidney disease).

Currently, evidence exist showing a positive association between post-mortem atherosclerosis lesions in youth and the presence of traditional CVD risk factors.

Specifically, the presence of one, two, three or four CVD risk factors (LDL, TC, BMI, and systolic and diastolic BP) increased significantly (~ 1.3% involvement with the presence of zero risk factors to ~ 11% with the presence of three or four risk factors) the percentage of intimal-surface involvement with fatty streaks in the coronary artery of participants who died at the ages of 2 – 39 years old (Berenson et al., 1998). In addition, the Pathobiological Determinants of Atherosclerosis in Youth Study proposed a CVD risk score derived from blood [lipids], BP, smoking, obesity and hyperglycaemia (i.e. modifiable risk factors) (McMahan et al., 2005). This score was developed to predict the likelihood of advanced arterial lesions in adolescents and young adults (<34 years old), rather than predict clinical CVD events (McGill et al., 2008). A higher risk score (> 11 points) was subsequently associated with ~ 10% probability of advanced atherosclerotic lesions (type III – IV) in the coronary artery of 15 – 19 years old individuals (McGill et al., 2008, McMahan et al., 2006). Collectively, these studies show a progressive association between atherosclerotic lesions and the number of traditional CVD risk factors in youth.

Numerous studies have described the presence of clustered CVD risk in apparently healthy youth (Raitakari et al., 1994, Andersen et al., 2006, Andersen et al., 2004, Andersen et al., 2008), measured as the cluster of two (Andersen et al., 2004) or three traditional CVD risk factors (Raitakari et al., 1994), with a prevalence of 2 – 15% (Andersen et al., 2003, Raitakari et al., 1994, Andersen et al., 2004) of clustered CVD risk in this population. This is problematic, due to the established positive associations between the number of clustered CVD risk factors and atherosclerotic lesions (Berenson et al., 1998). Furthermore, clustered CVD risk has been described to track into adulthood (Andersen et al., 2004), meaning an adolescent with clustered CDV risk is more likely to present clustered CVD risk across subsequent ages. These

studies provide a strong rationale to target modification of clustered CVD risk factors during childhood and adolescence.

Due to the childhood origin of atherosclerosis and its positive association with clustered CVD risk, the American Heart Association developed the concept of ideal cardiovascular health in 2010, aiming to determine metrics to measure, monitor and promote cardiovascular health (Lloyd-Jones et al., 2010). This concept stipulates seven cardiovascular health markers (four health behaviours: PA, diet, body composition and smoking, and three health factors: BP, blood [lipids] and GLU). An ideal cardiovascular health is identified with the presence of at least four ideal health markers. The number of adolescents, however, who achieve ideal cardiovascular health is concerning. For example, in Finland none of the adolescent participants (n = 1,098) presented all seven ideal markers, whilst the presence of five ideal markers decreased from 60.2% of participants at 15 years old, to around 34% at 19 years old (Pahkala et al., 2013). Notably, the health markers that participants mostly failed to achieve were PA and diet.

The consequences of a poor CVD risk profile observed in adolescence is reflected during adulthood. For example, the number of CVD risk factors measured at the age of 12 – 18 years old significantly ($P < 0.001$ for trend analysis between the groups with different number of CVD risk factors) predicted carotid intima-media thickness (cIMT), a non-invasive marker of atherosclerosis, in adulthood independently of adult CVD risk factors (Raitakari et al., 2003). Additionally, a poor ideal cardiovascular health profile in adolescents has been associated with an increased cIMT in adulthood (Laitinen et al., 2012), with the adolescents who score one ideal cardiovascular health marker presenting an advanced vascular age (~ 12 years) compared to adolescents

with six ideal cardiovascular health markers (Laitinen et al., 2012). These studies demonstrate the negative impact of CVD risk measured in youth on the preclinical arterial adaptations into adulthood.

In summary, atherosclerosis is a paediatric problem as its progression is positively associated with traditional CVD risk factors and clustered CVD risk during childhood and adolescence years (McGill et al., 2000). Early signs of a clustering of CVD risk factors are observed in the first decades of life which track into adulthood (Andersen et al., 2004), and predict preclinical atherosclerosis in adults (Pahkala et al., 2013). Therefore, strategies that reduce the burden of CVD should start by either preventing or modifying the development of CVD risk factors in young people (Magnussen et al., 2013). Importantly, prevention strategies are likely to be more impactful compared to risk factor modification, given that CVD risk measured in adolescents is associated with preclinical atherosclerosis markers in adults independently of adult CVD risk factors (Pahkala et al., 2013). Furthermore, one unit of increase in ideal cardiovascular health in youth is associated with a 25% decrease in the chances of having a cIMT above the 80th percentile in adulthood. These findings were controlled for the changes in the ideal cardiovascular health score between youth and adulthood (Laitinen et al., 2015), suggesting that CVD prevention in youth is paramount.

2.3 Cardiovascular risk reduction via physical activity, cardiorespiratory fitness and exercise

A lack of PA is considered a major cause of chronic disease, including CVD (Booth et al., 2012). In adults, as recently reviewed by the Physical Activity Guidelines Advisory Committee (Committee, **2018**), strong evidence demonstrates a significant inverse relationship between MVPA and CVD mortality. Importantly, the evidence discussed

by the Committee involved the inclusion of 10 systematic reviews including nine meta-analyses. The aim of this section is to focus on the paediatric literature supporting the role of PA intensities, CRF and exercise on CVD risk reduction.

2.3.1 Physical activity

Physical activity is defined as any bodily movement produced by skeletal muscles that results in increases in energy expenditure above baseline, and exercise is defined as a structured form of PA aiming to increase or maintain physical fitness (Caspersen, 1989). Physical activity and exercise can be further divided into different intensities such as light, moderate and vigorous. Estimates of PA can be done using direct and indirect measures which have been reviewed elsewhere (Loprinzi and Cardinal, 2011). However, the best method available considering a trade-off between feasibility and validity is accelerometry (Hallal et al., 2012). However, controversy exists in the literature regarding the stratification of PA intensities, with the best approach to define PA intensity still open to debate (Schaefer et al., 2014). Regardless of these limitations, the following section will highlight the evidence regarding PA intensities and CVD risk reduction in youth. Where possible, direct methods of PA assessment will be discussed, considering the well documented overestimation of PA using indirect self-report methods such as questionnaires (Kavanaugh et al., 2015).

Due to the lack of clinical manifestation of CVD in youth, the likely mechanisms by which PA decreases CVD risk in this population, and consequent future CVD risk in adulthood, is via modifying or preventing clustered CVD risk. A landmark investigation on the associations between PA and clustered CVD risk factors was conducted by Andersen et al. (2006). This study is interesting as it investigated the cross-sectional associations between objectively measured PA intensities and clustered CVD risk, on

the contrary to previous research investigating isolated CVD risk factors and using subjective estimates of PA. Furthermore, in this investigation a large cross-sectional sample of the European Youth Heart Study involving over 1,700 youth aged 9 – 15 years old was investigated. The results demonstrated that youth in the first, second and third less active quintiles for MVPA presented 3.29, 3.13 and 2.51 higher odds of clustering of systolic BP (SBP), TAG, ratio of total cholesterol to high density lipoprotein (HDL), insulin sensitivity, sum of four skinfolds, and CRF compared to the most active quintile.

These findings were later corroborated in other cross-sectional investigations by the European Youth Heart Study. In a sample of 1,769 youth aged 9 – 15 years old from three different countries, Andersen et al. (2008) showed that the least active quartile for mean acceleration counts obtained from accelerometers, presented ~ 80% higher chance of clustered CVD risk compared to the most active quartile. Importantly, the authors controlled the analysis for CRF and body fatness, meaning the observed associations were attributed to PA levels. Other studies have further corroborated an inverse association between total PA and clustered CVD risk in adolescents (Ekelund et al., 2007). Finally, evidence of the positive effects of PA on CVD risk reduction was obtained in a study with a pooled analysis of 14 cross-sectional investigations in which accelerometer data were available in 20,871 4 – 18-year olds. The results demonstrated that after controlling for age, sex, accelerometer wear time, body composition and sedentary time, MVPA was negatively associated with waist circumference (beta regression (β) = -0.54), SBP (β = -0.17), TAG (β = -0.03), and insulin sensitivity (β = -0.009) (Ekelund et al., 2012). Due to the inclusion of sedentary time as a covariate, the authors concluded that the associations between MVPA are independent of sedentary time, although sedentary behaviour was not considered.

In summary, these studies provide evidence of the cardio protective effects of PA, specifically MVPA. In general, these findings, amongst others (Janssen and Leblanc, 2010), have been used to support the recommendation that children should perform a minimum of 60 min·day^{-1} of MVPA (WHO, 2010). However, one important finding by the European Youth Heart Study was that 15 year old adolescents who presented a lowered clustered CVD risk performed ~ 88 min·day^{-1} of MVPA (Andersen et al., 2006), which exceeds the minimum recommended. This is especially concerning, as the literature shows that over 80% of 13 – 15 years old from 105 countries do not meet the minimum 60 min·day^{-1} of MVPA (Hallal et al., 2012). Furthermore, PA levels have been shown to decrease during adolescence (Reilly, 2016), and intervention studies designed to increase PA have only led to four min·day^{-1} increase in PA levels (Metcalf et al., 2012).

2.3.2 Physical activity intensity

Recently, studies have suggested that the negative associations between MVPA and CVD risk may reflect time spent performing VPA but not MPA (Barker et al., 2018, Fussenich et al., 2016). This is especially problematic as most of the literature investigating CVD risk reduction in youth have focused on MVPA levels, although guidelines also suggest youth to perform VPA at least three times per week to improve bone and muscular health (WHO, 2010). Unlike guidelines for adults, e.g.(Bull, 2010), suggestions for VPA are not specific in terms of minimum duration, and studies investigating VPA and CVD risk in youth are less in number compared to MVPA. The aim of this section, therefore, is to provide a justification for the role of VPA on CVD risk reduction.

Few key studies have investigated the associations between VPA and traditional CVD risk factors in apparently healthy youth. For example, in a cross-sectional observational study, Hay et al. (2012) demonstrated that 12 year old adolescents in the highest compared to the lowest tertile of VPA presented 57 and 64% lower chances of being classified as overweight and hypertensive, respectively. Further analysis of the data revealed that the risk reduction associated with VPA was obtained with only ~ 7 min·day^{-1}, but similar findings were not observed with ~ 46 min·day^{-1} of MPA. Although these results demonstrate associations between VPA, body weight status and BP, the authors did not investigate clustered CVD risk.

Recently Fussenich et al. (2016) investigated the cross-sectional differences in CVD risk between PA intensities. The authors divided 182 children between 9 – 11 years old into quintiles based on MPA and VPA levels. The results demonstrated a significant five times higher odds of clustered CVD risk in the least active children in terms of VPA, but not MPA. Interestingly, the sharpest decline in risk (50%) was observed in the second least active compared to the least active quintile for VPA. The least active quintile performed ~ 11 min·day^{-1} of VPA compared to ~ 17 min·day^{-1} for the second least active quintile, leading to the suggestion that 17 min·day^{-1} of VPA may be associated with 50% reduction in clustered CVD risk (Fussenich et al., 2016). Furthermore, another recent investigation has demonstrated that VPA (standardised β (stβ) = -0.159) but not MPA (stβ = -0.05) was negatively associated with clustered CVD risk in 534 adolescents aged 12 – 17 years old (Barker et al., 2018). Interestingly, the significant negative association between VPA and clustered CVD risk disappeared when CRF was inserted into the regression models, and to date the different contributions of CRF and VPA are unclear (Barker et al., 2018). However, it may be speculated that a shared variance exists between VPA and CRF given that increases

in CRF are determined mostly by the intensity of the exercise stimulus (Costigan et al., 2015).

Although the literature suggests that VPA is associated with CVD risk independently of MPA, causality cannot be inferred due to the cross-sectional nature of the investigations; for example, reverse causality may be present in that youth with a lower CVD risk perform more VPA. In contrast to cross-sectional investigations, longitudinal data may enable direction of causality to be inferred. In a two year longitudinal study involving 315 adolescents aged 9 – 15 years old, VPA measured at baseline was associated with better weight status (stβ ranging from -0.58 – -0.03 for the different VPA quartiles) and SBP (P = 0.06 for comparison between VPA quartiles) at follow up, whilst MPA was associated with weight status (P = 0.04 for comparison between MPA quartiles) (Carson et al., 2014). These results provide evidence of a casual association between VPA, weight status and SPB in youth. Several key aspects of Carson et al. (2014) study are worth noting. Firstly, the narrow range observed between the VPA quartiles (1 – 8 min·day^{-1}) shows pronounced effects of seven min·day^{-1} of VPA, as opposed to ~ 25 min·day^{-1} necessary between MPA quartiles; and secondly, all regression models were controlled for the other PA intensities, suggesting that the observed VPA associations are independent of MPA. However, the lack of inclusion of clustered CVD risk indicates that causality between VPA and decreased clustered CVD risk is yet to be observed.

Given the described associations and a possible causal effect of VPA, one attractive approach to deliver VPA is through high-intensity exercise. This can be achieved by isolating individual intensities via assessing an individual's metabolic transition points to determine moderate (below ventilatory threshold) and high (above ventilatory

threshold) intensity exercise. In high-intensity exercise protocols, to increase session volume, studies normally use an interval approach, described as high-intensity interval exercise (HIIE). In adults, the effects of HIIE training on the prevention and management of CVDs, such as coronary artery disease, heart failure, stroke and hypertension have been reviewed elsewhere (Hussain et al., 2016). The conclusion is that HIIE leads to a similar, or in some cases superior, benefits compared to moderate exercise in adults.

In youth, there is emerging research investigating the health benefits of HIIE training (Logan et al., 2014).A recent systematic review with meta-analysis investigated the effects of HIIE training compared to control or moderate-intensity interventions on CRF and body composition. In this review, 20 original studies were included, and the results were positive in favour of the HIIE training for improvements in CRF (pooled effect size (*d*) = 1.05; 95% confidence interval (CI) = 0.36 – 1.75), and body composition (pooled *d* = -0.37; 95% CI= -0.68 – -0.05). This meta-analysis provided level one evidence for the beneficial effects of HIIE training on body composition and CRF (Costigan et al., 2015), although the effects of HIIE training on clustered CVD risk are yet less clear. Nevertheless, Logan et al. (2014) in a narrative review concluded that the current body of evidence demonstrates the efficacy of HIIE training to improve a multitude of CVD risk factors, such as body weight status, BP, CRF, body composition, blood lipids and GLU. However, numerous research questions still await to be addressed such as the optimum HIIE protocol in terms of duration, frequency and intensity. Finally, it is important to highlight that the current evidence in youth gives support to the proposition that HIIE training is an important determinant of improvement in CRF (Costigan et al., 2015).

2.3.3 Cardiorespiratory fitness

Cardiorespiratory fitness can be defined as the integrated capacity of the cardiovascular and respiratory systems to supply oxygen to the contracting muscles, and the capacity of the muscles to use oxygen (Booth et al., 2012). Cardiorespiratory fitness is normally assessed as the maximum oxygen uptake ($\dot{V}O_2max$). Importantly, CRF is associated with CVD risk reduction independently of PA. This concept has been investigated in adults in a seminal meta-analysis involving 23 study cohorts and more than 1,300,000 participants per follow-up years in which the risk of coronary artery disease and CVD were the main outcomes. The results indicated that the risk of CVD and coronary artery disease drastically decreased in the first 25th percentile for CRF, and this decrease was not matched by the change in risk observed for PA levels reaching the 100th centile. The findings of the meta-analysis revealed a stronger impact of CRF compared to PA on CVD risk reduction (Williams, 2001).

In youth, the literature also indicates that independent of PA, CRF is associated with a decreased CVD risk (Ekelund et al., 2007, Ruiz et al., 2014). For instance, cross-sectional data from The European Youth Heart Study have shown a negative and significant association (stβ = -0.09) between CRF and clustered CVD risk in 1,709 youth aged 9 – 15 years old (Ekelund et al., 2007). This association was still present when PA levels were included in the regression model. In this study, CRF was estimated as peak power obtained in a cycling test and expressed as W per kg of fat free mass per minute. In a similar investigation using data from the European Youth Heart Study, Hurtig-Wennlof et al. (2007) demonstrated that CRF, expressed as peak power per kg of body mass, was significantly associated with CVD risk in youth aged 9-15 years old. From the canonical association it was demonstrated that 37% in the variance in CVD risk was explained by CRF. These findings were influenced by the

negative association between CRF and body fat (BF) expressed as the sum of biceps, triceps, subscapular, suprailiac and triceps surae skinfolds. Finally, a more recent study also corroborates the negative association between CRF and CVD risk in adolescents (Barker et al., 2018). In this later investigation, Barker et al. (2018) demonstrated that after controlling for PA levels an inverse association (stβ = -0.281) exists between clustered CVD risk and CRF, the later estimated from a 20m shuttle run test and expressed as $mL \cdot kg^{-1} \cdot min^{-1}$.

Cardiorespiratory fitness has also been associated with better ideal cardiovascular health scores. For example, a positive linear association exists between the number of ideal cardiovascular health metrics and CRF obtained from the 20m shuttle test in a sample of 510 European adolescents aged 12 – 17 years old (Ruiz et al., 2014). In this later investigation, it was demonstrated that an ideal cardiovascular health is associated with a CRF threshold of 43 and 34.6 $mL \cdot kg^{-1} \cdot min^{-1}$ for boys and girls, respectively. These suggested thresholds are particularly important to monitor cardiovascular health in youth, given the role of the ideal cardiovascular health on CVD risk progression (see section 2.2.1).

Longitudinal studies also evidence a possible effect of youth CRF on future CVD risk reduction. A systematic review conducted by Ruiz et al. (2009) provided strong evidence indicating that high levels of CRF during childhood predicts a better CVD risk profile in adulthood. Strong evidence was defined by the authors as evidence gathered from at least three studies deemed as high quality in the review. In addition to the longitudinal associations between CRF and CVD risk, a recent investigation has demonstrated that a one standard deviation increase in CRF (expressed as peak power per kg of body mass) measured in 743,498 adolescents at the age of 18 year

old was associated with an 18% decrease in risk of a myocardial infarction in a follow-up of 34 years (Hogstrom et al., 2014). Collectively, these studies indicate a strong role of CRF on CVD risk reduction and CVD events both in cross-sectional and longitudinal investigations.

The mechanisms by which CRF confers risk reduction via changes in traditional CVD risk factors is poorly understood. For example, in experimental exercise training studies, improvements in CRF are not paralleled by improvements in traditional CVD risk factors in adults with elevated CVD risk (Hartman et al., 2018), suggesting different pathways of cardiovascular protection. Additionally, the several different forms by which CRF is measured and expressed limits conclusions about CRF and CVD reduction. It has been argued that CRF *per se* may not be as important as body fatness. For example, when CRF is expressed against body mass using the ratio standard method (e.g. $mL \cdot kg^{-1} \cdot min^{-1}$) heavier children will inevitably present a lower CRF due to the phenomenon of 'over-scaling' (Loftin et al., 2016). Since lower CRF is associated with CVD risk, it seems plausible to question the inference of body fatness, which increases overall body weight and consequently decreases CRF. This scaling problem was recognized several decades ago (Armstrong and Welsman, 1994), and still clouds the interpretation of CRF in youth. Therefore, it is perhaps not surprising that Hurtig-Wennlof et al. (2007) found that adolescents with a lower CRF presented elevated CVD risk, as this is likely confounded by differences in BF content. Future investigations are encouraged to consider a size-free measure of CRF to investigate the mechanistic pathways by which CRF confers CVD risk reduction, either by scaling for fat free mass and/or using allometric approaches (Loftin et al., 2016).

In summary, the current evidence base indicates that MVPA, VPA and CRF all play an important role in modifying CVD risk in youth. However, it is not clear the duration and amount of VPA and HIIE important to consider when promoting CVD risk reduction in youth.

2.3.4 The risk factor gap

As evidenced above, PA intensities and CRF measured in youth are inversely associated with CVD risk factors in youth and later in adulthood. However, it is still debated whether improvements in traditional CVD risk factors fully translates into CVD risk reduction, with a risk factor gap being proposed in our current understanding of CVD risk reduction (Joyner and Green, 2009). The notion that improvements in traditional CVD risk factors do not fully explain risk reduction has been suggested in a narrative review (Swift et al., 2013). Swift et al. (2013) concluded that PA and CRF conferred CVD risk reduction between 12 – 60%, with traditional CVD risk factors accounting for 5 – 15% of the reduced risk (Swift et al., 2013). Importantly, the evidence presented by Swift et al. (2013) was gathered from longitudinal studies and systematic reviews, reinforcing the notion that improvements in traditional CVD risk factors does not fully explain CVD risk reduction.

In addition to the narrative review by Swift et al. (2013), the percentage of contribution from different CVD risk factors towards CVD risk reduction caused by PA has been previously examined in a seminal investigation by Mora et al. (2007). These authors were the first to investigate, in a sample of 27,055 women, how PA contributes to reduction in CVD and coronary heart disease via improvements in traditional CVD risk factors (and novel risk factors such as inflammatory markers). PA levels were estimated using questionnaires at baseline and participants were followed-up for

nearly 11 years. After controlling for smoking, diet habits, family history, menopause and hormone use, the results indicated that improvements in CVD risk factors accrued by PA explained 59% of the CVD risk reduction. The conclusion was the presence of 41% CVD risk reduction that could not be attributed to improvements in traditional CVD risk factors (Figure 2.2) (Mora et al., 2007).

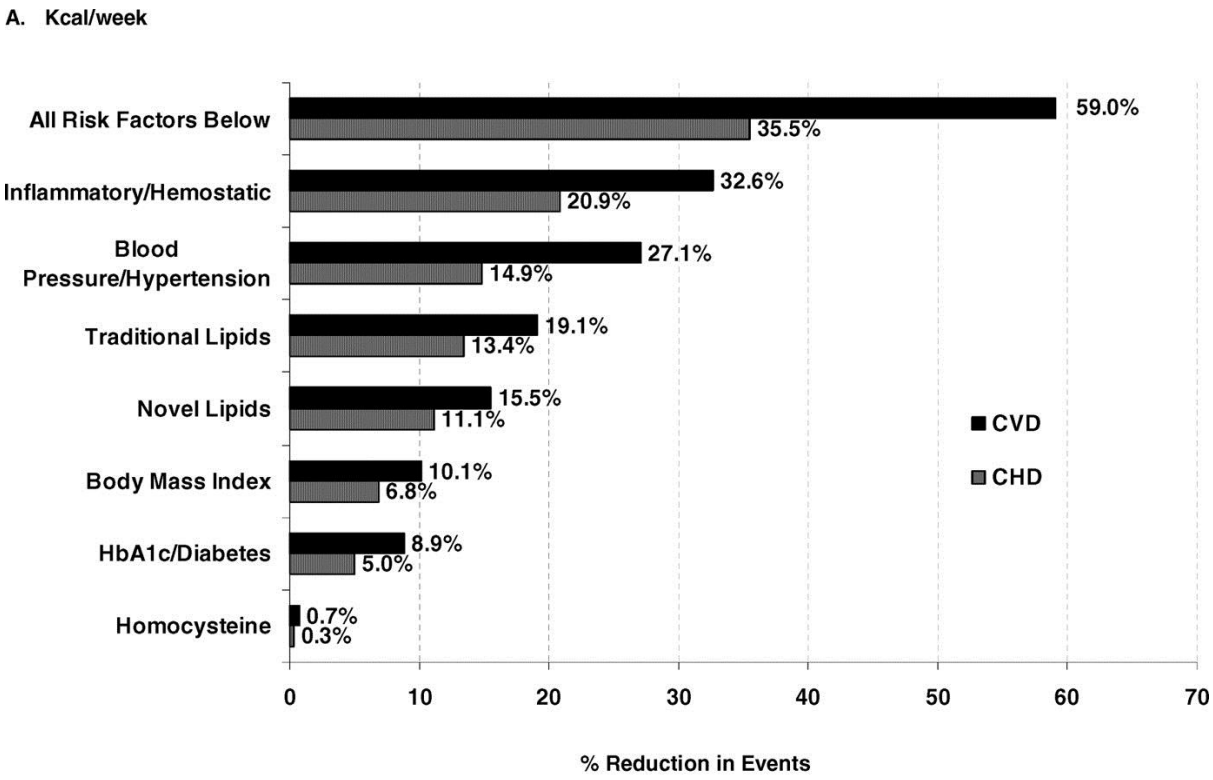

Figure 2.2: Reduction in cardiovascular disease associated with physical activity is only partially explained by traditional cardiovascular disease risk factors suggesting the existence of a risk factor gap. Reproduced from Mora et al. (2007) with permission.

Several limitations should be considered before generalisation of the findings by Mora et al. (2007). For example, despite the large sample size of over 27,000 participants, PA was estimated using questionnaires and only women were included in the analysis. Additionally, PA was represented as gross energy expenditure in kilocalories per week, meaning the effect of PA intensity was not investigated. Despite these

limitations, Mora et al. (2007) was the first original investigation to demonstrate that improvements in traditional CVD risk factors do not fully explain CVD risk reduction.

Over the last several decades, the arterial and autonomic nervous systems have emerged as putative novel CVD risk factors with improvements in these systems being proposed to explain benefits of PA on CVD risk reduction beyond the traditional risk factors (Green et al., 2008, Joyner and Green, 2009). Experimental data supporting the contribution of the arterial system to the risk factor gap have been gathered in a series of original investigations. These investigations demonstrated that in healthy adults, as well as adults with the presence of clinical CVD, a combination of aerobic and resistance exercise completed three times per week over a course of eight weeks, led to increases in arterial function (assessed non-invasively using flow mediated dilation (FMD)) with no concomitant improvements in traditional CVD risk factors (Green et al., 2003). To further demonstrate the important effects of exercise on FMD, the results of 13 exercise trials with a total of 183 participants were pooled and it was demonstrated that improvements in FMD were not significantly associated ($r < 0.25$) with improvements in traditional CVD risk factors such as CRF, blood GLU, BP, and body composition (Green et al., 2014). This is especially important, given the fact that arterial dysfunction is positively associated with mortality and CVD events in adults, and is considered the sentinel event into the atherosclerotic process (Green et al., 2011).

Similarly, the role of cardiac autonomic function on CVD risk in adults has been reviewed with the suggestion that cardiac parasympathetic activity is negatively associated with all cause of mortality, as well as CVD risk factors (Thayer et al., 2010, Thayer and Lane, 2007). A possible mechanism by which a higher cardiac autonomic

function may decrease CVD risk independently of improvements in traditional CVD risk factors is via a decreased resting HR, cardiac work and myocardial oxygen demand (Buch et al., 2002). Another likely mechanism is a decreased sympathetic influence on the vessel and consequent improved vasodilatory capacity and maintenance of BP (Charkoudian et al., 2006). However, on the contrary to vessel function, there are a lack of original investigations demonstrating a possible influence of PA on the autonomic system independently of traditional CVD risk factors.

The highlighted adult literature presents the possible role of the autonomic and arterial systems as components of the risk factor gap. In youth, the influence of these systems beyond traditional CVD risk factors has received little attention to date. A recent cross-sectional investigation included FMD into a clustered CVD risk score in 9 – 11 years old and identified decreases in the composite CVD risk score across VPA groups (Fussenich et al., 2016). However, the authors did not aim to investigate whether the inclusion of FMD increased the magnitude of association between VPA and CVD risk. This would help to identify the unique variance in CVD risk attributed to the inclusion of FMD and traditional CVD risk factors.

Another recent investigation in youth adds to the importance of autonomic and arterial functions on modification in CVD risk. It was demonstrated by Bond et al. (2015a) that improvements in both arterial and autonomic functions were observed after two weeks of HIIE training without concomitant improvements in traditional CVD risk factors and CRF (Bond et al., 2015a). The lack of significant relationships between the delta changes in arterial and autonomic function in this study showed that short-term adaptation in these systems may occur in an independent manner. However, further research is needed to investigate the possible contribution/associations between PA

intensities, CRF and the autonomic and vascular systems independently of traditional CVD risk factors, as well as a possible interdependence between these systems.

2.4 Autonomic function

The aim of this section is to introduce the reader to cardiac autonomic function. An overview of assessment approaches and physiological significance will be introduced, followed by a discussion on how PA intensities and CRF are associated with cardiac autonomic activity.

2.4.1 Assessment of autonomic function

The autonomic nervous system is divided into sympathetic and parasympathetic branches (Curtis and O'Keefe, 2002). For a more detailed review of the methods available to estimate autonomic function the reader is directed to Seals (2011). In this thesis, the specific autonomic effects on the heart will be addressed, with the sympathetic branch causing tachycardia whilst the parasympathetic branch leads to bradycardia. At rest, cardiac parasympathetic activity is predominant, and a diminished cardiac modulation by the parasympathetic activity has been positively associated with increased CVD risk and the progression of atherosclerosis in adults (Thayer and Lane, 2007, Thayer et al., 2010, Huikuri et al., 1999). In the following section, the use of heart rate variability (HRV) and heart rate recovery (HRR) to estimate the autonomic nervous system is discussed. Both HRV and HRR are particularly attractive outcomes due to their well-defined physiological determinants and prognostic value (Zulfiqar et al., 2010, Pecanha et al., 2017). The non-invasive nature of HRV and HRR measurements are also ideal for studies involving a paediatric population who have unique ethical considerations.

Although both HRV and HRR are mainly influenced by parasympathetic activity, the physiological determinants of each are distinct. While HRV is determined by the modulation of vagal tone, HRR is mainly determined by saturation of the cholinergic receptors in the heart (Malik and Camm, 1993, Dewland et al., 2007, Buchheit et al., 2007b). For example, increase in parasympathetic activity in the sinoatrial node by selective inhibition of acetylcholinesterase (enzyme responsible for the breakdown of acetylcholine) leads to an increase in HRR, but not HRV (Dewland et al., 2007). Additionally, HRR is also related to the sympathetic influence on the HR during the exercise bout, as well as the contribution of anaerobic metabolism, including the phosphocreatine utilisation and lactate production during exercise (Buchheit et al., 2007a). Reducing HRR to only a measure of parasympathetic reactivation is, therefore, questionable (Buchheit et al., 2007b). These studies also indicate that a comprehensive appraisal of cardiac autonomic function should involve measurements of both rest and recovery indices.

2.4.1.1 Heart rate variability

Heart rate variability is the measurement of the variations between the time for each ventricular depolarisation, which can be quantified using time and frequency domains, as well as nonlinear fractal approaches (Task-Force, 1996). Table 2.1 presents the HRV time and frequency domain indices and their physiological significance. Due to the well-known parasympathetic influence, two indices are highlighted. In the time domain, the square root of the mean of the sum of the squares of differences between adjacent heart beats (RMSSD) is derived from the mathematical average of the time in ms between each heartbeat. In the frequency domain, a mathematical approach (normally a Fast Fourier Transformation), is used to derive a frequency spectrum of the time in ms between each heartbeat. The frequency spectrum is then divided into

low (0.04 – 0.15 Hz) and high (0.15 – 0.40 Hz) frequencies, named LF and HF respectively. The power (ms^2) in the HF band is used as the parasympathetic determinant, whilst the power in the LF component can infer baroreflex control of BP (see section 2.6) and overall autonomic modulation (Rahman et al., 2011, Goldstein et al., 2011). Therefore, caution should be taken when interpreting the physiological significance of the LF band, and the LF/HF ratio, which is commonly used as an indication of the sympathetic/parasympathetic balance (Billman, 2013).

The parasympathetic contribution to the HF index has been described in studies using drug administration to block the autonomic influence on the heart. For example, administration of atropine, which blocks the acetylcholine receptors in the heart, abolishes the power in the HF band (Pomeranz et al., 1985, Akselrod et al., 1981). On the contrary, the administration of propranolol, a well-known beta blocker, does not change HF power. The RMSSD has been shown to present a strong and positive association ($r = 0.87$) with the HF component (Bigger et al., 1989) indicating similar parasympathetic determinants (Shaffer and Ginsberg, 2017).

The measurement of HRV can be performed using two valid approaches. The first approach is by using a long-term (24 h) recording and the second approach is by a short-term (5 min) recording. For short-term HRV measurements, well controlled conditions are encouraged (Task-Force, 1996). For example, previous exercise (i.e. 2 h before the measurements), caffeine and food ingestion, room temperature, length of the rest period preceding data collection, and breathing frequency all have the potential to alter HRV outcomes (Task-Force, 1996). However, it should be noted that debate exists (Shaffer and Ginsberg, 2017) on whether to control breathing frequency for HRV measures, with Williams and Lopes (2002) showing increases in HF when

breathing control was used in 15 year old adolescents. Table 2.1 provides an overview of HRV measurements using the short- and long-term approaches.

Table 2.1: Summary of time and domain indices of heart rate variability.

Indices	Domain	Physiological determinant	Data collection method
Very low frequency	Frequency (< 0.04 Hz)	Renin angiotensin system/ temperature control	Long-term
Low frequency	Frequency (0.04 – 0.15 Hz)	Parasympathetic and sympathetic	Long-term and short-term
High frequency	Frequency (0.15 – 0.4 Hz)	Parasympathetic	Long-term and short-term
Low frequency/ High frequency ratio	Arbitrary units	Parasympathetic and sympathetic	Long-term and short-term
Standard deviation of all inter-beat intervals.	Time	Parasympathetic and sympathetic	Long-term and short-term
RMSSD	Time	Parasympathetic	Long-term and short-term
Number of pairs of inter-beat intervals differing by more than 50 ms	Time	Parasympathetic	Long-term and short-term

RMSSD: square root of the mean of the sum of the squares of differences between adjacent heart beats.

Importantly, the control of confounding factors influences the reliability of HRV indices. For instance, previous food ingestion and exercise are known to decrease HRV (Bond et al., 2015a) which may lead to an erroneous risk stratification. Additionally, a recent meta-analysis involving 1,841 youth aged 5 – 18 years old showed that

inconsistencies in recording duration, prerecording acclimatisation, and frequency bandwidth selection, cloud the current interpretation of HRV reliability in youth (Weiner and McGrath, 2017). Nevertheless, HRV has been shown to present moderate (Fisher's Z = 0.62 and r = 0.55 from pooled meta-analysis for HF) to high (Fisher's Z = 1.00 and r = 0.76 from pooled meta-analysis for RMSSD) reliability obtained from a recent meta-analysis (Weiner and McGrath, 2017). However, despite being obtained from a meta-analysis including a large sample size (n = 1,841) based on 18 different studies, the pooled correlation reported by the authors provides an estimate of rank position and limits a comprehensive evaluation of mean bias (indicative for a learning/fatigue effect) and within-participant variability (variation at an individual level) of the HRV measures in the paediatric literature. The variability in HRV has recently been addressed in adolescents aged 14 – 19 years old, and the mean coefficient of variation (CV) obtained for RMSSD and HF was lower than 20% for short-term measurements (Farah et al., 2016).

2.4.1.2 Heart rate recovery

In addition to the resting HRV measurements, the post-exercise recovery indices of HRV and HRR can be used to measure parasympathetic recovery. During exercise there is a gradual intensity-dependent parasympathetic withdrawal, and in the first minute after exercise cessation there is a parasympathetic reactivation (Pecanha et al., 2017). Quantifying parasympathetic reactivation following exercise can be achieved using several measurements. For example, the number of heart beats recovered in the first minute of exercise (HHR60) (Cole et al., 1999), the slope of the linear association between heart rate recovery in the first 30 s (T_{30}) (Imai et al., 1994), the short-term (i.e. 5 and 10 min) recovery of the RMSSD (Goldberger et al., 2006),

and the time HR (HRRt)takes to achieve its asymptote in the first 10 min of recovery (Javorka et al., 2003).

The pros and cons of these measurements have been recently reviewed, as well as the physiological determinants and respectively prognostic value (Pecanha et al., 2017). Parasympathetic tone is the main determinant of the T_{30} following exercise, and an interplay between parasympathetic reactivation and sympathetic withdrawal is what determines HRRt (Pecanha et al., 2017). Heart rate recovery indices present moderate reliability in adults (intraclass coefficient of correlation (ICC) for T_{30} and HRRt of 0.62 – 0.77 and 0.71 – 0.74, respectively) (Pecanha et al., 2017). In youth, reliability of post-exercise measurements of HRR has been demonstrated in one investigation involving 17 adolescents aged 15 years old (Buchheit et al., 2008). The results showed acceptable reliability for the HRR60 (ICC = 0.70), HRRt (ICC = 0.86), and T_{30} (ICC = 0.73), although CVs, which represent variation at an individual level, were not reported.

The physiological factors during exercise that influence HRR in youth have been previously investigated. Buchheit et al. (2010) demonstrated that lactate concentration and blood pH throughout repeated sprints are significant related to HRRt in children aged nine years old. In this study, HRRt was positively associated with mean power output (r = 0.48), lactate concentration (r = 0.58), and blood pH (r = 0.48) obtained during repeated sprint exercise. Due to a lower mean output, lower lactate concentration and maintenance of pH values close to baseline, children presented a faster HRRt (~ 20 s) compared to adolescents (~ 40 s) and adults (~39 s), with no differences observed between adolescents and adults (Buchheit et al., 2010). However, it is not clear whether these findings of HRR obtained following repeated

sprints are replicable in other exercise modalities, such as HIIE and exercise to exhaustion. Given that the exercise characteristics influence HRR in youth, standardisation of the exercise bout is essential.

In summary, measurements of HRV and HRR provide non-invasive and reliable estimates of the parasympathetic modulation and are suitable to investigate the role of the autonomic system as part of the risk factor gap in adolescents.

2.4.2 Physical activity and autonomic function

The role of PA and exercise on autonomic function has been extensively documented in the adult literature. For example, in a meta-analysis conducted over a decade ago, 13 studies using exercise training interventions investigating whether exercise training alters autonomic modulation. A significant pooled effect was observed in favour of exercise training on resting HF (pooled d = 0.48) (Sandercock et al., 2005). In youth several original research studies have investigated whether PA and exercise training changes resting HRV, although less is known about HRR in this population. Estimates of PA by questionnaire show that daily PA or sports participation are positively related to an increase in resting HRV (Radtke et al., 2013a, Henje Blom et al., 2009). However, due to the overestimation of PA by self-reported methods (Corder et al., 2009), investigations using objective measurements of PA are encouraged. Using accelerometer data, Gutin et al. (2005) showed a positive association between MVPA and RMSSD (stβ = 0.18) in a sample of 304 adolescents aged 14 – 18 years old. Importantly, all associations were controlled for sex, race, body composition and CRF. However, the authors did not compare the possible associations between PA intensities and sedentary outcomes which would provide relevant information about PA intensity (see section 2.3.2).

Other studies have investigated the associations between HRV and PA intensities. For example, Radtke et al. (2013b) investigated associations between MPA, VPA and resting RMSSD in adolescents aged 15 years old. The authors showed a significant association between MPA and RMSSD (stβ = 0.448) after controlling for age, sex, maturity status and body fatness. Significant associations between VPA and RMSSD were not observed (stβ = 0.011). On the contrary, Buchheit et al. (2007c) in a sample of pre-pubertal 12 year old children showed significantly higher HF for children performing more than 60 min·week^{-1} of VPA (HF/HF+LF = 0.50 ± 0.1 ms) compared to children performing less (HF/HF+LF = 0.42 ± 0.1 ms). These findings were adjusted for age, sex, and body fatness. The differences between the age of the participants, and data analysis (i.e. RMSSD and HF/total power ratio) and data collection (min·week^{-1} vsmin·day^{-1}) approaches limits an overall conclusion to be achieved. Given that a considerable number of studies exist investigating PA, PA intensities and HRV in youth, a systematic review approach may help to elucidate the potential associations between PA, PA intensities and cardiac autonomic function in a paediatric population, as well as explore possible limitations in the current evidence base.

Another limitation on our current understanding is that no studies have measured autonomic recovery, which would provide insight into associations between different PA intensities, resting and recovery indices of autonomic function in youth. Similarly, it remains to be determined whether any possible associations between PA, HRV and HRR exist after accounting for traditional CVD risk factors, or whether the associations are stronger compared to the associations between traditional CVD risk factors and PA.

Regarding exercise training, a meta-analysis with the inclusion of only two studies investigating aerobic training (n = 29) against a control group (n = 28) in pre-pubertal children revealed no significant improvements in any of the parasympathetic HRV measures (da Silva et al., 2014a). The strict inclusion criteria of the meta-analysis limits the findings to only pre-pubertal children and no systematic review has included mid or post-pubertal adolescents. If there are differences in HRV training responses between pre-pubertal, mid-pubertal and post-pubertal children, it is currently unknown. However, due to a higher HF reported in 16 years old (2858 ± 540 ms^2) compared to 8 years old (1559 ± 332 ms^2) (Lenard et al., 2004), it is plausible that the training responses in different age/maturity groups may also differ. In a recent study investigating HIIE training on RMSSD in 13 – 14 years old boys and girls, a significant improvement was observed following two weeks of training (pre = 66.2 ± 23.6, post = 84.4 ± 27.2 ms) (Bond et al., 2015a). Research is still needed to elucidate exercise training characteristics such as intensity, duration, and frequency associated with HRV improvements in youth.

2.4.3 Cardiorespiratory fitness and autonomic function

The evidence for a possible association between CRF, HRV and HRR in youth is unclear. For example, Brunetto et al. (2005) showed that 15 year old adolescents divided into tertiles of $\dot{V}O_2max$, directly measured from an incremental running protocol and expressed as $mL \cdot kg^{-1} \cdot min^{-1}$,presented similar RMDDS values (1.7 ± 0.2; 1.8 ± 0.2, and 1.8 ± 0.1 ms) for the first, second and third tertile, respectively. On the contrary, in studies where confounders (i.e. age, sex, maturity status, and BP) were taken into consideration, positive associations were observed for CRF estimated indirectly and parasympathetic indices of HRV in youth (Gutin et al., 2005, Michels et al., 2013). These results highlight the existence of potential associations between CRF

and resting HRV in youth. However, contrary to the evidence base for the association between traditional CVD risk factors and CRF, which has been investigated in systematic reviews (Ruiz et al., 2009), no systematic review has addressed possible associations between CRF and autonomic function in youth.

In youth, the possible associations between CRF and HRR are less clear compared to resting HRV. On the contrary, in adults an investigation by Buchheit and Gindre (2006) elegantly demonstrated the interplay between CRF, PA levels, HRR and HRV. These authors showed that CRF in $mL \cdot kg^{-1} \cdot min^{-1}$ measured directly from a cycling test was moderately related to resting HRV ($r = 0.53$), but not to HRRt ($r = 0.01$). The opposite was observed for weekly PA estimated via questionnaire, which was moderately related to HRRt ($r = 0.55$) but not to resting HRV ($r = 0.01$). The mechanisms behind these findings were not examined in the study, and to date it is unclear why CRF and PA present different associations with HRV and HRR. Finally, no studies have yet identified the possible associations between CRF, PA, HRR and HRV in youth.

2.5 Arterial function

The aim of this section is to introduce the reader to arterial function, with focus on arterial compliance and distensibility. Currently there is a plethora of evidence linking arterial dysfunction and stiffness (inverse of distensibility) to traditional CVD risk factors. For example, in 11-year olds with familial hypercholesterolemia (a well described CVD risk factor) arterial distensibility and compliance are decreased by 15% and 19%, respectively (Aggoun et al., 2000). Similarly, in 11 – 14 year olds, elevated BP is a significant predictor of arterial stiffness ($\beta = 0.36$) assessed using pulse wave velocity (PWV), when sex, body weight status, maturity status, and HR are controlled

for, showing early signs of arterial stiffening in children with traditional CVD risk factors (Phillips et al., 2015). Likewise, a meta-analysis provided level one evidence of an increased arterial stiffness in 1,281 obese youth aged 4 – 24 years old compared to 956 healthy normal weight pairs (pooled *d* = 0.72; 95% IC = 0.29 – 1.42) (Cote et al., 2015b).

This section is structured with a short overview on the assessment and physiological significance of arterial stiffness, compliance and distensibility, followed by a discussion on how PA intensities and CRF are associated with arterial stiffness. Gaps in current knowledge will be highlighted.

2.5.1 Assessment of arterial stiffness

The arterial system is established not only as a conduit system to deliver blood, but also as a tissue that provides important physiological adjustment of blood flow and pressure (Wagenseil and Mecham, 2009). The arterial wall is structured in three distinct layers; the tunica intima, tunica media and tunica adventitia. The tunica intima is composed of a single layer of endothelial cells attached to a basal lamina (Wagenseil and Mecham, 2009). The endothelial cells are important for the production of endothelium-derived relaxing factors which possess atheroprotective roles (Vanhoutte et al., 2017). Nitric oxide (NO) is a well described endothelium-derived relaxing factor, and it is also an important determinant of arterial stiffness (Wilkinson et al., 2004).The tunica media is composed mostly of smooth muscle and elastin, which is responsible for changes in arterial tone via muscular contraction and relaxation. The tunica adventitia is the outmost layer and is composed mostly of collagen fibres (Wagenseil and Mecham, 2009). The different layers of the arterial wall ultimately give the elastic properties of the arterial tree. For instance, the large central

arteries such as the aorta are more elastic providing a cushioning to the blood flow, compared to stiffer conduit arteries, such as the radial artery (Oliver and Webb, 2003). Consequently, arterial compliance assessment can be altered depending on which segment of the arterial tree is used. For example a recent meta-analysis investigating the effects of an acute exercise bout on arterial stiffness revealed that in the first 5 min post-exercise upper body arterial stiffness increases, while arterial stiffness of the lower body decreases following the exercise bouts in adults (Mutter et al., 2017).

Assessment of arterial stiffness can be performed in several ways, including regional (i.e. PWV) and local (i.e. common carotid artery (CCA) distensibility) measures. The PWV method measures the speed in which the pulse wave travels across a given arterial segment (Laurent et al., 2006). The gold standard method for PWV assessment is the measurement of central PWV, normally in the central aorta. For central PWV, the pulse at the carotid and femoral arteries are obtained and the time delay between the nadir of the wave (i.e. end diastole) is measured. The distance between the two arterial segments is then obtained as the superficial distance between the two arterial points, normally measured as the difference between the external notch and the carotid artery and the external notch and femoral artery. PWV in then expressed in $m \cdot s^{-1}$(Laurent et al., 2006) (Figure 2.3). All superficial arteries can be used to assess PWV, however, most of the reference values in the adult literature, as well as the associations with CVD risk, have been obtained using central PWV (Reference Values for Arterial Stiffness, 2010). However, due the intrusive nature of assessing femoral pulse in youth, central PWV is often limited to laboratory conditions.

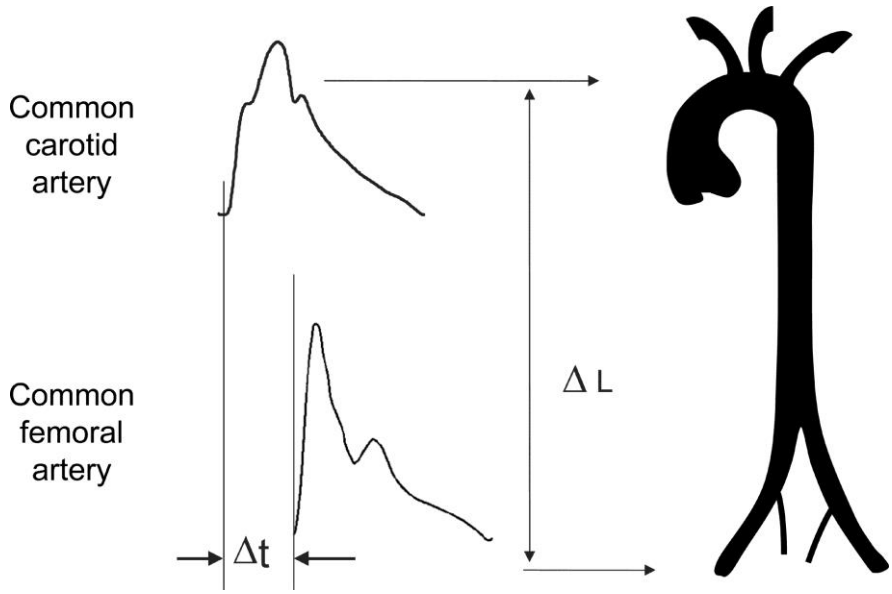

Figure 2.3: Central pulse wave velocity assessed from the carotid to femoral artery. The superficial location of these arteries allows measurement of the pulse on the body surface using waveform transducers. Changes in time (Δt) is then calculated between the foot-to-foot of the waves obtained at the carotid and femoral sites. The length of the segment is obtained as the difference between from the distance of the strongest pulse measured in the body surface (ΔL). PWV = Δt/ΔL. Reproduced from Laurent et al. (2006) with permission.

Due to their less intrusive nature, other peripheral arterial segments are often used in youth. Studies have used PWV from brachial to radial, carotid to ankle, and carotid to radial arteries (Mutter et al., 2017). The limitations of using different arterial segments is the lack of prognostic and reference values in the literature. Furthermore, a lower atherosclerotic manifestation exists in the muscular peripheral arteries compared to more elastic and central ones. Additionally, the reliability of peripheral PWV has not been addressed in youth, which can limit the interpretation of peripheral measures of PWV (Urbina et al., 2009). Finally, when assessing peripheral PWV, the specific arterial wall characteristics should be taken into consideration when interpreting the

results. This is because muscular peripheral arteries are stiffer compared to the central and more elastic ones (Urbina et al., 2009).

Measures of local arterial stiffness provide information about the strain stress relationship of a specific artery (Laurent et al., 2006). Any superficial artery can be used; however, studies highlight the clinical value of assessing local stiffness of the CCA in both adults and youth (Urbina et al., 2009, Laurent et al., 2006). Several indices can then be obtained from the assumption that the absolute change in volume (strain) is caused by changes in pressure (stress), which depends on the viscoelastic characteristics of the arterial wall. To measure local stiffness, measurements of BP are performed simultaneously with measures of the arterial diameter. According to specific guidelines, assessment of local pulse pressure (PP) (i.e. with applanation tonometry) rather than brachial pressure is desired (Laurent et al., 2006). High resolution images of CCA are then obtained using ultrasound during the cardiac cycle to obtain a longitudinal image of the artery with clear presentation of the IMT to quantify systolic and diastolic lumen diameters (SLD and DLD, respectively). Similar to PWV, there is also a lack of paediatric investigations on the reliability of CCA measures (Urbina et al., 2009), and further research is needed to determine the between and within-day variability of local arterial stiffness, which is paramount for interpretation of research findings.

The mechanisms by which arterial stiffness can occur are dependent on adaptations of the arterial wall to different stimuli, such as exercise or disease. As the collagen/ elastin ratio of the arterial wall reflects the stiffness of the vessel, changes in this ratio can explain arterial stiffness. This has been shown in animal models in which induced hypertension led to an over expression of collagen relative to elastin and a consequent

arterial stiffening (Xu et al., 2000). In addition, because elastin and collagen actively participate in the remodelling of the arterial wall induced by hemodynamic stimulus, it is likely that the inverse association between PA intensities and arterial stiffness (Ferreira et al., 2006, Ferreira et al., 2003, Ferreira et al., 2002) reflects adaptation of the artery towards an increase in elastin caused by a heightened stimulus on the arterial wall due to increases in shear stress during PA. The differences in elastin and collagen is also implicated in arterial stiffening with ageing (Zieman et al., 2005).

In addition to the changes in the elastic components of the arterial wall, external influences can also alter local and regional arterial stiffness. Such influences are sympathetic activity, angiotensin, blood lipids, shear stress and luminal diameter, amongst others (Zieman et al., 2005). For instance, an increased oxidative stress in the postprandial state following ingestion of high-fat and/or high-sugar meals (see section 2.7) impairs endothelial function with a consequent increase in arterial muscle constriction (Wilkinson et al., 2004, Zieman et al., 2005, Wang and Fitch, 2004). Additionally, an elevated vasoconstriction stimulated by sympathetic activity also leads to arterial stiffness (Wang and Fitch, 2004). These later mechanisms are more likely to alter arterial stiffness in the short-term (i.e. hours or days). Examples of short-term changes in arterial stiffness are following exercise (Naka et al., 2003), when endothelial function is increased (Bond et al., 2015c), and sympathetic activity to the vessel is diminished (Buckwalter and Clifford, 2001). Collectively, these results highlight that short-term effects on arterial stiffness are caused by external factors and long-term changes in arterial wall components may reflect an adaptation to stimuli, either exercise, disease or ageing. How exercise changes arterial stiffness in youth both in the short and long- term, and the associated mechanisms, are still unclear.

Because of the characteristics and physiological determinants of local and regional arterial stiffness, several factors should be controlled when using these measurements. For instance, due to the dependence of arterial stiffness on BP, factors such as room temperature, food intake in the hours before the measurements, standardization of resting period, body position, time of the day and white coat effect, should be considered (Laurent et al., 2006). In addition, local arterial stiffness is dependent on technical ultrasound imaging skills of the researcher. All the mentioned factors may decrease reliability of the measurements if not controlled. Investigations in 12 – 15 year old adolescents that did not aim to test reliability of the PWV stated that when the CV was higher than 20% the measurement was rejected (Boreham et al., 2004). Similarly, Ried-Larsen et al. (2014) reported CVs lower than 5% for intra-reader measures of CCA distensibility in 15-year old adolescents. Although these results do not provide specific information about reliability, they indicate that measures of arterial stiffness show the potential to be reliable, however, this is yet to be investigated.

In summary, arterial stiffness can be assessed using local and regional measures which are non-invasive and ideally suited to investigations in youth. There is still very little information regarding the reliability of arterial stiffness measures in youth, which is concerning because measures of reliability are necessary for interpretation of research findings.

2.5.2 Physical activity and arterial function

Due to the diverse mechanisms regulating arterial stiffness, the likely influence of PA intensities may be via changes in arterial wall structure (i.e. collagen/elastin) and/or function (i.e. NO dependent dilation). An interplay between function and structure appears to exist as evidenced by an increased cIMT observed only in adults with a combination of clustered CVD risk and impaired FMD, but not in adults with clustered CVD risk and maintained FMD (Juonala et al., 2004). These results suggest that impairment in endothelial function is a necessary step for arterial remodelling. On the contrary, in 10 year old healthy children, decreases in FMD obtained over four months (10.7 ± 4.3 at baseline vs 7.2 ± 3.5%) were not significantly associated (r = 0.14) with increases in cIMT observed after the 30 month follow-up (Hopkins et al., 2013). Collectively, these results appear to indicate the existence of a potential window of opportunity before arterial dysfunction leads to arterial remodelling in children. This is especially important, as PA and exercise can be used to prevent adverse arterial remodelling via maintenance of arterial function and stiffness.

In adults, exercise training has been shown as an important strategy to increase endothelial function. One mechanism implicated is an up-regulation of endothelial nitric oxide synthase, stimulated by the exercise induced shear stress and circulating factors (i.e. hormones, cytokines, adipokines) (Padilla et al., 2011). An important notion, however, is that increases in NO-dependent vessel function can occur independent of concomitant improvements in traditional CVD risk factors in both adults (Green et al., 2003) and adolescents (Bond et al., 2015a). Collectively, these results suggest that PA and exercise can alter vessel function via a different mechanistic pathway compared to their effects on traditional CVD risk factors.

In adolescents, the evidence for associations between PA and arterial stiffness has been mostly obtained from longitudinal investigations. For example, van de Laar et al. (2011) investigated the effect of habitual PA on arterial compliance in a 24 years longitudinal investigation. In this study, the first PA estimates were completed when participants were 13 years old using face-to-face interviews, and repeated at the ages of 14, 15, 16, 21, 27, 32 and 36 years old. At the age of 36, participants were divided into tertiles of arterial compliance measured at the brachial (tertile one = 0.27, tertile two = 0.15, and tertile three = 0.09 $mm \cdot kPa^{-1}$) and femoral (tertile one = 0.78, tertile two = 0.46, and tertile three = 0.29 $mm \cdot kPa^{-1}$) arteries. The results demonstrated that participants with higher brachial and femoral compliance spent significantly more time during the 24 years follow-up performing VPA, but not light-to-moderate PA. A follow-up analysis controlling for diet and traditional CVD risk factors did not alter the findings. In a similar investigation conducted by the same group, van de Laar et al. (2010) replicated the same findings for CCA compliance (tertile one = 0.72, tertile two = 0.97, and tertile three = 1.28 $mm \cdot kPa^{-1}$). These studies indicate that arterial compliance of central and peripheral arteries is increased due to VPA levels assessed longitudinally. Importantly, the decline in participants VPA observed after the age of 15 years was the main determinant of a decreased arterial compliance between the groups (Figure 2.4). Therefore, strategies targeting VPA in youth may preserve arterial compliance later in adulthood, reinforcing the importance of considering PA intensities.

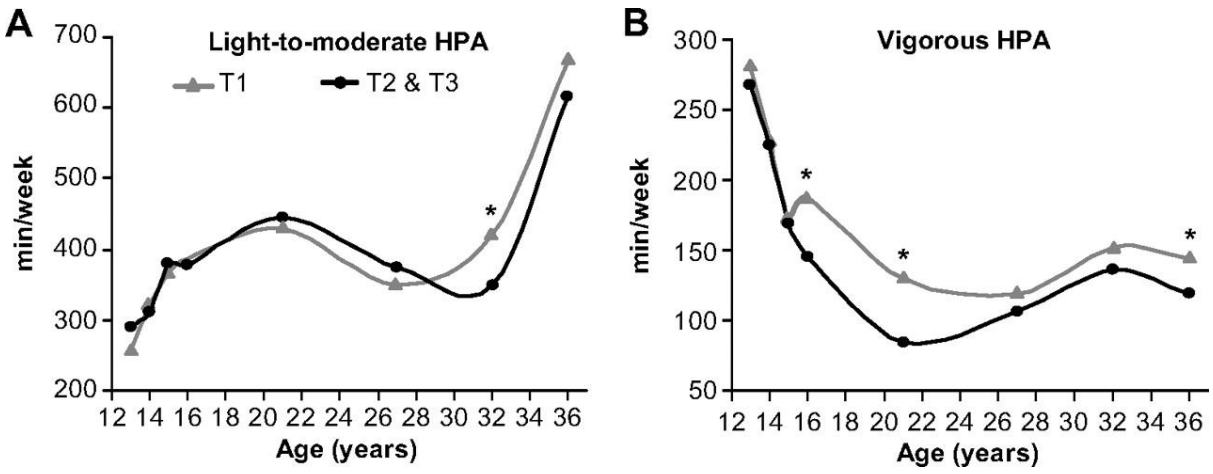

Figure 2.4: Longitudinal changes in A) light-to-moderate physical activity and b) vigorous physical activity for groups of high (triangles) and low (circles) values of common carotid compliance obtained at the age of 36 years old. In this analysis, only vigorous physical throughout follow-up was different between the tertiles of common carotid compliance at the age of 36 years old. This was attribute to the significant differences observed at the age of 15 despite no differences noted at the adult ages. Reproduced from van de Laar et al. (2010) with permission.

The possible effects of exercise interventions on arterial stiffness are less clear compared to the demonstrated associations with habitual PA. Exercise interventions are normally limited to populations with elevated CVD risk, such as obesity and metabolic conditions, and are often undertaken in the adult population (Fernhall and Agiovlasitis, 2008). A meta-analysis involving eight trials on obese adults (49 – 75 years old) revealed no significant effects of aerobic training on arterial stiffness compared to control (pooled *d* = -0.17; IC 95% = -0.39 – 0.06) (Montero et al., 2014a). A similar meta-analysis by the same group also revealed no significant effects of aerobic training on arterial stiffness for pre-hypertensive adults (pooled *d* = -0.19; IC 95% = -0.39 – 0.01) (Montero et al., 2014b). However, the lack of post-hoc analysis on training mode, intensity, duration, and frequency limits further conclusions and to

date the effects of these factors and mechanisms that exercise changes arterial compliance is open to investigation.

Extrapolating adult findings to healthy youth is challenging; however, due to a lack of decreases in arterial stiffness observed in two meta-analysis involving adults at elevated CVD risk, improvements in arterial compliance in healthy children due to exercise training may be difficult. However, this remains yet to be addressed.

2.5.3 Cardiorespiratory fitness and arterial function

Cardiorespiratory fitness measured during adolescence may not be longitudinally associated with CCA compliance in adulthood. For example, Ferreira et al. (2002) demonstrated that $\dot{V}O_2max$ in $mL \cdot kg^{-2/3} \cdot min^{-1}$, directly measured from a running treadmill test at the age of 13 – 16 years old was not significantly associated with CCA compliance (stβ = -0.007) obtained at adulthood. On the contrary, in a subsequent analysis of the same data, it was concluded that improvements in $\dot{V}O_2max$ from childhood to adulthood was positively associated with improvements in CCA compliance (β = 2.14), after controlling for adult traditional CVD risk factors (Ferreira et al., 2003). These studies are interesting as CRF is expressed using allometric scaling to adjust for the influence of body size and isolate the influence of CRF.

In summary, VPA measured during childhood present important longitudinal associations with arterial compliance in adults and decreases in VPA after the age of 15 years old negatively influences CCA compliance at the age of 36 years old. These results highlight that strategies targeting VPA are encouraged. Furthermore, CRF measured in adolescence is not associated with arterial compliance measured in adults; however, increases in CRF from adolescence to adulthood is positively associated with increases in CCA compliance. It can be speculated that the positive

influence of VPA on CRF (Aires et al., 2010) is a link between the studies. The likely mechanisms are unclear, as most of the studies are observational by design. Moreover, there is a paucity of information about PA characteristics such as frequency, duration and intensity, and possible training effects on arterial compliance.

2.6 Interaction between autonomic and vascular systems

The aim of this section is to introduce the reader to the BRS and how the baroreflex provides unique information about the interaction between the autonomic and vascular systems. A short overview of assessment methods and gaps in the literature will be addressed. At the end of the section, the effects of acute exercise and exercise training on BRS will be discussed.

2.6.1 Baroreflex sensitivity

An interesting approach to investigate how PA and exercise acutely and chronically changes the autonomic and arterial systems is to explore the arterial baroreflex by measuring BRS. The baroreflex is a reflex mechanism involved in the homeostatic regulation of arterial BP by triggering a series of mechanisms aimed at modifying cardiac output ($\dot{Q}$) and total peripheral resistance (TPR). This reflex mechanism is orchestrated by a series of inputs from peripheral baroreceptors monitoring changes in arterial BP. Increases in BP results in decreased sympathetic activity to the heart and vessels and an increase in the parasympathetic drive to the heart. This leads to a diminished TPR, venous return, HR, and $\dot{Q}$. By contrast, when BP decreases, a reduced baroreceptor activity leads to an increased sympathetic drive to the heart and vessels causing an increased TPR, HR and $\dot{Q}$ (Benarroch, 2008, La Rovere et al., 2008).

Important peripheral receptors orchestrating BRS are the baroreceptors located in the aorta and carotid arteries. Increased arterial BP causes the baroreceptors embedded in the adventitia of the carotid sinuses and arc of the aorta to stretch due to the mechanical deformation of the artery wall. This mechanical deformation (e.g. arterial stretch) leads to an increased firing rate of the baroreceptors and excitatory input to the central nervous system (Kornet et al., 2002, Bonyhay et al., 1996). The increased firing rate of the baroreceptors result in central adjustments in the autonomic nervous system.

The adjustments in the autonomic responses to the baroreflex stimuli are performed in the autonomic centres located in the brain stem, which eventually will lead to changes in the sympathetic and parasympathetic branches of the autonomic nervous system (Pilowsky and Goodchild, 2002). Cardiac BRS has been shown to be exclusively dependent upon the fast action of the parasympathetic activity to the heart and is also known as cardiovagal baroreflex (Keyl et al., 2001). The sympathetic baroreflex reflects changes in the sympathetic drive to the vessels caused by BP stimuli.

Cardiac BRS can be assessed by measuring the effects of changes in BP on adjustments in RR intervals, such as increases in BP are expected to lead to increases in the RR interval (decreases in HR). The degree of BP influence on HR is normally defined as the gain of the BRS and expressed as units of change in RR intervals in ms by units of change in BP ($ms \cdot mmHg^{-1}$). An elevated BRS gain indicates that the vagal system responds promptly to changes in BP. To quantify sympathetic BRS gain, it is common to measure the effects of changes in beat-to-beat BP on the sympathetic

activity in superficial nerves, and to express the decreases in the sympathetic activity caused by units of changes in BP.

From the association between changes in BP and HR, it is possible to model the full baroreflex arc at rest (Figure 2.5). Important information from the full baroreflex arc can be drawn by investigating the threshold, saturation, operating and centering points. The threshold point is the BP needed to cause a change in HR and the saturation point is the point where increases in BP lead to no more decreases in HR. The operating point is the pressure at which the baroreflex system operates and the centering point is the point at which increases in BP leads to an equal pressor and depressor response. The gain at the threshold and saturation is small (Figure 2.5 B), whereas the maximum gain is obtained at the centering point. It is important to notice that in Figure 2.5 B the operating point of high fit participants is located at a lower mean arterial pressure (MAP). The gain (BRS) of the association between BP and RR intervals can be calculated at any given point of the baroreflex curve as proposed by Kent et al. (1972).

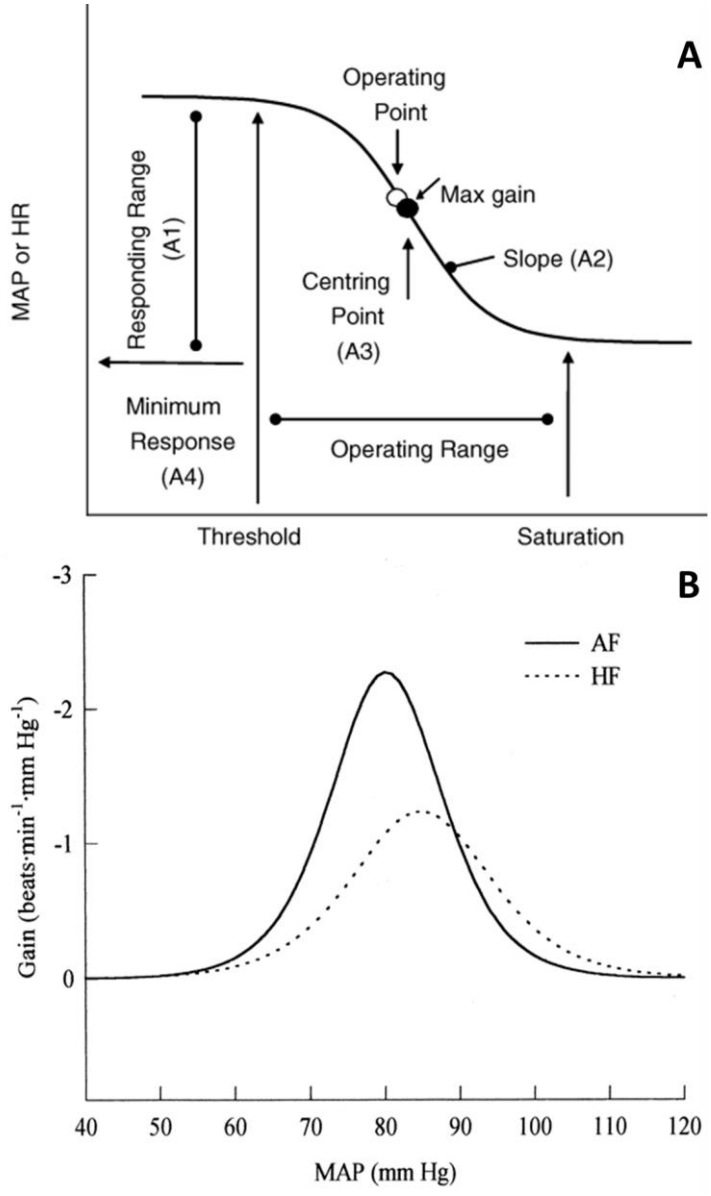

Figure 2.5: A) representation of the full baroreflex curve. B) gain (BRS) of the baroreflex along the full baroreflex curve. The highest BRS gain is obtained at the operation point. HF = high fit participant, AF = average fit participants. Reproduced from A) Raven et al. (2006) and B) Smith et al. (2000) with permission.

Due to the invasive nature of sympathetic BRS assessment, work conducted so far exploring the baroreflex in youth has been performed by measuring cardiovagal BRS, thus limiting current knowledge to the parasympathetic branch of the autonomic nervous system. Similarly, the focus of the present work is on the cardiovagal

baroreflex; its strengths and limitations, reliability, and associations with exercise will be discussed in the following subheadings.

2.6.1.1 Assessment of baroreflex sensitivity

Several techniques have been used to assess both cardiovagal and sympathetic BRS in humans. Although no gold standard exists amongst different BRS methods, an informative approach is the modified Oxford method. The Oxford method consists of infusion of vasodilator and vasoconstrictor drugs to decrease and increase BP, respectively. The changes in BP are then plotted against the correspondent values of RR intervals (Persson et al., 2001, Di Rienzo et al., 2001, La Rovere et al., 2008). One of the advantages of the Oxford method is the assessment of changes in HR over a wide range of BP. However, a major limitation of the Oxford method is its invasive nature, which limits application to studies involving youth. Additionally, due to artificial changes in BP via drug administration, the ecological validity of the method is questionable, and side effects, such as dizziness and syncope may occur. Besides the Oxford method, neck suction applied with neck collars provide important estimates of BRS over a wide range of BP (Raven et al., 2006). Figure 2.5 A provides an example of the full baroreflex arc obtained with the neck suction method. A limitation of the neck suction method is that it is impossible to obtain CCA ultrasound images in conjunction with BRS assessment (later in this section) due to the position of the neck collar.

An alternative non-invasive approach is to measure spontaneous BRS. This method quantifies the spontaneous oscillations in BP and RR intervals using time and frequency domains analyses (Persson et al., 2001). Time domain measurement (also known as sequence method), is obtained by plotting the natural increases or decreases in BP against the correspondent increases or decreases in the RR interval.

Frequency domain methods (or spectral methods) use the assumption that oscillations in BP at one determined frequency will cause oscillations in RR intervals at the same frequency (Robbe et al., 1987). The spontaneous method, however, has limitations, such as it does not estimate the saturation and threshold points (Figure 2.5), and the BRS gain obtained from spontaneous methods only reflects the gain at the operating point (Schwartz et al., 2013). This limitation should be considered when interpreting results using spontaneous methods. Nevertheless, the spontaneous method has been shown to be valid compared to the Oxford method in adults (Persson et al., 2001, Parlow et al., 1995).

Although informative, the spontaneous method does not distinguish between the vascular and autonomic determinants of baroreflex. However, the development of techniques based on CCA ultrasound images (see section 2.5.1) acquired simultaneously with measurements of BRS, provides the foundation to quantify the contribution of the arterial and autonomic determinants of the baroreflex (Taylor et al., 2014, Tzeng, 2012). The underpinning assumption is that changes in CCA diameter and compliance during the BRS assessments provide information regarding arterial wall deformation and consequently the degree of baroreceptors stimuli (Hunt et al., 2001a, Bonyhay et al., 1996). With the implementation of ultrasound images it is possible to non-invasively divide spontaneous cardiovagal BRS into three different components in youth: the BRS gain (the association between changes in BP and RR intervals obtained either with the sequence or frequency methods and expressed as $ms \cdot mmHg^{-1}$); the vascular component (estimated as CCA compliance and expressed as $\mu m \cdot mmHg^{-1}$); and the autonomic component (estimated as the division between the BRS gain and the CCA compliance and expressed as $ms \cdot \mu m^{-1}$) (Lenard et al., 2004). Thus, the simultaneous measurement of carotid images and BRS can provide

unique non-invasive insights into the BRS gain at the operating point and the contribution of the vascular and autonomic branches of BRS (Taylor et al., 2014, Reneman et al., 2005). This non-invasive method although suitable to use in a paediatric population has not yet been validated and can be only used as a surrogate method of invasive measures of autonomic and vascular determinants of BRS.

The possible role of CVD risk factors on BRS in youth has been recently reviewed (Honzikova and Zavodna, 2016). Specifically, spontaneous BRS has been shown to be lower in cross-sectional studies comparing children with obesity, hypertension, and diabetes to healthy pairs. For instance, Fitzgibbon et al. (2012) have shown that BRS is significantly lower in adolescents with elevated (10.5 ± 6.8 ms·mmHg^{-1}) compared to adolescents with normal (15.1 ± 6.8 ms·mmHg^{-1}) BP. Whether this is due to changes in the vascular and/or autonomic components of the BRS is currently unclear (Honzikova and Zavodna, 2016), and casualty cannot be determined due to the cross-sectional design of the studies. However, results suggest that a decreased BRS is a sentinel event for hypertension development. For example, in normotensive young adults aged 22 years old who are offspring of hypertensive parents, BRS has been shown to be lower (~ 8.5 ms·mmHg^{-1} vs ~ 9.5 ms·mmHg^{-1}) before manifestation of elevated BP (Boutcher et al., 2011). These results indicate that BRS not only provides information about the interaction of the arterial and autonomic systems but is also associated with the development or presence of CVD risk factors in youth.

The reliability of spontaneous BRS has been investigated in youth. For example, in 11 years old, spontaneous BRS measured with the frequency method has a test-retest CV of 14% (Dietrich et al., 2010). However, no studies have addressed the reliability of BRS and its autonomic and vascular determinants in adolescents. Similarly, less is

known regarding the within day reliability, which is concerning as a previous adult investigation has demonstrated diurnal variation in BRS and its autonomic and arterial determinants (Taylor et al., 2011), suggesting within-day measures may present different reliability compared to between-days.

In summary, the BRS gain reflects the association between changes in BP and HR. Spontaneous BRS estimates are valid and their non-invasive nature makes this approach attractive for BRS measurement in youth. Furthermore, due to the arterial and autonomic determinants of the BRS, exploring BRS can provide further understanding about the effects of PA and exercise on CVD risk reduction that cannot be accounted for by traditional risk factors. Likewise, because baroreflex impairment is related to diverse health conditions (La Rovere et al., 2008), investigating BRS during childhood and adolescence, and how PA and exercise influences it, can increase our current pathophysiological understanding of CVD. There is, however, a lack of studies investigating the reliability of BRS and its autonomic and vascular determinants in youth.

2.6.1.2 Physical activity and baroreflex

The effect of habitual PA on the BRS gain during childhood has only been examined in two studies. Lucini et al. (2013) investigated BRS in 105 children aged 11 years old taking part in football training. The participants were divided into groups of overweight (n = 11) and normal weight (n = 94) and their PA levels were estimated using questionnaires. This investigation demonstrated that overweight children (~ 19 $ms \cdot mmHg^{-1}$) had impaired BRS compared to normal weight pairs (~ 30 $ms \cdot mmHg^{-1}$). However, the estimated PA levels was similar between the groups meaning any conclusion about the effects of PA on BRS were not possible. In another study

exploring the effects of PA on BRS of preadolescents aged 10 – 13 years old, Dietrich et al. (2006) failed to find a statistically significant relationship (r = 0.05) between PA and BRS and concluded the main determinants of BRS are sex, age and body composition. Caution, however, should be taken when interpreting these results as PA was estimated using questionnaire. Additionally, the role of PA intensities was not addressed.

2.6.1.3 Acute exercise

Up to 24 h following exercise, arterial BP decreases below resting values characterising a state of post-exercise hypotension (Halliwill et al., 2013). The exact definition of post-exercise hypotension is unclear, but studies have defined this phenomenon as a decrease in BP below baseline values or below control when no exercise is performed (Kenney and Seals, 1993). The magnitude of post-exercise BP is normally 8 – 9 mmHg for healthy adults and is higher when BP before exercise is elevated, such as in those with hypertension(MacDonald, 2002). Post-exercise hypotension has been well described in the adult literature and the diverse mechanisms which underpin the changes in BP have been reviewed elsewhere (Halliwill et al., 2013). However, for this thesis a special focus will be given to the arterial and autonomic changes following exercise, which ultimately influences BRS. Given that the hours following exercise are proposed as a potential stimulus for training adaptation (Luttrell and Halliwill, 2015), how exercise acutely alters autonomic function, vascular compliance and the interplay between these systems (i.e. BRS), may provide unique mechanistic information about CVD risk reduction.

Given that arterial stiffness at rest is dependent on BP (Oliver and Webb, 2003), it is likely that following exercise arterial stiffness decreases concomitantly with BP. A

recent meta-analysis including 43 investigations which assessed arterial stiffness following exercise in adults (20 and 35 years old) revealed an increase in central arterial stiffness in the first five min post-exercise, with a consequent decrease after five min (Mutter et al., 2017). Several limitations hamper interpretation and conclusions from this meta-analysis, however. For example, the multitude of exercise intensities and protocols (varying from HIIE, continuous cycling, maximal exercise to exhaustion and repeated short-duration sprints), the difference in exercise dose (from 10 min up to two hours), the different approaches to measure arterial stiffness (central PWV, applanation tonometry, arterial compliance), the time of follow-up after exercise (from immediately after exercise, all the way up to one hour post), and the number of assessments in the post-exercise period.

The extent to which adult findings can be translated to children is also questionable. For instance, Melo et al. (2016), investigated the effects of a maximal exercise treadmill test on arterial compliance 10 min following exercise in children aged seven years old compared to adults aged 25 years old. The authors showed that after adjustments for PP, MAP and stature, children presented elevated CCA distensibility at rest (adjusted means = 0.068 ± 0.004 vs 0.041 ± 0.003 $mm^2 \cdot KPa$) and following exercise (adjusted means = 0.053 ± 0.003 vs 0.035 ± 0.002 $mm^2 \cdot KPa$). Furthermore, adults presented an accentuated decline following exercise in CCA distensibility compared to children, and children also presented a distinct change in PP, which was always significantly higher compared to adults (data not shown). These results showed that the hemodynamic response to maximum exercise, as well as arterial compliance can be different between children and adults. The impact of these findings on the BRS and its autonomic determinant is unknown.

Additionally, it has not yet been identified whether there is an effect of exercise intensity on the acute changes in BRS and its autonomic and arterial components in youth. In 14 year old adolescents, Bond et al. (2015c) showed that HIIE exercise led to improvements in NO dependent endothelial function in a biphasic manner, using FMD. Specifically, immediately following HIIE, brachial artery FMD decreased significantly from ~8 to ~ 4%. On the contrary, at one (from ~8 to ~ 12%) and two hours (from ~8% to ~ 12%) following HIIE, FMD was significantly improved compared to baseline values for boys and girls. These findings were not observed for a bout of work-matched continuous moderate-intensity cycling. The decreased NO dependent dilation observed by Bond et al. (2015c) immediately following exercise may also explain the observed decreases in arterial compliance 10 min following maximal exercise in Melo et al. (2016). This, however, remains speculative. Additionally, future research is needed to investigate the possible impact of the reported intensity-dependent changes in endothelial function on BRS and its autonomic and vascular determinants.

In adults, the interplay between the vascular and autonomic components of BRS following exercise has been demonstrated. In a seminal investigation by Studinger et al. (2003), it was demonstrated that immediately after maximal cycling exercise CCA compliance was decreased compared to baseline (16.4 ± 1.2 vs 27.3 ± 2.7 $\mu m \cdot mmHg^{-1}$). This was closely followed by a decrease in spontaneous BRS gain immediately post compared to baseline (5.8 ± 1.2 vs 17.1 ± 2.7 $ms \cdot mmHg^{-1}$). However, 60 min following exercise CCA compliance increased compared to baseline (17.1 ± 2.7 vs 33.9 ± 1.4 $\mu m \cdot mmHg^{-1}$), which was paralleled with a recovery of BRS gain to baseline values. The results also revealed that changes in BRS gain and CCA compliance were significantly associated immediately and at 60 min post-exercise (r = 0.74 – 0.83). The

authors suggested that the vascular component was responsible for the observed changes in overall BRS gain. However, it can be argued that the lack of improvement in BRS at 60 min post, despite increases in CCA compliance, reflects a lowered autonomic determinant.

2.6.1.4 Exercise adaptation on BRS and its autonomic and vascular determinants

Whether the aforementioned post-exercise changes in the autonomic and vascular determinants of BRS influences training adaptations is not clear. In adults, the adaptation on the BRS and its autonomic and vascular determinants have been described. For example, Hunt et al. (2001b) have shown using the Oxford method in conjunction with ultrasound CCA images, that the BRS gain is lower in untrained 63 years old compared to untrained 25 years old (6.8 ± 1.2 vs 15.7 ± 1.8 ms·mmHg^{-1}). These results were attributed to decrements in both vascular (old untrained = 9.1 ± 1.0 vs young untrained = 17.1 ± 2.4 µm·mmHg^{-1}) and autonomic (old untrained = 0.6 ± 0.1vs young untrained = 0.9 ± 0.1 ms·µm^{-1}) determinants of the BRS. However, BRS gain of 59 years old who were trained was similar to the BRS of the young untrained men (13.3 ± 2.4 vs 15.7 ± 1.8 ms·mmHg^{-1}), due to a maintained autonomic (1.0 ± 0.2 vs 0.9 ± 0.1 ms·µm^{-1}), but not vascular (12.1 ± 1.4 vs 17.1 ± 2.4µm·mmHg^{-1}) determinant. The authors concluded that regular PA in trained old men protected against impairments in BRS by improving its autonomic determinant. Other studies have shown an elevated autonomic determinant of the BRS in trained young men compared to non-trained pairs (Komine et al., 2009). However, the cross-sectional design of these studies impedes casualty about the effects of training on BRS adaptations.

2.6.1.5 Translational of adult findings to young people

Because growth and maturation has been suggested to alter BRS gain, extrapolating adult BRS research findings to paediatric groups may be limited, highlighting the need for paediatric specific studies in this area. Lenard et al. (2004) have shown that 12-year olds presented significantly lower BRS compared to 16-year olds (8.1 ± 0.7 vs 16.2 ± 1.4 ms·mmHg^{-1}). Because CCA compliance was smaller in the older group (25.1 ± 1.1 vs 23.0 ± 1.0 μm·mmHg^{-1}) the authors attributed the observed increase in the BRS gain to an improved autonomic determinant. The authors, however, did not consider the effect of maturity, which has recently been shown to affect BRS in boys but not girls (Chirico et al., 2015). Chirico et al. (2015) demonstrated no differences between sexes when participants were grouped by age. By contrast, when participants were grouped according to maturity groups (pre, early, peri, late, and post measured by the Tanner scale), boys post maturity (~ 15.0 ms·mmHg^{-1}) presented a significantly lower BRS gain compared to the early mature ones (~ 30 ms·mmHg^{-1}). This was not observed for the girls. Chirico et al. (2015) however did not investigate the autonomic and vascular determinants of BRS.

In summary, how exercise and the intensity of exercise alters BRS and its autonomic and vascular determinants post-exercise and following a period of exercise training is currently unclear in adolescents. Similarly, the possible role of the post-exercise responses on training adaptations have not been investigated in youth.

2.7 Cardiovascular risk in the postprandial state

The important role of PA, CRF and exercise on CVD risk reduction is reported in youth, both via improving traditional CVD risk factors and vascular function (Fernhall and Agiovlasitis, 2008, Ekelund et al., 2007, Andersen et al., 2011b). However, most of the

reduction in CVD risk accrued by PA, exercise and CRF in the youth population have been described in the fasted state. The aim of this section is to briefly describe evidence supporting the role of exercise, PA and CRF on postprandial outcomes, mainly the autonomic and vascular systems.

Fasting measurements of blood markers provide the foundation linking traditional CVD risk factors to atherosclerosis and CVD outcomes. However, the transient increase in blood [lipids], blood [GLU] and BP during the postprandial state provide valuable information about CVD risk, even when controlling for fasted measurements of traditional CVD risk factors (Ansar et al., 2011). For example, increases in blood [TAG] after the ingestion of a HFM have been linked to the atherosclerotic process (Hyson et al., 2003). Similarly, postprandial lipaemia may have influences on CVD risk in early adolescence as evidenced by a longitudinal study showing that non-fasting [TAG] measured at the age of 15 years old, predicts CVD events in the fourth and fifth decades of life after adjustments for adult CVD risk factors (Morrison et al., 2009). Furthermore, in children postprandial GLU intolerance predicts adult mortality independently of adult CVD risk factors (Franks et al., 2010). Therefore, since humans spend most of the waking day in a postprandial state, it is important to explore non-fasting CVD risk and examine whether PA, PA intensities, exercise and/or CRF may confer any risk reduction.

The possible mechanism by which postprandial lipaemia and glycaemia changes vascular and autonomic functions may be via increases in oxidative stress. There is evidence of the atherogenic role of oxidative stress (Stocker and Keaney, 2004), suggesting a causal pathway, or at least postprandial oxidative stress as a CVD risk factor (Nakamura et al., 2016). The potential mechanisms by which the postprandial

state is linked to endothelial dysfunction has been reviewed elsewhere (Lacroix et al., 2012). From the diverse mechanisms, it is believed that a lower NO bioavailability is the result of an overproduction of free radicals, such as super oxide, in the mitochondria (Figure 2.6). This lower NO bioavailability consequently limits the ability of the endothelial cell to stimulate smooth muscle relaxation via the NO pathway, leading to a constricted and stiff vessel. As discussed in the section 2.6, increases in arterial stiffness may be linked to a decreased BRS due to a lowered vascular component, which may be heightened in the postprandial state. Alternatively, blood [GLU] and insulin levels are known to alter central autonomic regulation which may also lead to decreases in BRS due to a decreased central nervous system modulation of parasympathetic tone (Wan and Browning, 2008). The influences of the postprandial state on vascular stiffness, autonomic function and BRS, as well as the mechanisms associated are less clear in youth.

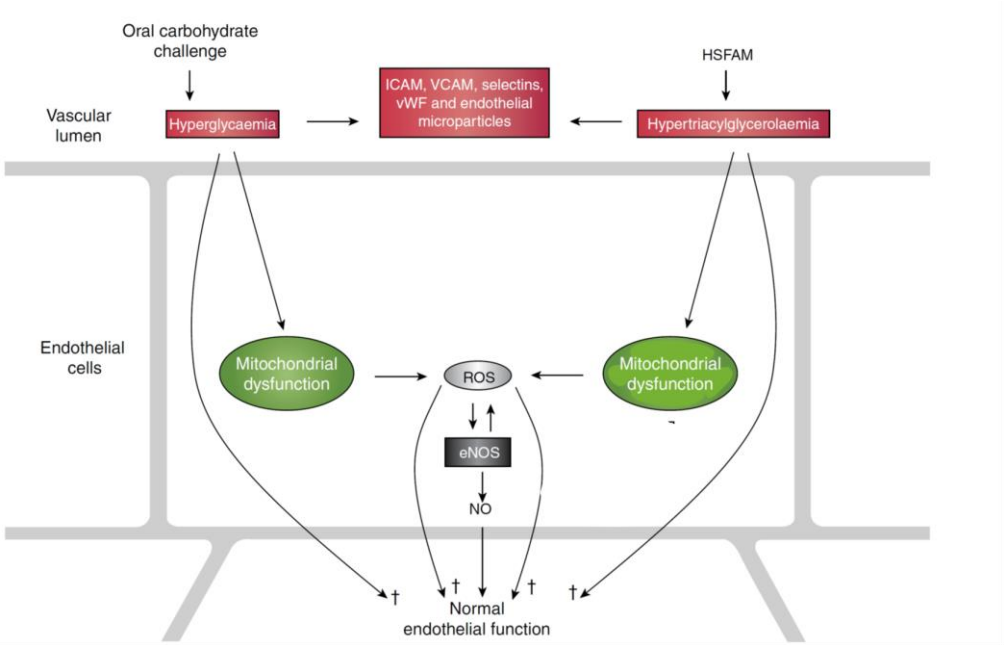

Figure 2.6: Mechanistic link between postprandial state and endothelial dysfunction. ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule; vWF, von Willebrand factor; HSFAM, high-saturated fat meal; ROS, reactive oxygen species; eNOS, endothelial NO synthase; VSMC, vascular smooth muscle cell. ł indicate negative effect on normal endothelial function. Increases in reactive oxygen species due to mitochondrial dysfunction are well established mechanisms of postprandial endothelial dysfunction. Reproduced from Lacroix et al. (2012) with permission.

2.7.1 Hyperglycaemia

Several methodologies exist to investigate postprandial hyperglycaemia in adolescents. The overall aim is to induce increases in blood [GLU] and to measure the time until glucose (and/or insulin) returns to baseline. Amongst several methods, the oral glucose tolerance test (OGTT) is a safe and reliable method (between-day CV of 5.6% for the area under the curve of glucose concentration (Cockcroft et al., 2017a)) to investigate the physiological effects of acute rises in blood [GLU] in youth(Brown and Yanovski, 2014). The OGTT consists of the ingestion of up to 75 g of GLU, whilst

blood samples are collected at specific points either in a short (two hours with seven blood samples) or a long (four hours with 11 blood samples) version of the test (Dalla Man et al., 2005).

2.7.1.1 Hyperglycaemia, autonomic and arterial systems

In 23 years old young adults, the parasympathetic system has been shown to be depressed 120 min after an OGTT compared to pre-OGTT values (RMSSD = 53 ± 10 ms vs 73 ± 11 ms, respectively) (Holwerda et al., 2015). Similar effects of [GLU] have been demonstrated on the BRS in adults (LFgain = 28 ± 4 ms·mmHg^{-1} at pre-OGTT compared to 14 ± 2 at 120 min) (Holwerda et al., 2015). Interestingly, the decreased BRS during hyperglycaemia is mainly driven by the changes in [GLU], because when insulin increases but GLU is maintained at a fixed concentration using a euglycemic insulin clamp, BRS is maintained similar to baseline during the OGTT (Holwerda et al., 2015). However, when blood [GLU] increases, BRS significantly decreases 30min into the OGTT (Holwerda et al., 2015). These results highlight the important role of [GLU] on the overall BRS. However, research is needed to investigate how the vascular and autonomic determinants of BRS respond to increases in [GLU].

The depression in vascular compliance and cardiovagal modulation caused by [GLU] may contribute to the observed autonomic dysfunction and arterial stiffness in insulin resistant and diabetic adults and adolescents (McCloskey et al., 2014, Shin et al., 2010). However, most of the findings about the effects of OGTT on autonomic and vascular systems have been obtained in adults, and studies in youth are needed.

2.7.1.2 Physical activity and hyperglycaemia

Exercise can alter postprandial hyperglycaemia up to 24 h following the exercise bout. For example, in healthy adolescents both continuous moderate-intensity exercise and

HIIE have been shown to lower glucose and insulin excursions, and insulin resistance in the two and 24 h following the exercise session (Cockcroft et al., 2015, Cockcroft et al., 2017b). The mechanisms underpinning the observed two and 24 h effects of exercise on GLU excursions during the OGTT may also be different. For example, short-term influences of exercise can be attributed to differences in muscular insulin tolerance reflecting short-term glycogen repletion, as well as a lowered blood flow to the gastric tract and decreased gastric emptying of glucose. Whereas long-term action can be attributed to the slow phase of glycogen repletion that can last up to 40 h(Price et al., 1999), and improvements in insulin action.

The effects of exercise on postprandial glycaemia can also alter postprandial autonomic and vascular functions, as well as BRS. The possible role of habitual PA in altering arterial function following glucose ingestion has been described in one recent adult study involving decreases in daily step count for five days (< 5000·day^{-1}). Credeur et al. (2018) measured femoral and brachial arterial compliance 60 and 120 min into the OGTT pre and post five days of reduced PA levels. At pre, the OGTT did not cause increases in femoral arterial stiffness; however, post five days of reduced daily steps, decreases in femoral compliance were observed at 60 and 120 min following the OGTT. However, using a similar approach to decrease daily steps, Holwerda et al. (2015) did not find any influence of decreasing daily steps on arterial BRS following glucose ingestion. These results highlight that PA levels may offer a protective effect on arterial compliance, which is not translated into BRS. In youth, the possible long and short-term effects of PA on BRS and autonomic and vascular functions during the postprandial hyperglycaemic state remains to be elucidated.

2.7.2 Hyperlipaemia

To test the effects of hyperlipaemia, a similar approach to OGTT may be used. This involves the ingestion of a HFM in which blood samples are collected to measure TAG hourly after the HFM ingestion (Kolovou et al., 2011). Although there is no gold standard method to assess postprandial outcomes using a HFM challenge, standardisation between studies is encouraged as the fat content is known to influence the postprandial responses (Kolovou et al., 2011). Similarly, other factors may alter the responses to the meal such as the time of the day and the fat content of the last meal (Kolovou et al., 2011). Furthermore, although no guidelines exist for the assessment of postprandial lipaemia, the use of four hour protocols is encouraged as the time TAG takes to achieve peak and return to baseline also provides valuable information (Kolovou et al., 2011). In youth a HFM delivered as a milk shake has been shown to provide important information on postprandial risk factors and the role of exercise intensity (Bond et al., 2014).

2.7.2.1 Hyperlipaemia and autonomic and arterial functions

Similar to the effects of [GLU], elevations in blood [TAG] following the ingestion of a HFM may negatively impact the arterial and autonomic systems. In a systematic review, Wallace et al. (2010) investigated the effects of a HFM on endothelial function and the associated mechanisms. The authors identified 20 studies investigating postprandial lipaemia and arterial function in adults. The results of the review presented level one evidence showing increases in [TAG] and oxidative stress and a consequent depressed vascular function in adults (Wallace et al., 2010). These results have been replicated in healthy adolescents, where the ingestion of a HFM delivered as a milk-shake led to a significant decrease in vascular function, measured using

FMD, three h after the ingestion of the meal (~ 7% compared to pre ~ 9%) (Bond et al., 2015b).

Due to the physiological link between endothelial function and arterial stiffness via decreases in NO-dependent smooth muscle relaxation (Wilkinson et al., 2004), it is possible that a decrease in endothelial function following the ingestion of a HFM also increases arterial stiffness (Wilkinson et al., 2004); however, this has yet to be confirmed in adolescents. On the contrary, Augustine et al. (2014) reported an increased PWV of peripheral but not central arteries following the ingestion of a HFM in adults. The increases in PWV were moderately related (r = 0.44,) to increases in [TAG], suggesting that factors other than [TAG] may also be implicated in the observed augmented PWV. Furthermore, increases in arterial stiffness following a HFM appear to be depend on the type of measurement applied. For instance, local arterial stiffness assessed at the CCA in adults did not significantly change following a HFM (Murray et al., 2015). However, contrary to the acute effect of the meal, a chronic elevation in blood lipids may be associated with increases in local arterial stiffness, as an evidenced by I an increased CCA stiffness in children with dyslipidaemia (Nunez et al., 2010).

Less is known about the effects of blood [TAG] on the autonomic function and BRS. One study involving healthy adolescents has reported a decreased RMSSD following the ingestion of a HFM (Bond et al., 2015a), which shows that acute increases in [TAG] also alters autonomic modulation. However, the mechanisms behind the effect of a HFM on autonomic function and BRS of youth are yet to be explained.

2.7.2.2 Physical activity and hyperlipaemia

The possible effects of PA on postprandial lipaemia can be divided into two components: the short-term effect which is normally observed in the hours following the exercise bout; and the long-term effect which is normally observed 12 – 18 h following the exercise bout (Maraki and Sidossis, 2013). The mechanisms behind the short and long-term effect of exercise are likely to be different. For example, Tolfrey et al. (2014) reviewed the literature and found that when exercise is performed 12 – 16 h before a HFM, the postprandial lipaemic response is reduced in adolescent boys and girls suggesting that the up-regulation of lipoprotein lipase, which peaks 4 – 18 h following the exercise bout (Maraki and Sidossis, 2013), is responsible for the postprandial TAG clearance.

The short-term (i.e. up to four hours after exercise completion) effect of exercise on postprandial lipaemia is dependent on other mechanisms. For example, an increased hepatic oxidation rather than re-esterification of fatty acids culminates in a lower secretion of very-low density lipoprotein following exercise, despite a greater TAG availability (Magkos et al., 2006). Consequently, when exercise is performed an increased very-low density lipoprotein clearance maintains low plasma TAG concentration (Magkos et al., 2006). Recently, in 12 – 15-year-old adolescents high- but not moderate-intensity exercise has been shown to increase hepatic fatty acid oxidation, showing that exercise also influences this mechanism of TAG clearance in youth (Bond et al., 2015b), evidenced by the association (r = 0.61) between 3-hydroxybutyrate, a marker of hepatic fat acid oxidation, and a lowered TAG concentration over a 3-h postprandial analysis. Another mechanism by which exercise alters short-term postprandial lipaemia is via an increase in the uptake and metabolism of TAG in the skeletal muscle (Horton et al., 2002).

Interestingly, in youth the short and long-term mechanisms of the exercise effects on postprandial lipaemia have been shown to be differently altered by sex. For example, in 12 – 15 years old, girls presented an elevated postprandial lipaemia compared to boys, however, high-intensity exercise performed 12 – 18 h before the HFM lowered postprandial lipaemia independent of sex (Thackray et al., 2018). On the contrary, when exercise is performed 1-h before the ingestion of a HFM, girls present a lowered postprandial response compared to boys(Bond et al., 2014), indicating that sex influences the mechanisms by which exercise alters postprandial lipaemia in youth. However, the effects of habitual physical activity on possible sex differences in postprandial lipaemia is currently unknown.

As exercise acutely lowers postprandial lipaemia, it is reasonable to hypothesise that habitual PA and exercise training would be associated with a lowered postprandial lipaemia. In 70 year olds, postprandial lipaemia was 38% lower for the participants classified as active compared to the participants classified as inactive based on MVPA levels measured with accelerometers (Miyashita et al., 2011). These results highlight potential influences of habitual PA which may also be present in youth. However, in adolescents no investigation has identified whether habitual PA, PA intensities and CRF are associated with postprandial lipaemia. Similarly, the possible effects of PA and CRF, as well as PA intensities on the changes in the autonomic and vascular functions following a HFM is unclear.

On the contrary the short-term effect of exercise intensity on arterial function has been demonstrated following the ingestion of a HFM in adolescents. For example, performing moderate-intensity cycling exercise one hour before the ingestion of a HFM protects the vascular dysfunction observed when exercise in not performed (FMD of

~9% for moderate-intensity compared to ~7% for control). However, when HIIE is performed, FMD is improved (~11%) following the ingestion of HFM compared to moderate-intensity exercise and control (Bond et al., 2015b). Similar protective effects of exercise intensity were observed for changes in BP after the ingestion of a HFM (Bond et al., 2014). These results highlight potential effects of exercise intensity to acutely protect against the postprandial changes in traditional and non-traditional CVD risk factors in adolescents. Given that endothelial dysfunction can also increase arterial stiffness (Wilkinson et al., 2004), it is possible that an increased vascular function following exercise may be able to maintain arterial compliance in the postprandial state. However, it is still unclear how habitual PA alters the postprandial effects on autonomic and vascular functions in adolescents.

In summary, the literature highlights that the postprandial period is linked to decreases in autonomic function and BRS, as well as an increase in arterial stiffness. Most of the evidence on changes in autonomic and arterial functions have been gathered using hyperglycaemia challenges and in adult populations. The postprandial effects of [GLU] and [TAG] on autonomic function and vascular stiffness have been partially demonstrated in adolescents. However, the underpinning mechanisms are still unclear. Given atherosclerotic progression may be associated with postprandial outcomes (Hyson et al., 2003), understanding the postprandial state in youth is paramount for a better management of the disease.

2.8 Theses rationale and aims

It is well established that atherosclerosis may have its origins in childhood (McGill et al., 2000) and that changes in traditional CVD risk factors due to PA might not fully explain reductions in CVD risk (Mora et al., 2007, Bond et al., 2015a). Adult data show

that the autonomic and vascular systems are important candidates for the risk factor gap (Joyner and Green, 2009), and the BRS provides information about the interplay between these systems. In adolescents, improvements in these systems with no concomitant changes in traditional CVD risk factors implies that the risk factor gap may also exist in youth (Bond et al., 2015a). Furthermore, the identification of novel CVD risk factors is needed to help the management of the initial process of atherosclerosis in this population (Balagopal et al., 2011). This thesis, therefore, addresses the role of PA, PA intensity, CRF, and acute and chronic exercise in modifying the autonomic and arterial systems of adolescents. In addition, the postprandial state heightens CVD risk (Su et al., 2009) and measurements of traditional and novel CVD risk factors during the postprandial period may provide further valuable information about CVD risk reduction via PA and exercise in youth. Thus, this thesis also addresses the role of PA, exercise intensities, and/or CRF on traditional CVD risk factors, autonomic and arterial functions, and the possible interplay between these systems by measuring the BRS in the postprandial state. For this purpose, a series of novel experimental chapters were performed, and the aims of each chapter were:

Chapter 4: To systematically review the literature to evaluate the potential associations between PA, PA intensities, CRF and cardiac autonomic function in adolescents.

Chapter 5: To investigate the associations between habitual PA, PA intensities, CRF, HRV and HRR in health adolescents. In addition, this chapter also examined whether adding indices of autonomic function to a clustered CVD risk factor score based on traditional risk factors improved the strength of associations between CVD risk PA and CRF.

Chapter 6: To investigate the associations between PA intensities, CRF and postprandial TAG, HRV, and arterial stiffness.

Chapter 7: To determine the between- and within-day reliability of the autonomic and vascular determinants of baroreflex sensitivity in adolescents.

Chapter 8: To address how moderate and high-intensity interval running alters the post-exercise recovery of BP, BRS and its autonomic and vascular determinants.

Chapter 9: To investigate whether the autonomic and vascular determinants of BRS are altered following an oral glucose challenge. This chapter also investigates whether exercise performed before the glucose challenge modifies the postprandial outcomes and is dependent on exercise intensity.

Chapter 10: To investigate the autonomic and vascular adaptations of BRS in response to four weeks of HIIE training and two weeks of detraining. In addition, the acute changes in BRS following exercise and its autonomic and vascular determinants were also examined at before and after four weeks of HIIE training.

Chapter 3: Methods

The aim of this chapter is to provide a general overview of the methodological approaches used in this thesis. The emphasis of the chapter is to provide additional information about study design, recruitment and data handling that are not presented in the forthcoming experimental chapters. Further details on the methods used are presented in Chapters 4 – 10.

3.1 Study designs

Chapter 4 is a systematic review of the literature and its methods have been presented in detail in Chapter 4 and as a registered study protocol (International Prospective Register for Systematic Review; reference CRD42015023614). Chapters 5 and 6 were cross-sectional observational studies. Chapter 7 – 10 were cross-over controlled trials with repeated measures. Chapter 10 was a randomised controlled trial.

3.2 Inclusion/exclusion criteria and ethics

All data collection within this thesis received institutional ethics approval from the Sport and Health Sciences Ethics Committee (Appendix 1, page 344). Exclusion criteria included the presence of any contraindications towards maximal exercise, any relevant allergies (i.e. lactose intolerance), use of any supplement or medication known to influence fat or carbohydrate metabolism, cardiac autonomic function, BP, and vascular function. For Chapters 5 and 6 there were no specific inclusion criteria apart from age (12 – 15 years). For Chapter 10 only boys were included.

3.3 Recruitment

A convenient sampling method was used in Chapters 5 – 10. For this, local secondary schools were contacted and after consent form the Head Teacher and the Physical Education department, the recruitment procedures took place. Assemblies were conducted explaining the aims, benefits and risks of the project and at the end of each assembly, envelopes were delivered to potential participants. The envelopes contained information about the study, study design, rationale and procedures, contact details of the research team, a parent/guardian consent form, a participant assent form and a health screening form. A sample of the recruitment documents is presented in Appendix 2, page 346. After the return of the envelopes, parents and guardians were contacted and once agreement about study involvement was achieved, the procedures for data collection took place.

For Chapter 5 and 6 a total of 260 envelopes were delivered. Eighty-eight (34%) were returned indicating an interest in taking part in the study. From 88 potential participants, 80 (31% from the initial number of envelopes) gave consent to take part in the study. From the 80 volunteers, 18 (22%) dropped out decreasing the final sample size to 62 (25 girls). Figures 5.1 and 6.1 provide the flow diagram of the recruitment process and the final sample size in Chapters 5 and 6.

For Chapters 7 – 9 a total of 160 envelopes were delivered from which 16 (10%) were returned indicating an interest in taking part in the study. All 16 potential participants gave consent/assent. From the 16 initial participants one (6% of included participants) dropped out and the final sample size of Chapters 7 – 9 was 15 (two girls). Initially the study aimed to recruit 12 girls to compare the possible effects of sex, however, due to

lack of volunteers the study ended with the inclusion of 13 boys. The final sample size is described in the experimental Chapters 7 – 9.

For Chapter 10, a total of 70 envelopes were delivered from which 21 (30%) were returned showing an interest in taking part in the study. All 21 participants gave consent/assent to take part in the study. From the 21 volunteers, two (10%) dropped out decreasing the final sample size to 19. In this study, only boys were included.

3.4 Participants characteristics

Anthropometrics, body composition and pubertal status measures were collected in Chapters 5 – 10. Body mass was obtained to the nearest 0.1 kg using commercial body mass scales (SECA, UK). For determination of stature a stadiometer was used to the nearest of 0.1 cm (SECA, UK). Pubertal status was determined using age from peak height velocity (PHV) in Chapters 5 and 6. For this, sitting height was obtained to the nearest of 0.1 cm (SECA, United Kingdom) and Equations 3.1 and 3.2 were used to determine the age from PHV for girls and boys, respectively (Mirwald et al., 2002). The standard error of estimates for equation 3.1 and 3.2 are 0.57 and 0.59 years, respectively. Assessment of PHV has strong (r^2 = 0.89 for girls and boys) criterion-related validity with longitudinal measures of PHV (Mirwald et al., 2002). Participants were then classified as pre (-1 year), circa (-1 to +1 year), or post (+1 year) PHV. In Chapters 7 – 10, pubertal status was determined by the self-assessment of secondary sexual characteristics using adapted drawings of pubic hair development (Morris and Udry, 1980).

PHV (years) = -9.376 + 0.0001882(leg length · sitting height) + 0.0022(age · leg length) + 0.005841(age · sitting height) - 0.002658·(age · body mass) + 0.07693(age/stature)·100

Equation 3.1: Peak height velocity determination for girls.

PHV (years) = -9.236 + 0.0002708(leg length · sitting height) + -0.001663(leg age · leg length) + 0.007216(age · sitting height) + 0.02292(body mass/stature)·100

Equation 3.2: Peak height velocity determination for boys.

For BF%, triceps and subscapular skinfold thickness were obtained in triplicate to the nearest of 0.1 mm (Holtain Ltd, Crymych, UK), and the average of the closest values calculated to determine BF % (BF%) using validated population specific equations 3.3 – 3.6 (Slaughter et al., 1988). Assessment of BF% from skinfolds has strong (r^2> 0.80) criterion-related validity with direct determination of body density and the error of estimate is 3.8% (Slaughter et al., 1988). In Chapter 10, body composition was obtained using air displacement plethysmography (BodPod®, Concord, California, USA). The BodPod® is a double chamber unit, with the chambers separated by a diaphragm which is electronically controlled and measures perturbations in pressurisation of the chamber for volumetric measurement. Prior to testing, the system was calibrated following the manufacturer's instructions. For this, five measurements of a 49.887 L cylinder were performed, and the average and standard deviation obtained. The acceptable range for the obtained average was 49.787 to 49.987 L, with standard deviation lower than 75 mL. Participants wore a swimsuit and a swim cap, sat still in the chamber and body volume in L was determined in duplicate. When the duplicate measures differed more than 75 mL, a third measurement was obtained. After accounting for lung volume using the software sex specific equations, body

composition was obtained using equation 3.7 (Siri, 1993). Assessment of BF% from the BodPod has a strong (r^2 = 0.82) criterion-related validity with body composition obtained from dual-energy X-ray absorptiometry (Ferri-Morales et al., 2018).

$$BF\ (\%) = -1.7 + 1.21(\text{Sum of skinfolds}) - 0.008(\text{Sum of skinfolds}^2)$$

Equation 3.3: Body fat % determination for pre-pubertal boys.

$$BF\ (\%) = -3.4 + 1.21(\text{Sum of skinfolds}) - 0.008(\text{Sum of skinfolds}^2)$$

Equation 3.4: Body fat % determination for pubertal boys.

$$BF\ (\%) = -5.5 + 1.21(\text{Sum of skinfolds}) - 0.008(\text{Sum of skinfolds}^2)$$

Equation 3.5: Body fat % determination for post-pubertal boys.

$$BF\ (\%) = -2.5 + 1.33(\text{Sum of skinfolds}) - 0.013(\text{Sum of skinfolds}^2)\ \text{girls}$$

Equation 3.6: Body fat % determination for girls.

$$BF\ (\%) = (495 / \text{Body Density}) – 450$$

Equation 3.7: Siri's equation.

3.5 Cardiorespiratory fitness

In Chapters 5 and 6 CRF was determined using a validated steep ramp test (Bongers et al., 2013). This test was chosen due to feasibility in a school-setting. For this, participants cycled to exhaustion on an electromagnetic braked cycle ergometer (Lode, The Netherlands). After three min of warm-up at 25 W, participants started the test, which consisted of a predetermined increment in work-rate per minute. The work-rate increments were determined according to participant stature: 60 W if <120 cm, 90

W if between 120 and 150 cm, and 120 W if >150 cm. Participants were asked to maintain a pedalling frequency of 80 revolutions per minute. The protocol ended when participants dropped the pedalling frequency for five s below 60 revolutions per min despite strong verbal encouragement. Maximal effort was considered when participants showed subjective signs of intense effort (e.g., unsteady cycling, sweating, and clear unwillingness to continue despite encouragement). This method has been shown to have strong (r^2 = 0.92) criterion-related validity with directly measured CRF (Bongers et al., 2013). The peak power in W obtained at the end of the ramp test was used to estimate peak $\dot{V}O_2$ in mL using equation 3.8 with an error of estimate of 237 mL.

$$\dot{V}O_2max\ (mL \cdot min^{-1}) = (8.262 \cdot \text{peak power}) + 177.096$$

Equation 3.8: Determination of maximum oxygen uptake using power output from the steep ramp test.

For Chapters 8 and 9 CRF was determined as the $\dot{V}O_2$max obtained from a combined incremental and supramaximal test to exhaustion (Barker et al., 2011), on a motorised treadmill (Woodway GmbH, Germany). The incremental test started at 6-km·h^{-1} with 1% inclination after a three min warm-up at 4-km·h^{-1}. Increments of 0.5-km·h^{-1} were completed every 30 s until participants reached exhaustion. At exhaustion, MAS was determined and participants completed a three min cool down period at 4 km·h^{-1}. The cool down was followed by 10 min of recovery. Participants then completed a running bout to exhaustion with 5% inclination at the MAS obtained in the incremental test. $\dot{V}O_2$max in L·min^{-1} was determined as the highest value obtained during the incremental or supramaximal test. An example of the $\dot{V}O_2$max obtained with this protocol is presented in Figure 3.1.

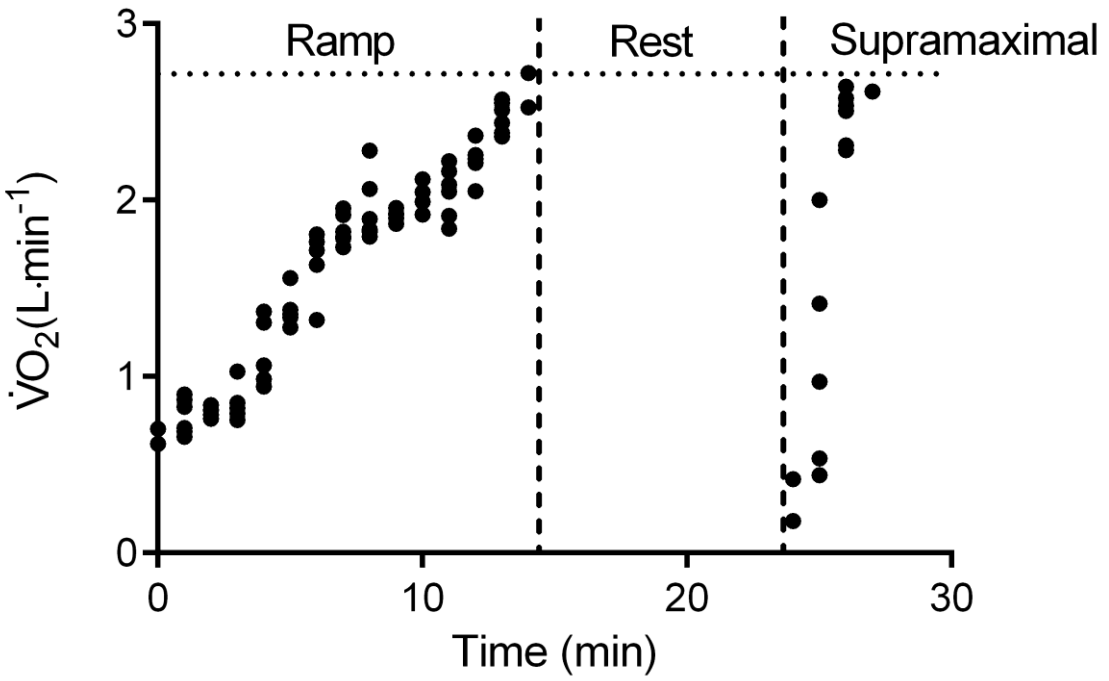

Figure 3.1: Representative oxygen consumption obtained from the incremental and supramaximal test. From this oxygen uptake trace, maximal oxygen uptake is determined as the maximum value obtained from either the ramp or the supramaximal test.

Pulmonary oxygen uptake and carbon dioxide production ($\dot{V}O_2$ and $\dot{V}CO_2$, respectively) were obtained breath-by-breath throughout the incremental and supramaximal test (Cortex Metalyzer III B; Cortex, Germany). Before testing, the equipment was calibrated according to the manufacturer instructions. Breath-by-breath data were exported in bins of 10 s and the gas exchange threshold (GET) identified as the disproportionate increase in $\dot{V}CO_2$ relative to $\dot{V}O_2$. The latter was performed by two independent researchers.

3.5.1 Normalisation of cardiorespiratory fitness

Cardiorespiratory fitness taken as absolute $\dot{V}O_2$in $L \cdot min^{-1}$ is positively associated with body size (r = 0.58; Figure 3.2 A). To account for the effects of body size, CRF is normally is expressed relative to body mass using a ratio standard approach (i.e. $mL \cdot kg \cdot min^{-1}$). However, as shown in Figure 3.2 B, CRF relative to body mass does not produce a size free measurement of CRF, but rather leads to a lower CRF in heavier participants (r = -0.55). To overcome this problem of 'over-scaling', CRF was further expressed in Chapters 5 and 6 using an allometric scaling approach. Allometric scaling was obtained by calculating a sample specific scaling exponent using log-linear regression between body mass and CRF controlling for sex. Allometric scaling with this new size exponent (β = 0.58), better controlled for the influence of body mass on CRF, as evidenced by the non-significant association between CRF and body mass (r = 0.07; Figure 3.2 C).

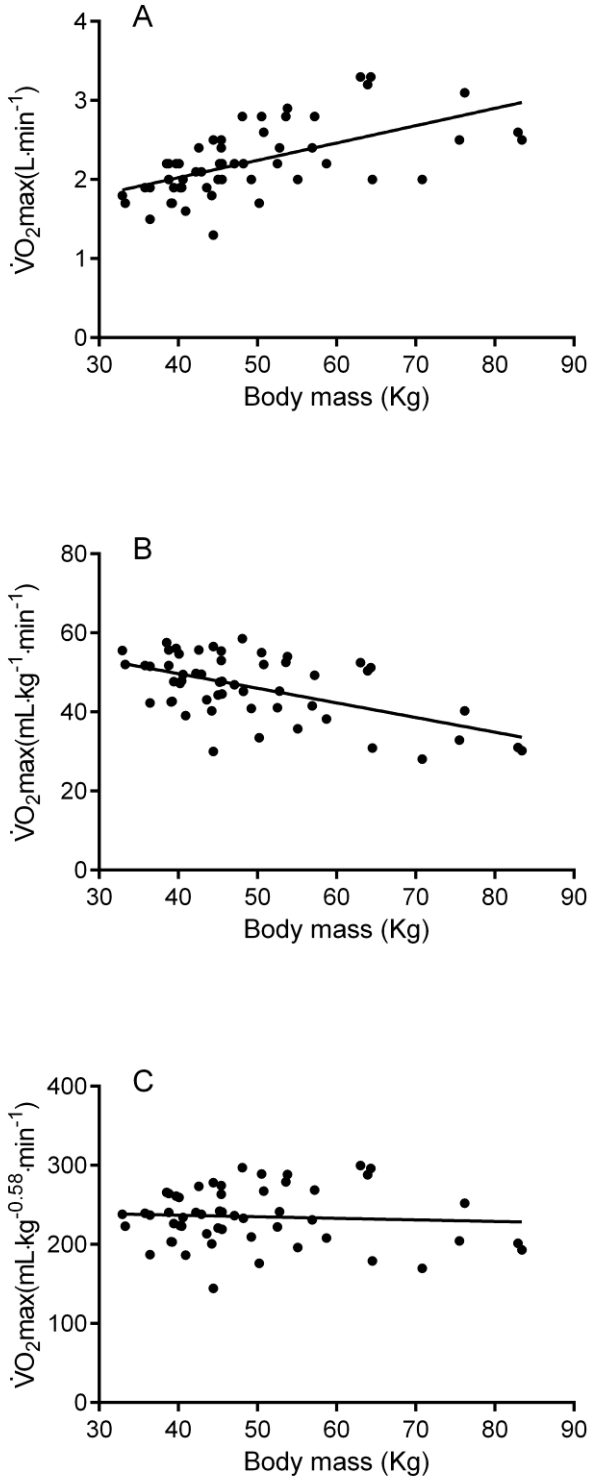

Figure 3.2: Dependence of CRF on body size. A) positive association between body mass and CRF; B) negative association between ratio expressed CRF in $mL{\cdot}kg^{-1}{\cdot}min^{-1}$ and body mass; C) lack of association between allometric expressed CRF in $mL{\cdot}kg^{-0.58}{\cdot}min^{-1}$ and body mass. Data presented are obtained from Chapters 5 and 6.

3.6 Physical activity

In this thesis, PA was measured using accelerometers either to characterise habitual PA levels of the participants (Chapter 5 and 6), or to standardise testing procedures (Chapters 7 – 9). Physical activity was objectively measured using a wrist-worn accelerometer (GENEActiv, Cambridge, UK). The device was set to record PA for seven (or three when used to test standardisation) consecutive days at a frequency of 100 Hz. Participants were instructed to wear the device on their non-dominant wrist, including sleeping h and water activities. The device was then retrieved, and the raw acceleration data transformed into epochs of 60 s using the GENEActiv software (version 2.9, GENEActiv; UK). The 60 s epoch files were then imported into a freely available spreadsheet (available at: https://open.geneactiv.org/) to calculate the time spent performing sedentary time, and LPA, MPA and VPA. Age-specific cut-off points of <420 $g \cdot min^{-1}$ for sedentary time, between 420 and 1,140 $g \cdot min^{-1}$ for LPA, between 1,140 and 3,600 $g \cdot min^{-1}$ for MPA and >3,600 $g \cdot min^{-1}$ for VPA were used (Phillips et al., 2013). Participants were included in the final analysis with a minimum of three days wear time defined as 12 h per day of valid data which has been shown to produce reliable assessment of PA levels in a large cohort of British children (Rich et al., 2013).

3.7 Experimental manipulations

3.7.1 High-fat meal and oral glucose tolerance test

In Chapter 6, participants consumed within 15 min a HFM consisting of a milkshake of three parts Cornish ice cream and one-part double cream, providing 1.50 g of fat (70% total energy), 1.20 g carbohydrate (25%) and 0.21 g of protein (5%) per kg of body mass (80 $kJ \cdot kg^{-1}$). This meal replicated previous studies from our research group, which demonstrated impairments in endothelial function and postprandial lipaemia in

healthy adolescents (Bond et al., 2015b, Bond et al., 2014, Bond et al., 2015d). To test the effects of the meal on postprandial lipaemia, [total cholesterol], [TAG], [HDL] and [GLU] were determined in whole capillary blood using a validated portable system (CardioChek, Polymer Technology Systems, IN, USA) (Panz et al., 2005). For this, 40 (for lipid profile) or 25 µL (for [GLU]) of blood were collected into micro-tubes (CardioChek, Polymer Technology Systems, IN, USA) and pipetted into straps that determined either [lipid] or [GLU]. The range for each outcome using the CardioChek are as follows: TAG = 0.57 – 5.65 mmol·L^{-1}; TC =2.59 – 10.36 mmol·L^{-1}; HDL =0.39 – 2.59, and GLU =1.11 to 33.3 mmol·L^{-1}. Samples were measured in duplicate and the CVs between the duplicate measures was lower than 7%.

In Chapter 9 participants completed an OGTT according to previous work from our research group (Cockcroft et al., 2015, Cockcroft et al., 2017b). For this, 75 g of GLU was diluted in 300 mL of water and participants ingested the drink within five min. Seven capillary blood samples were collected via fingertip capillary sampling at 0, 10, 20, 30, 60, 90 and 120 min post GLU ingestion. These time points are known to provide an accurate estimate of insulin secretion and action (Dalla Man et al., 2005). Capillary blood samples (~ 600 µL) were collected into heparin fluoride coated microvettes (CB 300 tubes, Sarsted Ltd, UK). After collection, blood samples were promptly analysed for blood [GLU] (YSI 2300 Stat Plus, Yellow Springs, OH, USA). The YSI measures GLU within the range of 0 to 30 mmol·L^{-1} from a 25 µL sample. As GLU data were obtained over time, the total area under the curve (tAUC) and the incremental area under the curve (iAUC) were calculated using the trapezium rule (GraphPad prism, CA, USA). Glucose tAUC and iAUC responses to an OGTT have been shown to be reliable (CVs from 5 – 7%) in a sample of healthy adolescents (Cockcroft et al., 2017a).

3.7.2 Exercise conditions

In Chapters 8 and 9, participants completed HIIE and moderate-intensity interval exercise (MIIE) on a motorised treadmill (Woodway GmbH, Germany). The HIIE protocol, consisting of 8 bouts of high-intensity work intervals, was based on previous work in our group (Bond et al., 2015b, Bond et al., 2015c, Cockcroft et al., 2015, Cockcroft et al., 2017b), except in the present thesis running exercise was chosen as it better reflects typical PA of adolescents. The HIIE protocol consisted of three min warm up at 4-km·h^{-1} followed by the completion of eight bouts of 1-min at 90% of the MAS obtained from the incremental test (section 3.3.2), interspersed by 75 s of active recovery at 4 km·h^{-1}. Participants finished the protocol with two min cool down at 4 km·h^{-1}. The duration of the HIIE was 23 min.

The MIIE protocol was prescribed so the participants would complete the work-intervals of for the exercise bout below the GET determined from the incremental test (see section 3.5). For this, participants performed bouts of 1-min at 90% of GET interspersed by 75 s of active recovery at 4 km·h^{-1}. The number of bouts in MIIE was tailored to each participant to match the total distance covered in the HIIE condition. MIIE was also preceded by three min warm-up and followed by two min cool down at four km·h^{-1}.

In Chapter 10, participants performed a training intervention involving the completion of 12 training sessions using a similar HIIE protocol as for Chapters 8 and 9. Maximum aerobic speed was obtained from a 20 m shuttle run test performed at the sports hall at the University of Exeter. For this, participants ran back and forth to cones set 20 meters apart with the speed guided using a pre-recorded audio. Speed increased by 1-km·h^{-1} at the end of each stage. Maximum aerobic speed was obtained for training

guidance as previously performed in a paediatric population (Mandigout et al., 2002). Participants completed the HIIE training running back and forth to the cones with the distance individualised to match the speed associated with 90% of the MAS obtained from the shuttle run test. In each training session, participants completed three min warm up at 4 $km \cdot h^{-1}$, followed by the completion of 8 – 12 (from week one to four) 1-min boutsat 90% of the MAS. The bouts were interspersed by 75 s of active recovery, which consisted of walking between the cones. The speed was controlled using whistles. During all training sessions in Chapter 10, participants wore heart rate monitors.

3.7.3 Chapters checklist

The experimental manipulations used in each experimental chapter is described in Table 3.1.

Table 3.1: Checklist of experimental manipulations in each experimental chapter.

	Chapter Five	Chapter Six	Chapter Seven	Chapter Eight	Chapter Nine	Chapter Ten
High-fat meal		X				
OGTT					X	
Exercise conditions				X	X	X

OGTT: oral glucose tolerance test.

3.8 Outcomes

3.8.1 Standardisation

All protocols for determination of HRV, BRS and arterial stiffness followed strict standardised procedures. In all experimental chapters, participants were transported to the laboratory following an overnight fast and were instructed to avoid extraneous exercise in the 48 h preceding data collection. In Chapters 7 – 9, participants were

also asked to keep a similar diet in the 24 h preceding the protocols, according to food diaries obtained in the first visit. Food diaries were analysed for the determination of total calories, and the absolute and relative contribution from carbohydrates, lipids and proteins using validated nutrition software (CompEat Pro, Nutrition Systems, UK). All participants in the experimental chapters reported compliance with the standardisation procedures.

In Chapters 5 and 6 before HR data collection, participants were given five min of supine rest, followed by five min of data acquisition according to published guidelines (Task-Force, 1996). In Chapters 7 – 10, participants rested supine for 10 min before data collection took place. During HRV and BRS data acquisition in Chapters 5 – 10, participants maintained a breathing frequency at 12 cycles per min (0.2 Hz). This breathing frequency is known to increase autonomic modulation of heart rate in adolescents (Williams and Lopes, 2002). This breathing frequency was also used for BRS determination because it shifts breathing frequency above the frequency at which BRS influences HR (0.04 – 0.15 Hz) (Keyl et al., 2001), as suggested when examining spontaneous BRS (Bothova et al., 2010, Tzeng et al., 2009).

Arterial stiffness assessment with PWV was conducted following 15 min of supine rest in Chapters 5 – 6. In Chapters 7 – 10, arterial stiffness assessment was preceded by 10 min of supine rest. For both arterial stiffness assessments, measurements of BP were obtained. In Chapter 5 and 6, BP was measured three times after a 10 min supine rest (A&D Medical Co., 140 LTD, Japan). The average of the two closest SBP and diastolic BP (DBP) was used to calculate PWV. In Chapters 7 – 10, arterial BP was reconstructed from finger plethysmography (Finometer PRO, Netherlands) as

occurred simultaneously with arterial images for determination of local arterial stiffness.

3.8.2 Heart rate variability and recovery

Heart rate variability was calculated in all experimental chapters, except Chapter 7. To calculate HRV, beat-by-beat HR was obtained. In Chapters 5 and 6, HR was obtained using HR transmitters (Polar Team2, Polar, Kempele, Finland). This device measured HR at a frequency of 1,000 Hz, and inter-beat intervals in ms were obtained using specific software (Polar Precision Performance 5.0, Polar, Kempele, Finland). In Chapters 8 – 10, HR was obtained using a three-led ECG device. Data were recorded at a frequency of 1,000 Hz using the ECG module for PowerLab (PowerLab, ADInstruments). Inter-beat intervals were then automatically obtained by an R wave detection algorithm using purposely build macros in Chart 5 (Chapters 7 – 9) or Chart 8 (Chapter 10) (PowerLab, ADInstruments).

Inter-beat intervals were exported to Excel (Microsoft, USA), where data were visually checked for ectopic beats, and all errors were manually corrected by linear interpolation using adjacent intervals. Signals with more than 3% of errors were discarded from analysis. Once manually edited, data were exported to .txt files and uploaded to Kubios v 3.0 (Biosignal Analysis and Medical Imaging Group at the Department of Applied Physics, University of Kuopio, Kuopio, Finland) for HRV analysis. In Chapters 7 – 10, RR intervals used in Kubios were exported to .txt files and saved for BRS analysis.

HRV was obtained in both time and frequency domains. The time domain indices obtained were RMSSD and SDNN (standard deviation of all RR intervals). For the frequency domain, data were interpolated at 4 Hz and Fast

Fourier Transformation using a Welsh's periodogram with hamming windows of 300sand 50% overlapping applied (Tarvainen et al., 2014). The area under the low frequency (LF = 0.04 – 0.15 Hz) and high frequency (HF = 0.15 – 0.50 Hz) bands were obtained and calculated in absolute (ms^2) and normalised units (nu), as well as a ratio (LF/HF).

Heart rate recovery was obtained in Chapter 5 following the steep ramp test. The same procedures of error correction for the HRV data were applied. Error free HR traces were analysed using two different methods. For the first, a regression between the natural logarithm of HR and time in s was obtained in the first 30 s of recovery, and the inverse of the beta coefficient (i.e. -1/slope) expressed as the T_{30} (Imai et al., 1994). For the second, a mono-exponential function was fitted to the 10 min HRR trace. For this, beat-by-beat HR was interpolated into one beat per second and the mono-exponential curve obtained according to equation 3.9 using GraphPad prism (GraphPad prism, CA, USA). The time constant (t)reflecting 63% of the time HR took to reach its asymptotic value, was used as an indicator of the HRRt (Javorka et al., 2003).

$$HR = HRmin + A^{exp[-time/tau]}$$

Equation 3.9: Mono-exponential model for HRR. Where HR is the dependent variable; HRmin is the HR at which asymptote is obtained; A is the amplitude between maximum heart rate and asymptote; and tau (t) is the time constant reflecting 63% of the time HR took to its asymptotic value.

In addition to the HRR, HRV at the recovery period was calculated using the time domain analysis. For this, RMSSD was calculated every 30 s throughout the full 10 min of recovery ($RMSSD_{30}$). $RMSSD_{30}$ was calculated in Kubios as described above.

$RMSSD_{30}$ was transformed into natural logarithm and a median filter was applied following recommendations (Goldberger et al., 2006). Figure 3.3 presents the HRR of a representative participant including the calculation of all HRR indices.

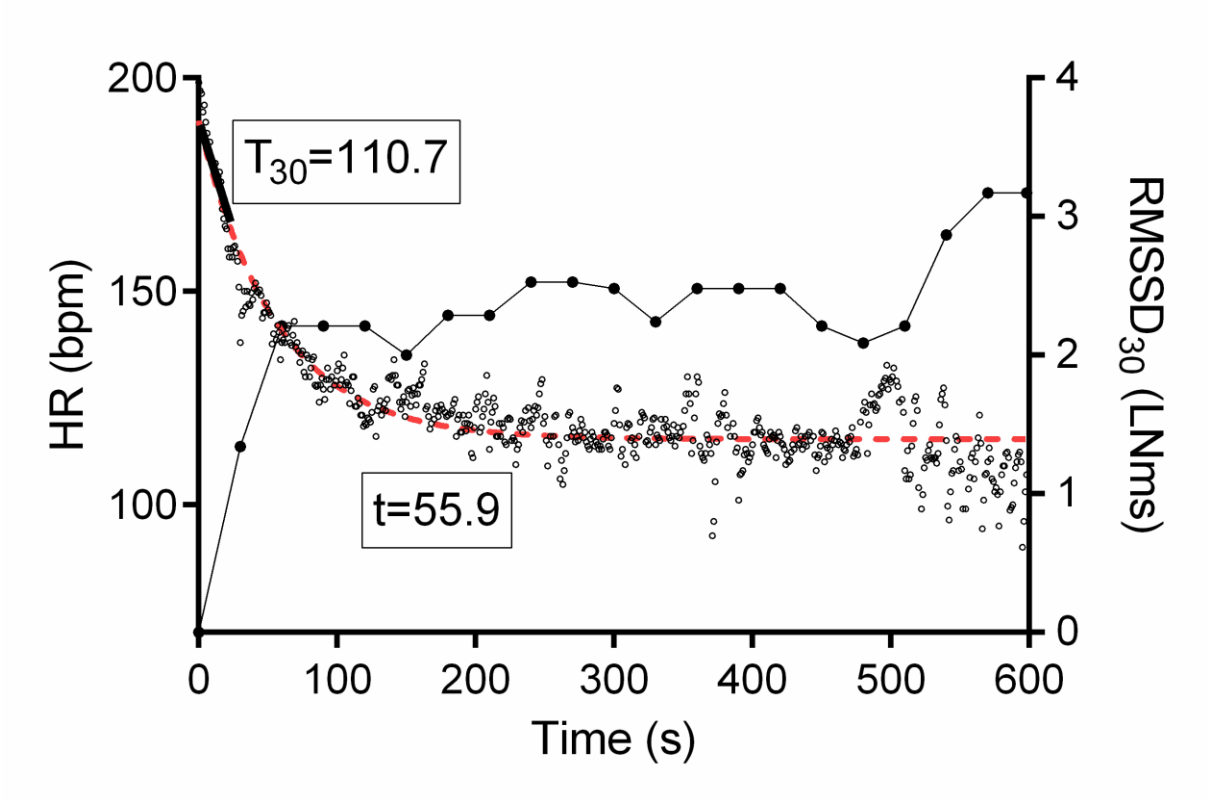

Figure 3.3: Representative sample of a heart rate recovery analysis. The black line represents T_{30} linear fit and the red dashed line represents the mono-exponential curve from which HRRt (t) was obtained. Open circles: heart rate in beats per minute. In B) the natural logarithm (LN) of the RMSSD was obtained for the whole 10 min recovery.

3.8.3 Baroreflex protocol

To investigate the autonomic and vascular determinants of BRS, a BRS protocol was adopted based on previous work (Lenard et al., 2004). For this, simultaneous measures of arterial BP, ECG and CCA images were obtained. The BRS protocol started after 10 min of supine rest (see section 3.8.1) and consisted of: 1) a

measurement of brachial BP; 2) CCA images recorded for 15 cardiac cycles; and 3) five min of simultaneous ECG and BP recordings while participants paced breathing frequency at 12 cycles per minute. The procedures were completed in the described order, lasting for a total duration of ~ 20 min (including the standardisation period).

For the BRS protocol, beat-to-beat BP was measured continuously using finger plethysmography (Finometer PRO, Netherlands). The Finometer uses the volume-clamp method to continuously measure BP. For this, the diameter of the digital artery is kept constant by adjusting pressure in the cuff surrounding the artery. Changes in diameter are measured using infrared photo-plethysmography, and small increases in diameter leads to a counter pressure applied by the cuff to avoid arterial distension (Truijen et al., 2012). The artery can be clamped at any diameter, however, for a precise estimation of BP, the artery needs to be clamped at the diameter where the transmural pressure is decreased, and the internal and external arterial pressures are equivalent. Because smooth muscle tone changes during BP monitoring, the Finometer adjusts the ideal diameter by applying a Physiocal function (Truijen et al., 2012). Physiocal stops continuous monitoring of BP and changes the cuff pressure to a constant pressure regardless of arterial diameter. As such, Physiocal constantly (i.e. every minute) adjusts the ideal volume clamp for BP estimation. Physiocal procedures can last up to three cardiac cycles, and therefore, the continuous beat-to-beat BP monitoring is lost. For this reason, during BRS (last five min of simultaneous BP and ECG), Physiocal function was turned off. A recent investigation has shown that in well controlled situations (i.e. supine rest), Physiocal does not influence the outcomes of BP analysis (Kiviniemi et al., 2014).

Because the BP measured at the finger differs from the value obtained at the brachial artery, the Finometer was calibrated using a measurement of brachial pressure before data collection took place. This was performed automatically two times using Finometer return to flow calibration, according to manufacturer's instructions. During data collection, participants kept their hands at heart level. For data analysis, the reconstructed brachial pressure obtained from the Finometer was used (Guelen et al., 2008). The Finometer has been validated in children against the auscultatory method (Tanaka et al., 1994).

Simultaneously with beat-by-beat BP, ECG signals were obtained. Both BP and ECG signals were collected using a Power Lab system (PowerLab, ADInstruments) which acquired data at a frequency of 1,000 Hz. Figure 3.4 shows an example ECG and BP trace. Beat-by-beat SBP in mmHg and RR intervals in ms were automatically obtained in Chart 5 (Chapters 7-9) or Chart 8 (Chapter 10) (PowerLab, ADInstruments). Data were exported to Excel (Microsoft, USA) and manually checked for errors in data extraction and ectopic beats. Systolic BP errors were manually interpolated, and RR intervals were obtained from Kubios (section 3.3.5.2) after HRV analysis was completed.

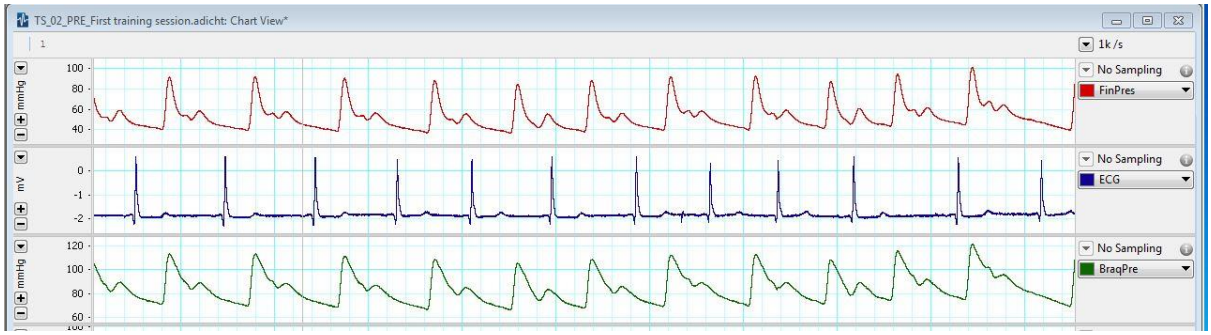

Figure 3.4: Beat-by-beat electrocardiographic and blood pressure trace obtained from Power Lab. Red trace indicates finger blood pressure. Blue trace indicates electrocardiography signal. Green trace indicates reconstructed brachial pressure.

In Chapters 7 – 10, BRS was determined as the transfer function between the SBP and RR. For this, the gain between the SBP and RR data was calculated as the LFgain, from the final five min of the BRS protocol. For this purpose, beat-to-beat RR intervals and brachial reconstructed SBP were interpolated at 2 Hz and a Fast-Fourier Transformation similar to HRV analysis was applied to obtain the power spectrum in the low frequency band for both signals (LF =0.04 – 0.15 Hz). A cross-spectral transfer function was then applied and the mean cross-spectrum (LFgain) in the range where the coherence was > 0.5 was expressed as the baroreflex gain (BRS) in ms $mmHg^{-1}$. This index was chosen due to its established validity compared to BRS assessment using vasoactive drugs (Robbe et al., 1987). LFgain was calculated using a homemade routine (MatLab R2017a).

In Chapter 10, BRS was also obtained as the sequence method. For this, sequences of three or more beats where SBP and RR interval increased (Seq++) or decreased (Seq--) more than one mmHg and five ms were computed. The coefficient of the linear regression when $r^2 > 0.9$ between SBP and RR was used as the sequence method. The sequence method was obtained using freely available software (CardioSeries v2.4, http://www.danielpenteado.com).

3.8.4 Arterial imaging

In Chapters 7 – 10 local arterial compliance and distensibility were obtained at the CCA in accordance with established guidelines (Urbina et al., 2009). All arterial images were acquired during the BRS protocol.

CCA images were obtained ~ 2 cm distal from the carotid bulb using a high-resolution (13 MHz) linear array transducer (Apogee, 1000, SIUI, China). The images were obtained over 15 cardiac cycles recorded at 15 Hz. Subsequently CCA images were

analysed using validated wall tracking software (Carotid Analyzer - Medical Imaging Applications LLC) (Mancini et al., 2004) for determination of DLD and SLD. The average of 3 – 7 cardiac cycles with clear definitions of the near and far walls were used. Figure 3.5 shows a representative carotid scan with the respective analysis of DLD and SLD.

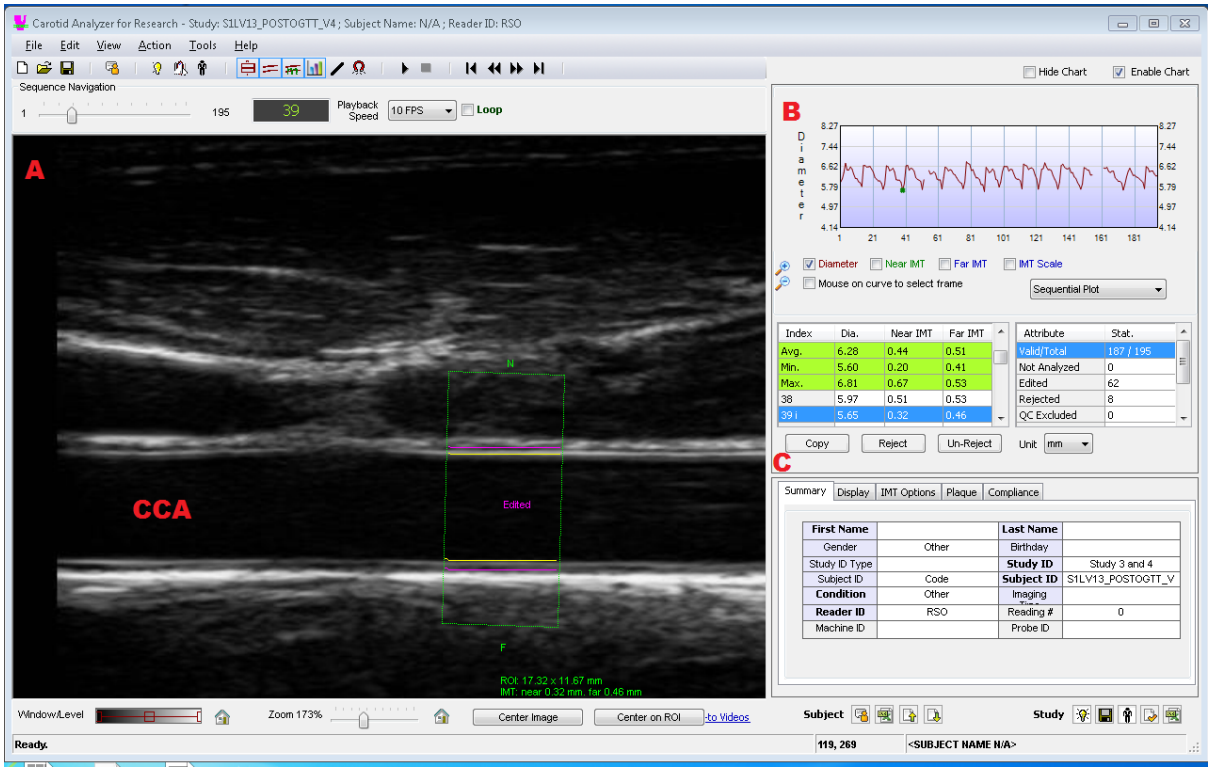

Figure 3.5: Example of a common carotid artery and the respective intra-media thickness and arterial diameter during 15 cardiac cycles. In A) a sample image is obtained, and the region of interest is shown in green. The arterial diameter is then obtained using the purple lines and lumen diameter using the yellow lines. The near (N) and far (F) intra-media thickness are obtained automatically by the software as the distance between the purple and yellow lines. In B) continuous diameter trace is obtained frame-by-frame from the monitoring of the region of interest from A. In C) the automatic track provides the values of carotid intra-media thickness and arterial diameter.

During the 15 cardiac cycles, beat-to-beat brachial reconstructed systolic and diastolic BP were obtained from the Finometer and averaged to determine PP. Equations 3 – 10 and 3 – 11 were used to determine CCA compliance and distensibility.

$$AC\ (\mu m \cdot mmHg^{-1}) = \Delta D/PP$$

Equation 3.10: Arterial compliance. Where ΔD is SLD minus DLD and PP the measures pulse pressure.

$$AD\ (mmHg \cdot 10^{-3}) = \Delta CSA/PP \cdot CSAmin$$

Equation 3.11: Arterial distensibility. Where CSA in the cross sectional CCA artery calculated as $CSA = \pi r^2$ being r = diameter/2 and ΔCSA the systolic CSA minus diastolic CSA (CSAmin).

In addition, in Chapter 10 the young elastic modulus was calculated using equation 3.13.

$$Young\ elastic\ modulus\ (mmHg{:}10^{-3}) = [3(1+CSAmin/WIMT)]/AD$$

Equation 3.12: Young elastic modulus. Where WIMT is the IMT cross sectional area in mm^2 obtained as $\pi(IMT^2)/4$ and AD is the calculated arterial distensibility.

3.8.5 Pulse wave velocity

In Chapters 5 and 6 PWV was calculated between the carotid and radial arteries (Complior SP, Artech Medical, France). Two probes were positioned on the region with the strongest pulse on these arteries and the distance from the external notch to the carotid probe was subtracted from the distance from the external notch to the radial probe and used to calculate PWV. The difference in time between the upstroke of the

waveform recorded at the carotid and radial arteries was obtained by the software. Only waveforms with clear baseline, maximum amplitude, and a reasonable configuration were accepted by the software for PWV calculation. Measurements were taken three times with the two closest values averaged and retained for analysis.

3.8.6 Chapter checklist

The outcome measurements using in each experimental Chapter are described in Table 3.2.

Table 3.2: Checklist of the outcomes in each experimental chapter.

	Chapter Five	Chapter Six	Chapter Seven	Chapter Eight	Chapter Nine	Chapter Ten
HR monitors	X	X				
ECG			X	X	X	X
BP			X	X	X	X
Arterial imaging			X	X	X	X
PWV	X	X				

ECG: electrocardiography. BP: blood pressure. PWV: pulse wave velocity. HR: heart rate.

3.9 Statistics analyses

All statistical analyses were performed on SPSS (Chicago, USA) and Excel spreadsheets available at (http://www.sportsci.org/resource/stats/). HHRt, tAUC and iAUC were obtained using GraphPad (Prism, GraphPad Software, San Diego, California, USA). Data are presented as mean and standard deviation, unless otherwise stated. In addition to the null hypothesis testing used in the experimental chapters, effect sizes (ES) were obtained as the difference in means divided by the pooled standard deviation. Effect sizes are an important addition to null hypothesis as

it describes the magnitude and direction of changes as previously suggested (Hopkins et al., 2009). All relevant statistical analysis and interpretation are presented in the experimental chapters.

Chapter 4: Is Cardiac Autonomic Function Associated with Cardiorespiratory Fitness and Physical Activity in Children and Adolescents? A Systematic Review of Cross-Sectional Studies

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4.1 Abstract

This Chapter aimed to systematically address the associations between HRV, PA and CRF in children and adolescents. Data sources Medline, EMBASE, SportDISCUS and CINAHLPlus were searched on 5th September 2015 and updated on 4th August 2016. Eligibility criteria Observational studies comparing HRV in different groups of PA and CRF, and/or studies investigating the association between PA, CRF and HRV. Sports practices and PA intensities were also included. The RMSSD, HF, LF, and the LF/HF ratio were included. Risk of bias was assessed using the adapted Newcastle-Ottawa Scale (NOS). The results demonstrated that heterogeneity exists in the assessment of the exposures and outcomes, and sample characteristics. Risk of bias was observed in most of the studies. Studies with low risk of bias showed positive associations between moderate-to-vigorous PA and RMSSD. The evidence for the associations between PA and frequency indices is weak. Similarly, the evidence for the association between CRF and HRV is weak. Conclusions Despite the heterogeneity in the studies, moderate-to-vigorous PA is positively associated with

RMSSD, but less clear are the associations between CRF and HRV, as well as other PA intensities. Further research is needed to clarify the role of PA and CRF on HRV in children and adolescents.

4.2 Introduction

Cardiovascular diseases are the main cause of mortality worldwide and the process of atherosclerosis has been found to originate in childhood (McGill et al., 2000, Berenson et al., 1998, Berenson et al., 1992). Strong evidence exists for the benefits of PA and CRF on CVD risk reduction in children and adolescents via modifying traditional risk factors such as body fatness, BP, blood lipids, and insulin resistance (Janssen and Leblanc, 2010). Although improvements in traditional CVD risk factors related to PA and CRF are associated with a decreased CVD risk, changes in traditional CVD risk factors do not fully explain CVD risk reduction (Mora et al., 2007). This has created a 'risk factor gap' in our knowledge of how PA and CRF confer CVD risk reduction (Green et al., 2008). In addition, the American Heart Association recognises that further research is needed to explore novel CVD risk factors in youth in order to advance pathophysiological understanding and CVD management in this population (Balagopal et al., 2011).

Cardiac autonomic function, assessed by HRV, has been suggested as a potential candidate which may help explain the risk factor gap (Joyner and Green, 2009). In contrast to the research base for traditional CVD risk factors which have been explored in systematic reviews (Janssen and Leblanc, 2010, Andersen et al., 2011b, Ruiz et al., 2009), the evidence for the associations between childhood HRV, PA and CRF have yet to be systematically evaluated. A systematic approach is crucial, as while some studies have shown relationships between PA, CRF and HRV in children and

adolescents (Michels et al., 2013, Gutin et al., 2005), others have found no relationships (Krishnan et al., 2009, Cayres et al., 2015, Brunetto et al., 2005), meaning conclusions are not yet clear.

The aim of this study was to systematically review observational studies to investigate the following question: is HRV related to PA and CRF in children and adolescents? Examining this association will increase our current knowledge of how PA and CRF are related to HRV indices of cardiac autonomic function in children and adolescents, as well as providing level one evidence that may be used to inform current PA guidelines for health in children and adolescents. Evidence shows that HRV is inversely related to CVD risk factors, decreased in children with congenital heart disease (Zhou et al., 2012, Massin et al., 1999), and inversely related to CVD mortality in adults (Thayer et al., 2010). In addition, HRV has been suggested as a factor that may provide further evidence into CVD risk reduction accrued by PA (Joyner and Green, 2009), and therefore the present hypothesis is that HRV will be positively associated with CRF and PA in children and adolescents.

4.3 Methods

The review was conducted following best practice (Dissemination, 2008), and reported here in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations (Liberati et al., 2009). The protocol for the review was registered with PROSPERO (International Prospective Register for Systematic Review; reference CRD42015023614).

4.3.1 Search

A systematic search was originally completed on 5th September 2015 and later updated on 4th August 2016 in the following databases: Medline [Ovid], EMBASE

[Ovid], SportDISCUS [Ebscohost] and Cumulative Index to Nursing and Allied Health Library (CINAHL Plus) [Ebscohost]. The search was restricted to studies published in English, but with no date restriction. A copy of the search strategy used in Medline is shown in Appendix 3, page 359. Supplementary searching involved forward and backward citation of the included studies.

4.3.2 Participants, exposures, comparators and outcomes – PECO

4.3.2.1 Participants

Participants were healthy children and adolescents aged between 5 – 18years old. This age range was chosen to reflect current PA guidelines for health in children and adolescents (Bull, 2010). Studies exclusively investigating obese and overweight children were eligible for inclusion as previous research suggests that PA and CRF are related to HRV independently of body fatness in children (da Silva et al., 2014b, Gutin et al., 2005). Furthermore, body weight status was included as a possible mediator of associations between PA, CRF and HRV in the current review. However, children and adolescents with any specific diseases or long-term conditions, such as diabetes, hypertension, congenital heart disease, metabolic syndrome, amongst others were excluded. Exposures: physical activity and cardiorespiratory fitness

Studies assessing PA and CRF using either objective or subjective measures were included. We considered accelerometers, pedometers, doubly labelled water, global positioning system (GPS) devices, and heart rate monitors as objective measures of PA. Questionnaires and interviews were considered subjective measures of PA. Time spent in subdivisions of PA intensities, including light PA (LPA), MPA, VPA, and MVPA, were included. Sedentary time and behaviour were also considered but none of the studies measured these exposures. Sports practice was considered as a

subcomponent of PA and was treated as an independent exposure. Studies examining the influence of sports practice without the inclusion of a control group of non-athletes for comparison were not included. Sports practices as quantified through training loads with GPS and heart rate monitors were considered as objective methods, whereas the use of questionnaires or interviews was considered as subjective. For the inclusion of CRF studies, both direct and indirect assessments were included. Measurements of peak oxygen uptake ($\dot{V}O_2peak$) with direct quantification of gas exchange were considered as a direct measurement, whereas indirect estimation using either validated equations or submaximal tests were considered. Surrogate markers of CRF such as performance in field-based tests (e.g. 20 m shuttle run test) were also included in the analysis.

4.3.2.2 Outcomes: heart rate variability

Studies were considered eligible when HRV measurements were conducted in accordance to published guidelines (Task-Force, 1996). Time and/or frequency domain indices were considered for analysis but other HRV measurements, such as non-linear, fractal analysis amongst others were not included. We decided *a priori* to limit the analyses to the RMSSD, HF and LF using either absolute (ms^2) or normalized units (nu), and the LF/HF ratio. The RMSSD and HF are known to reflect parasympathetic modulation and the LF is considered a marker of overall autonomic activity. The LF/HF ratio is normally used as a marker of the sympatho-vagal balance (Task-Force, 1996). We used these four indices to aid data pooling as well as due to the established prognostic value of these indices, albeit in adults (Thayer and Lane, 2007).

4.3.2.3 Study designs

Quantitative studies allowing mean comparison or relationships between the exposures and HRV were included in the review. The definition described by the Centre for Reviews Dissemination guidance for undertaking reviews in health care for study designs was used (2008). Observational studies examining HRV in children using correlational/regression analyses to investigate associations with PA levels and CRF, and cross-sectional investigations comparing different groups according to PA levels, sports practice or CRF were included. Case studies and reports with insufficient data, such as abstracts and conference papers, were not considered eligible for inclusion.

4.3.3 Study selection

Titles and abstracts were reviewed and studies not meeting the eligibility criteria were discarded. The remaining studies were kept for a second round, where the full texts were obtained. Two reviewers (RSO, KMW) independently performed these stages. All disagreements were discussed, and a consensus formed. EndNote reference manager was used to complete these steps. Kappa coefficient of agreement between the reviewers was 0.78 (95% CI = 0.72 – 0.84).

4.3.4 Data extraction and categorization

Using a bespoke form, the following information was extracted: authors, aim, design, sample characteristics, recruitment procedures, criteria for exclusion and inclusion of participants, measurement of outcomes and exposures, confounders and how the studies dealt with them, statistical approach and main results. Studies were divided according to the exposures (i.e. CRF, total PA, PA intensities and sports practice). A post-hoc division was performed aiming to classify the studies according to the

analysis into: regression analysis; bivariate relationships; and group comparisons. Several investigations were categorized into more than one of these categories.

4.3.5 Quality assessment

Risk of bias was evaluated using a modified version of NOS (Appendix 4) previously used by Perera et al. (2015). Agreement was achieved between two researchers (RSO and ARB). The NOS contained four domains of risk assessment. In each domain, the scale measures the likelihood of bias with four possible scores ranging from 0 – 3 representing high and low risk of bias, respectively. The following domains were included:

Selection bias: This domain contained one subdomain regarding the source of the population. Low risk of bias was considered when random sampling was used, and high risk of bias when a convenience sample was used without explanation of the recruitment procedures undertaken.

Performance bias: This domain contains two subdomains: one regarding the sample size and one about confounders. Low risk of bias was considered when the study provided an appropriate power analysis for sample size calculation, and when the study controlled for important confounders using appropriate statistical methods (see detection bias below).

Detection bias: This domain contained two subdomains: one regarding the statistical approach and one about missing data. Low risk of bias was considered when an appropriate statistical approach was used, and the authors properly described how missing cases were handled. Studies not mentioning missing cases were considered to have a low risk of bias.

Information bias: This domain contains two subdomains: one about the appropriate assessment and the other about the objective measurement of the exposures. Low risk of bias was considered when the exposures were objectively measured, and sufficient details provided to enable the measurement to be replicated by the reader. In addition, the appropriate subdomain for the measurement of HRV was duplicated. In this case, low risk of bias was considered when studies presented sufficient details of the procedures taken before and during the measurement of HRV according to published guidelines (Task-Force, 1996).

The score of the individual subdomains were added to create a risk of bias score. The highest score was 24 (low risk of bias) including three from selection bias, six from performance bias, six from detection bias, and nine from the information bias (six from the exposure and three from the HRV assessment). The scores of each subdomain are presented in Figure 4.2. Studies were not excluded based on the risk of bias, but bias was considered in the synthesis of results and interpretation of the findings.

4.3.6 Data analysis

From the studies using regression analysis the beta coefficient (raw or standardised) and the significance of the association were extracted. For the studies using bivariate correlation, the coefficient of relationship and the significance of the association were extracted. Finally, from the studies comparing groups, data were pooled to report whether a significant difference existed between the groups and the direction of the difference. Due to the differences in the measurement of PA, CRF and HRV and the different designs employed between studies, a meta-analytical approach was not possible in the current review. Rather, a narrative synthesis of the data is provided. In order to investigate possible factors modifying the associations, subgroup analyses

were performed based on age, sex and body weight status. Body weight status subgroup analysis was performed using the studies that explicitly included overweight and obese participants.

4.4 Results

4.4.1 Study selection

A PRISMA flow diagram is presented in Figure 4.1. The original search yielded 4,915 studies of which, after screening for eligibility and duplicate removal, 17 studies met the criteria for inclusion. The search update identified a further 300 studies, from which one eligible study was found. Subsequently 18 studies were included in the review. All studies were published after the year 2000. Ten studies investigated the association between PA and HRV (Buchheit et al., 2007c, Cayres et al., 2015, Farah et al., 2014, Gutin et al., 2000, Gutin et al., 2005, Henje Blom et al., 2009, Iwasa et al., 2005, Krishnan et al., 2009, Michels et al., 2013, Radtke et al., 2013a), four between CRF and HRV (Brunetto et al., 2005, da Silva et al., 2014b, Gutin et al., 2000, Michels et al., 2013), and six between sports practice and HRV (Alom et al., 2011, Cayres et al., 2015, Nagai and Moritani, 2004, Radtke et al., 2013a, Vinet et al., 2005, Sharma et al., 2015). From the PA studies, eight used regression models (Buchheit et al., 2007c, Farah et al., 2014, Gutin et al., 2000, Gutin et al., 2005, Henje Blom et al., 2009, Krishnan et al., 2009, Michels et al., 2013, Radtke et al., 2013b), six used bivariate relationships (Cayres et al., 2015, Chen et al., 2012, Gutin et al., 2000, Henje Blom et al., 2009, Iwasa et al., 2005, Krishnan et al., 2009), and two divided the participants according to different levels of PA (Buchheit et al., 2007c, Radtke et al., 2013b). From the CRF studies, two used regression models (Gutin et al., 2005, Michels et al., 2013), one used bivariate relationships (da Silva et al., 2014b), and one divided the

participants according to tertiles of CRF (Brunetto et al., 2005). From the sports practice studies, one used regression models (Cayres et al., 2015), one used bivariate relationships (Cayres et al., 2015), and six divided the participants according to sports practice groups (Alom et al., 2011, Cayres et al., 2015, Nagai and Moritani, 2004, Radtke et al., 2013a, Vinet et al., 2005, Sharma et al., 2015). A summary of the 18 studies is presented in the Table 4.1.

4.4.2 Risk of bias

The risk of bias is presented in Figure 4.2. The median risk of bias was 12, with the highest and lowest being 22 and six out of 24. The main source of bias was in the participants' recruitment, with only three studies reporting random sampling (Buchheit et al., 2007c, Krishnan et al., 2009, Michels et al., 2013). Additionally, nine studies did not control for confounders (Alom et al., 2011, Brunetto et al., 2005, Chen et al., 2012, da Silva et al., 2014b, Henje Blom et al., 2009, Iwasa et al., 2005, Krishnan et al., 2009, Nagai and Moritani, 2004, Vinet et al., 2005, Sharma et al., 2015), and eleven presented bias in the assessment of the exposure, either by not providing sufficient information or by using subjective assessment methods (Alom et al., 2011, Cayres et al., 2015, Chen et al., 2012, Farah et al., 2014, Gutin et al., 2000, Henje Blom et al., 2009, Iwasa et al., 2005, Nagai and Moritani, 2004, Vinet et al., 2005, Radtke et al., 2013a, Sharma et al., 2015). Five studies did not explain with detail the procedures preceding the HRV measurements (Gutin et al., 2000, Iwasa et al., 2005, Radtke et al., 2013a, Radtke et al., 2013b, Vinet et al., 2005).

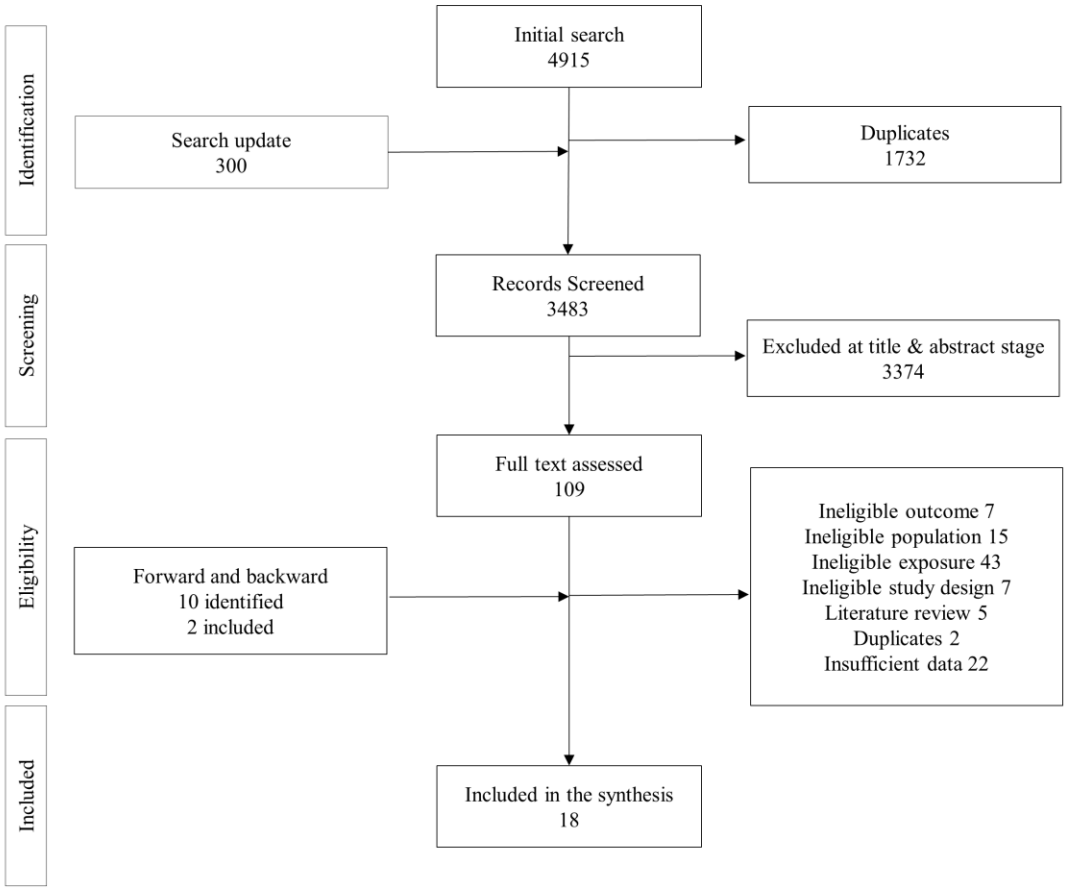

Figure 4.1: PRISMA flow diagram of the included studies.

4.4.3 Participants

The average age of the participants ranged from 7.5 (Iwasa et al., 2005) to 16.5 years old (Farah et al., 2014). The age of the participants included in one study was not clearly stated (Chen et al., 2012). Most of studies included both males and females, but three investigated males only (Alom et al., 2011, Farah et al., 2014, Vinet et al., 2005). Three studies investigated exclusively healthy weight children (Brunetto et al., 2005, Radtke et al., 2013a, Radtke et al., 2013b), three investigated exclusively obese/overweight children (Chen et al., 2012, da Silva et al., 2014b, Gutin et al., 2000), and one included both obese and healthy weight children (Nagai and Moritani, 2004).

In 10 studies it was not clear whether overweight or obese participants were included (Alom et al., 2011, Buchheit et al., 2007c, Cayres et al., 2015, Farah et al., 2014, Gutin et al., 2005, Henje Blom et al., 2009, Iwasa et al., 2005, Krishnan et al., 2009, Michels et al., 2013, Vinet et al., 2005).

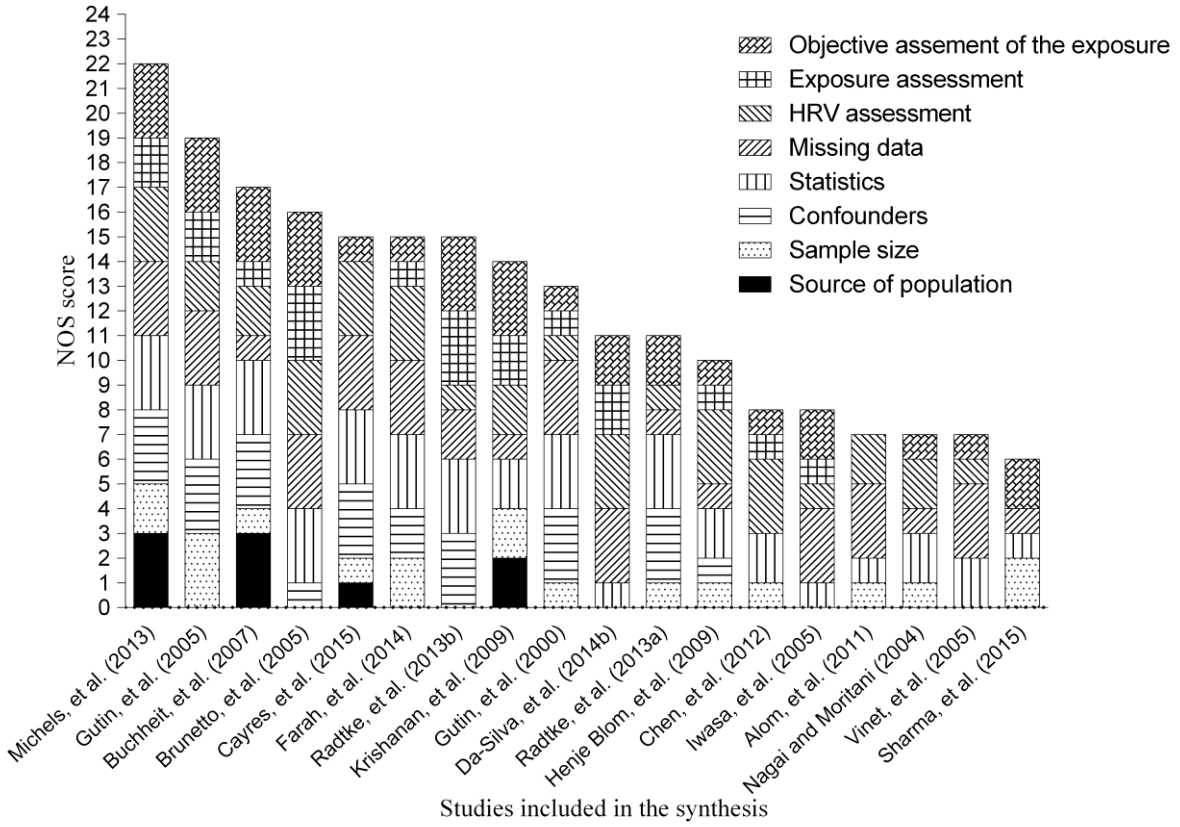

Figure 4.2: Risk of bias of the individual studies. The maximal value of 24 reflects the lowest risk of bias. Studies are ranked according to the observed risk of bias.

Table 4.1: Summary of the included studies.

Author/aims	Study design	Participants	Exposure measurement	HRV measurement	Risk of bias
Physical activity					
Michels et al. (2013) Aims: To investigate the association of age, sex, time point, body composition, PA and CRF to HRV in children	Multiple regression models to investigate the associations of PA and HRV	Healthy children n=460 n girls? (age=8.0±?) n boys? (age= 8.1±?)	Uniaxial accelerometer 15 s epochs. 3 consecutive days Wear time? MPA >2296 counts·min^{-1} VPA >4012 counts·min^{-1} MVPA used for analysis Indirect assessment of $\dot{V}O_2$max using Leger equation from 20 m shuttle test performance	**Duration:** 5-min **Time of the day:** 9am-6pm **Position:** Supine **Breathing control:** No **Pre measurement:**No VPA on the measurement day **Day of measurement:** Each child was individually examined in a quiet room in the supine position for 10 min **Device:** Polar Wearlink 31 **Error correction:** The RR series detrended with Smoothness priors (alpha = 300). Interpolation at 4 Hz. Quality and stationarity checked: no large RRI outliers, an equidistance between consecutive RRI points, minimal variation, stable mean and unimodal, Gaussians RRI and HR distribution graphics **Time domain:** RMSSD **Frequency domain:** LF (0.04-0.15 Hz), HF (0.15-0.4 Hz) LF/HF ratio	22/24
Gutin et al. (2005) Aims: To determine the association between HRV, race, sex, free-living PA, CRF, %BF, subcutaneous and abdominal adiposity in adolescents	Multiple regression models to investigate the associations of PA and HRV	Healthy children n=304 n girls=171 (age: white=16.2±1.1 y; black=16.3±1.3 y) n boys=133 (age: white=16.4±1.3 y; black =16.0±1.1 y)	Uniaxial accelerometer 1-min epochs 5 days Wear time? MPA 3–6 METs VPA 6–9 METs MVPA used for analysis CRF: $\dot{V}O_2$ recorded at heart rate of 170 bpm during a multistage treadmill test	**Duration:** 256 RR intervals **Time of the day**? **Position:** Supine **Breathing control:** No **Pre measurement**? **Day of measurement:** Measures performed after 10 min of quiet rest **Device:** ECG **Error correction:** Any ectopic beats automatically identified and rejected **Time domain:** RMSSD **Frequency domain:** HF (0.15-0.4 Hz); LF (0.05-0.15 Hz) and LFnu/HFnu	19/24

Buchheit et al. (2007c) Aims: To evaluate in 12-year-old the association between PA intensities and health-related indexes	Multiple regression models to investigate association between PA and HRV indices. Participants divided into groups according to criteria of MPA and VPA levels	Healthy children n=67 (age=11.5±0.8 y) n girls=42 n boys=25 Groups of PA n low MPA= 16 (boys? girls?) n high MPA= 52 (boys? girls?) n low VPA= 21(boys? girls?) n high VPA= 56 (boys? girls?)	Triaxial accelerometer Epoch length? Days? Wear time? MPA > 4 METs VPA > 6 METs	**Duration:** Short 5-min **Time of the day:** 8-9am **Position:** Supine **Breathing control:** No **Pre measurement?** **Day of measurement:** Avoid running or other VPA when coming to school. Light breakfast. HR recorded in a comfortable quiet room **Device:** Polar 810s **Error correction:** Ectopic beats replaced with interpolated RR interval **Time domain:** RMSSD **Frequency domain:** LF (0.04-0.15 Hz); HF (0.15-0.4 Hz); HF/(LF+HF) ratio	17/24
Radtke et al. (2013b) Aims: To evaluate the relationship of different levels of PA intensity on CRF, microvascular endothelial function, and HRV in adolescents	Multiple regression models to investigate association between PA and HRV indices. Participants divided into groups according to criteria of MVPA and VPA levels	Healthy children n = 45 Girls=28 (age=14.5±0.7 y) Boys=24 (age= 14.5±0.7 y) Groups of PA n low MVPA=22 (boys=7; girls=15) n high MVPA=22 (boys=9; girls=13) n low VPA=22 (boys=7; girls=15) n high VPA=23 (boys=10; girls=13)	Uniaxial accelerometer 5 s epoch averaged in 60 s 5 days including one weekend day >9 h of wear time MVPA >3,000 counts·min^{-1} VPA >5,200 counts·min^{-1}	**Duration:** 24-h **Time of the day:** 24-h **Position:** Not applied **Breathing control:** Not applied **Pre measurement**? **Day of measurement**? **Device:** ECG **Error correction:** Sinus beat identified and eliminated. **Time domain:** RMSSD **Frequency domain:** Not used	15/24
Farah et al. (2014) Aims: To determine the relationship between HRV measures and the clustering of RFs for cardiovascular disease in adolescent boys	Multiple regression models to investigate the associations of PA and HRV	Healthy children n=1152 n girls=0 n boys=1152 (age=16.6±1.2)	Subjective Face-to-face interview: 7-days recall How many days active for a total of at least 60 minutes?	**Duration:** 5-min **Time of the day**? **Position:** Supine **Breathing control**? **Pre measurement:** No caffeinated beverages 12 hours prior. No PA 24 prior **Day of measurement:** Approximately 30 minutes of supine rest **Device:** Polar RS800cx **Error correction:** Stationary periods of the tachogram with at least 5 minutes **Time domain:** RMSSD **Frequency domain:** LF (0.04-0.15 Hz); HF (0.15-0.4 Hz) and LF/HF	15/24

Krishnan et al. (2009) Aims: To evaluate gender differences in the association between HRV and adiposity in children	Multiple regression models to investigate the associations of PA and HRV. Parametric relationships between PA and HRV	Healthy children n=208 n girls=101 (age=9.0±0.3 y) n boys=107 (age=9.0±0.3 y)	Accelerometers 60 s epochs Seven days Wear time? LPA < 1000 counts·min^{-1} MPA <2500 counts·min^{-1} VPA >2500 counts·min^{-1}	**Duration:** 5-min **Time of the day**? **Position**? **Breathing control:** No **Pre measurement**? **Day of measurement:** Subjects rested for 10 min before the start of each recording **Device:** ECG **Error correction:** Absence of ectopic beats and stationarity of the time series **Time domain:** RMSSD **Frequency domain:** LF (0.05-0.15 Hz) and HF (0.15-0.40 Hz)	14/24
Gutin et al. (2000) Aims: To determine the relations of pre training HRV to body composition and PA in obese children	Multiple regression models to investigate the associations of PA and HRV. Parametric relationship between PA and HRV	Obese children n=78 (age=9.5±1) n girls=53 (age?) n boys=26 (age?) Groups of PA n MPA=71 (age? boys? girls?) n VPA=71 (age? boys? girls?)	Subjective Face-to-face interview: 7-days recall Activities >10-min in duration of: MPA (walking) VPA (running) >VPA (between walking and running). Time spent in VPA and >VPA were summed to derive a VPA index	**Duration:** Over 256 RR intervals **Time of the day**? **Position:** Supine **Breathing control**? **Pre measurement**? **Day of measurement:** 10-min of quiet rest **Device:** ECG **Error correction**? **Time domain:** RMSSD **Frequency domain:** Not used	13/24
Henje Blom et al. (2009) Aims: To investigate whether there is an impact of lifestyle (PA, eating habits, sleeping pattern and smoking) on HRV	Multiple regression models to investigate the associations of PA and HRV. Parametric and non-parametric relationships between PA and HRV	Healthy children n=71 (age=16.5±?) n girls=47 (age?) n boys=24 (age?)	Subjective Five-point scale: The frequency of exercising with hard breathing and sweating (“never”, “seldom”, “once a week”, “twice a week” and “more than twice a week”)	**Duration:** 2-min **Time of the day**? **Position:** Sitting **Breathing control**? **Pre measurement:** Tobacco, caffeine intake, and beta stimulant asthma medication not allowed 1 h prior **Day of measurement:** 15 min of quiet rest **Device:** ECG **Error correction:** Ectopic beats and artefacts replaced with cubic spline interpolation **Time domain:** Not used **Frequency domain:** HF (0.15-0.4 Hz) and LF (0.04-0.15 Hz)	10/24

Chen et al. (2012) Aims: To explore the effects of PA on HRV for those who have an abnormal autonomic nervous system function	Simple linear regression to investigate the association between PA and HRV in the pubertal overweight participants	Overweight children n=21 n girls? (age?) n boys? (age?)	Subjective Physical Activity Questionnaire of Children 7-days recall Nine items: Sports and games, physical activities in school, and leisure activities Frequency: (none) to five (>7 times/week) PA measured by determining the mean score for the 9 items	**Duration:** 5-min **Time of the day:** Morning **Position:** Supine **Breathing control:** No **Procedures:** **Pre measurement:**No VPA and caffeinated beverages 2 h prior **Day of measurement:** 15 min of quiet rest **Device:** ECG **Error correction:** Automatically and manually inspected **Time domain:** Not used **Frequency domain:** LF (0.04-0.15 Hz); HF (0.15-0.4 Hz)	8/24
Iwasa et al. (2005) Aims: To investigate the association of HRV at night and PA in children	Non-parametric relationships between PA and HRV	Healthy children n=29 (age=7.5±1.4) n girls=12 (age?) n boys=17 (age?)	Pedometer 11 grade exercise levels 2 min intervals The amounts of energy consumption (kCal) every 4 s The most frequent value over 2 min classified into: Rest: 0–0.5 Gait, 1–3 Fast gait, 4–6 Intense exercise, 7–9	**Duration:** Overnight **Time of the day:** Midnight- 5am **Position:** Supine **Breathing control:** No **Pre measurement**? **Day of measurement**? **Device:** ECG **Error correction**? **Time domain:** Not used **Frequency domain:** LF (0.04-0.15 Hz); HF (0.15-0.4 Hz); and LF/HF	8/24
Cardiorespiratory fitness					
Brunetto et al. (2005) Aims: To systematically evaluate the effects of gender and aerobic fitness on resting and head-up tilt HRV in healthy adolescents	Participants divided into groups according to tertiles of fitness	Healthy children n=41 n girls=21(age=15.2±1.1) n boys=20 (age=15.4±0.8) Tertiles of fitness n low=14 (age? boys? girls?) n mod=13 (age? boys? girls?) n high=14 (age? boys? girls?)	CRF: Objective assessment of $\dot{V}O_2$max using Bruce treadmill protocol	**Duration:** 5-min **Time of the day:** Between 2-5pm **Position:** Supine **Breathing control:** Yes **Pre measurement:** No caffeinated and/or alcoholic beverages on the day. No VPA on the day prior **Day of measurement:** 15 min of quiet rest **Device:** Polar S810 **Error correction:**R-R intervals that differed by ±20 beats from the mean of the analysed period deleted. Recordings that required filtering of more than 10% of the R-R intervals were discarded **Time domain:** RMSSD **Frequency domain:** LF (0.04-0.15 Hz); HF (0.15-0.4 Hz); and LF/HF	16/24

da Silva et al. (2014b) Aims: To investigate HRV and its association with CRF,PA, insulin, and hemodynamic profile in overweight and obese adolescent boys and girls	Non-parametric relationship between CRF and HRV indices	Healthy children n=28 n girls=18 (age=14±2 y) n boys=10 (age=13.2±2 y)	CRF: Indirect assessment of $\dot{V}O_2$max using Leger equation from 20 m shuttle test performance	**Duration:** 5-min **Time of the day:** Between 4-5pm **Position:** Sitting **Breathing control:** No **Pre measurement:** No VPA or beverages containing caffeine 24 h prior. No food 2 h prior **Day of measurement:** 10 min of quiet rest. Controlled temperature (23 °C) **Device:** Polar RS800 CX **Error correction:** Ectopic beats (deviation higher than 20 % of adjacent intervals) interpolated by adjacent R–R intervals **Time domain:** RMSSD **Frequency domain:** LF (0.04-0.15 Hz); HF (0.15-0.4 Hz), and LF/HF	11/24
Sports practice					
Cayres et al. (2015) Aims: To analyse the relationship among sports practice, physical education class, habitual PA and cardiovascular risk in adolescents	Participants divided into groups according to sports practice. Multiple regression models to investigate association between sports practice and HRV	Healthy children n=120 n girls? (age?) n boys? (age?) Sports practice =60 (age=12±1) Inactive=60 (age=11±1)	Sports practice: Subjective assessment Do you participate in sports activities outside of the school environment? Number of days (1-5) in the week used for analysis Habitual PA was evaluated by a pedometer over a period of 7 days	**Duration:** 1000 RR intervals **Time of the day:** Morning **Position:** Supine **Breathing control:** No **Pre measurement:** No VPA and caffeinated beverages for 2 h prior to testing **Day of measurement:** 30-min quite rest **Device:** Polar RS800 **Error correction:** Digital filtering, visually checked and removed abnormal intervals **Time domain:** RMSSD **Frequency domain:** Not used	15/24
Radtke et al. (2013a) Aims: To compare the benefits of high-volume sports club participation vs. low-volume sports club participation on HRV in active, normal-weight children	Participants divided into groups according to sports practice	Healthy children n= 49 (age=11±1.0) n girls=29 n boys=20 Sports practice =23 n girls=14 (age?) n boys=9 (age?) Inactive=26 n girls=15 (age?) n boys=11 (age?)	Sports practice: Subjective defined as sports club participation (min/week) and leisure-time PA within the last year based on their individual weekly volume of PA within a sports club Low volume: <180 min/week High volume: >180 min/week	**Duration:** 5-min **Time of the day:** Between midnight and 5am **Position:** Supine **Breathing control:** No **Pre measurement**? **Day of measurement**? **Device:** ECG **Error correction**? **Time domain:** Not measured **Frequency domain:** LF (0.04-0.15 Hz) and HF (0.15–0.4 Hz)	11/24

Nagai and Moritani (2004) Aims: To investigated whether HRV was altered in obese and/or in physically inactive children	Participants divided into groups according to sports practice and weight status	Healthy and obese children n=96 n of girls=34 n of boys=62 Sports practice: n lean=24 (age=9.6±1.3; boys=23; girls=1) n overweight=24 (age=9.5±1.4; boys=23; girls=1) Inactive: n lean=24 (age=9.4±1.8; boys=8; girls=16) n overweight=24 (age=9.3±1.7; boys=8; girls=16)	Sports practice: Subjectively defined as frequency sports activities higher than three times a week, and more than 60 min each time. 'Intensive activity' was defined as 'intensive practice or exercise with heart thumping' Inactive: No children regularly engaged in various sports activities or exercises	**Duration:** 5-min **Time of the day:** Morning **Position: S**itting **Breathing control:** No **Pre measurement**? **Day of measurement:** Controlled temperature (25 °C) quite and comfortable **Device:** ECG **Error correction**? **Time domain:** Not used **Frequency domain:** LF (0.03-0.15 Hz); HF (0.15-0.5 Hz)	7/24
Alom et al. (2011) Aims: To measure resting HRV in healthy adolescents' male athletes who were exposed to regular physical exercise and also in healthy adolescent male with sedentary lifestyle	Participants divided into groups according to sports practice	Healthy children n=92 Spots participation=30 boys (age=14.9±2.2) Inactive=62 boys (age=15.1±2.5)	Sports practice: Subjectively defined as regular exercise for at least one year Inactive: Participants recruited due to sedentary lifestyle	**Duration:** 5-min **Time of the day:** Between 9-11 am **Position:** Supine **Breathing control:** No **Pre measurement:** No coffee or tea prior to measurement **Day of measurement:** 20-min of quiet rest prior **Device:** ECG **Error correction?** **Time domain:** Not used **Frequency domain**: HF (?), LF (?)	7/24
Vinet et al. (2005) Aims: To compare HRV parameters in highly trained swimmer boys and untrained counterparts	Participants divided into groups according to sports practice	Healthy children 20 n girls= 0 Sports practice =11(age=11.9±0.9) Inactive=9 (age=11.6±1.1)	Sports practice: Swimmers for 4 years 4–5 sessions/week (1h 30 min) Inactive: Did not practice more than 2 h of PA per week and formal training or organized sport	**Duration:** 6-min **Time of the day:** Overnight **Position:** Supine **Breathing control:** No **Pre measurement**? **Day of measurement**? **Device:** ECG **Error correction:** Automatically and manually edited **Time domain:** RMSSD **Frequency-domain:** LF (0.04-0.15 Hz) and HF (0.15-0.4 Hz)	7/24

Sharma et al. (2015) Aims: To provide normative data for HRV for adolescents based on sex and sports practice	Participants divided into groups according to sports practice	Healthy children 439 n girls=189 Sports practice =79(age? boys=45; girls=34) Inactive=360 (age? boys=205; girls=155)	Sports practice: Represented the school at state, national or international level athletic interscholastic sport event and was undergoing supervised physical training Inactive?	**Duration:** 5-min **Time of the day**? **Position:** Supine **Breathing control:** No **Pre measurement:** No VPA for 24 hours and no caffeinated beverages or stimulant 12 hours prior **Day of measurement**:Empty bladder and sit comfortably in a dim lighting and temperature controlled room (24–26 °C) **Device:** ECG **Error correction:** Artefacts and ectopic beats excluded **Time domain:** RMSSD **Frequency-domain:** LF (0.04-0.15 Hz); HF (0.15-0.4 Hz)	6/24

? = Information could not be retrieved. HRV: heart rate variability; RMSSD: square root of the mean of the sum of the squares of differences between adjacent RR intervals; HF; high frequency; LF: low frequency; ECG: electrocardiogram; CRF: cardiorespiratory fitness; PA: physical activity; LPA: light physical activity; MPA: moderate physical activity; VPA: vigorous physical activity; MVPA moderate-to-vigorous physical activity.

4.4.4 Physical activity and heart rate variability

4.4.4.1 Regression analyses

The results of the studies using regression models are presented in Table 4.2. The RMSSD was positively and significantly associated with MVPA in all three studies investigating this association (Gutin et al., 2005, Michels et al., 2013, Radtke et al., 2013b). Additionally, RMSSD was positively and significantly associated with total PA in the only study investigating this association (Farah et al., 2014). In contrast, RMSSD was not significantly associated with LPA, MPA or VPA in the three studies investigating these separate PA intensities (Gutin et al., 2000, Krishnan et al., 2009, Radtke et al., 2013b). The HF (normalized and absolute) was not significantly associated with MVPA in the two studies investigating this association (Gutin et al., 2005, Michels et al., 2013). PA as measured using total energy expenditure was not associated with HF (nu) in the only study investigating this association (Buchheit et al., 2007c). The HF (nu) was significant and positively associated with total PA in one study (Farah et al., 2014), but not in the other (Henje Blom et al., 2009). The LF (nu) was significant and negatively associated with total PA in the two studies investigating this association (Farah et al., 2014, Henje Blom et al., 2009). The LF/HF ratio was positively and negatively associated with MVPA in the two studies investigating this association (Gutin et al., 2005, Michels et al., 2013), but was not associated with total PA in the only study that investigated this association (Farah et al., 2014).

4.4.4.2 Correlational analyses

The studies assessing bivariate relationships are presented in Table 4.3. From two studies, RMSSD was positively and significantly associated with total PA in one (Krishnan et al., 2009), but not with steps per day in another (Cayres et al., 2015). The RMSSD was not significantly associated with VPA and MPA in the only study

examining these associations (Gutin et al., 2000). Both HF and LF (normalized and absolute) were significant and positively associated with total PA in the two studies examining these associations (Chen et al., 2012, Henje Blom et al., 2009). The HF (ms^2) and LF/FH were not significantly associated with VPA in the only study investigating this association (Iwasa et al., 2005).

4.4.4.3 Group analyses

The studies dividing participants in groups of PA are presented in Table 4.4. RMSSD was significantly higher for the group performing more compared to the group performing less MVPA, but not for VPA according to the specific cut-off points (Radtke et al., 2013b). Another study did not find significant differences for RMSSD and HF (nu) between the groups performing more or less than 210 $min \cdot day^{-1}$ of MPA (Buchheit et al., 2007c). HF(nu) was higher for the groups performing more compared to the group performing less than 60 $min \cdot day^{-1}$ of VPA (Buchheit et al., 2007c).

Table 4.2: Predictors of heart rate variability in the studies using multiple linear regression.

	Author	Predictors	HRV indices	Controlled for	Results	Risk of bias
PA	Michels et al. (2013)	MVPA (min·day^{-1})	RMSSD (ms), HF (ms^2), LF/HF	Boys and girls analysed in separate models; Age; Heart rate; %BF; Time point	Boys **MVPA and RMSSD: ß? (*P*<0.05)** MVPA and HF: ß? (*P*>0.05) **MVPA and HF/LF: ß? (*P*<0.05)** Girls MVPA and RMSSD: ß? (*P*>0.05) MVPA and HF: ß? (*P*>0.05) MVPA and HF/LF: ß? (*P*>0.05)	22/24
	Gutin et al. (2005)	MVPA (min·day^{-1})	RMSSD (ms); HFnu; Ln HF/LF	Sex; Age; Tanner stage; %BF; BP; Race; Heart rate	**MVPA and RMSSD: ß=0.18 (*P*<0.05)** MVPA and HF: ß? (*P*>0.05) **MVPA and HF/LF: ß=-0.0018 (*P*<0.05)**	19/24
	Buchheit et al. (2007b)	PAEE (kcal·day^{-1}); PAL (kcal·day^{-1})	HF(nu)	Sex; Age; %BF	PAEE and HF: ß? (*P*>0.05) PAL and HF: ß? (*P*>0.05)	17/24
	Radtke et al. (2013b)	MVPA; VPA (min·day^{-1})	RMSSD (ms)	Sex; Age; Tanner stage; Sum of skinfolds	**MVPA and RMSSD: ß=0.553 (*P*<0.05)** VPA and RMSSD: ß=0.018 (*P*>0.05)	15/24
	Farah et al. (2014)	PA (day·w^{-1})	RMSSD (ms); HFnu; LFnu; HF/LF	Age; Period of the day when HRV was collected	**PA and RMSSD: ß=1.54 (*P*<0.05)** **PA and HF: ß=0.56 (*P*<0.05)** **PA and LF: ß=-0.56 (*P*<0.05)** PA and LF/HF: ß=-0.03 (*P*>0.05)	15/24
	Krishnan et al. (2009)	LPA(au); MPA (au); VPA (au)	RMSSD (ms)	Heart rate; Boys and girls analysed in separate models	Boys and girls LPA and RMSSD: ß? (*P*>0.05) MPA and RMSSD: ß? (*P*>0.05) VPA and RMSSD: ß? (*P*>0.05)	14/24
	Gutin et al. (2000)	VPA; MPA (h·w^{-1})	RMSSD (ms)	Sex; Age; %BF; BP; Race; Heart rate	VPA and RMSSD: ß? (*P*>0.05) MPA and RMSSD: ß? (*P*>0.05)	13/24
	Henje Blom et al. (2009)	PA (Frequency index)	Log HFnu; log LFnu	Heart rate; Glucose	First measurement **PA and LF: ß? (*P*<0.05)**	10/24

					PA and HF: ß? (*P*>0.05) Second measurement **PA and LF: ß? (*P*<0.05)** PA and HF: ß? (*P*>0.05)	
CRF	Michels et al. (2013)	CRF (ml·kg·min^{-1})	RMSSD (ms), HF (ms^2), LF (ms^2), LF/HF	Boys and girls analysed in separate models; Age; Time point	Boys: **CRF and RMSSD: stß=0.17 (*P*<0.05)** **CRF and HF: stß=0.16 (*P*<0.05)** CRF and LF: stß=0.12 (*P*>0.05) CRF and HF/LF: stß=-0.06 (*P*>0.05) Girls: CRF and RMSSD: stß=0.10 (*P*>0.05) CRF and HF: stß=0.06 (*P*>0.05) CRF and LF: stß=0.14 (*P*>0.05) CRF and HF/LF: stß=-0.12 (*P*>0.05)	22/24
	Gutin et al. (2005)	CRF (ml·kg·min^{-1} at 170 bpm)	RMSSD (ms); HFnu;	Sex; Age; Tanner stage; %BF; BP; Race; Heart rate	**CRF and RMSSD: ß=0.85 (*P*<0.05)** CRF and HF: ß? (*P*>0.05)	19/24
Sports practice	Cayres et al. (2015)	Sports practice (day·w^{-1})	RMSSD (ms)	Sex; Age; Race; Peak growth velocity; maturation, age, %BF	**Sports practice and RMSSD: ß=0.039(*P*<0.05)**	15/24

? = Information could not be retrieved. RMSSD: square root of the mean of the sum of the squares of differences between adjacent RR intervals; HF; high frequency; LF: low frequency; CRF: cardiorespiratory fitness; PA: physical activity; LPA: light physical activity; MPA: moderate physical activity; VPA: vigorous physical activity; MVPA moderate-to-vigorous physical activity.

Table 4.3: Relationship coefficients between physical activity, cardiorespiratory fitness, sports practice and heart rate variability.

	Author	Predictors	HRV indices	Results	Risk of bias
PA	(Cayres et al., 2015)	PA (steps·day^{-1})	RMSSD (ms)	PA and RMSSD: r=0.01(*P*=0.78)	15/24
	(Krishnan et al., 2009)	Total PA (au)	RMSSD (ms)	Boys **PA and RMSSD: r=0.364 (*P*=0.002)** Girls PA and RMSSD: r? (*P*=0.525)	14/24
	(Gutin et al., 2000)	VPA; MPA (h·week^{-1})	RMSSD (ms)	VPA and RMSSD: r= -0.03 (*P*>0.05) MPA and RMSSD: r=0.13 (*P*>0.05)	13/24
	(Henje Blom et al., 2009)	PA (Frequency index)	Log HFnu; log LFnu	First measurement **PA and LF: r=0.35 (*P*<0.05)** **PA and HF: r=0.26 (*P*<0.05)** Second measurement **PA and LF: r=0.29 (*P*<0.05)** **PA and HF: r=0.30(*P*<0.05)**	10/24
	(Chen et al., 2012)	PA?	Log HF(ms^2); log LF(ms^2)	**PA and LF: r=0.62 (*P*<0.05)** **PA and HF: r=0.49 (*P*<0.05)**	8/24
	(Iwasa et al., 2005)	VPA (min·day^{-1})	HF (ms^2), LF/HF	VPA and HF: r? (*P*>0.05) VPA and LF/HF: r? (*P*>0.05)	8/24
CRF	(da Silva et al., 2014b)	CRF (ml·kg·min^{-1})	RMSSD (ms), HF (ms^2), LF (ms^2), LF/HF	**CRF and RMSSD: r=0.42 (*P*<0.05)** **CRF and HF: r=0.38 (*P*<0.05)** **CRF and LF: r=-0.38 (*P*<0.05)** **CRF and HF/LF: r=-0.37 (*P*<0.05)**	11/24
Sports practice	(Cayres et al., 2015)	Sports practice (day·w^{-1})	RMSSD (ms)	**Sports practice and RMSSD: r=0.22(*P*<0.05)**	15/24

? = Information could not be retrieved. RMSSD: square root of the mean of the sum of the squares of differences between adjacent RR intervals; HF; high frequency; LF: low frequency; CRF: cardiorespiratory fitness; PA: physical activity; LPA: light physical activity; MPA: moderate physical activity; VPA: vigorous physical activity.

4.4.5 Cardiorespiratory fitness and heart rate variability

4.4.5.1 Regression analyses

The results of the studies using regression models are presented in Table 4.2. RMSSD was significantly and positively associated with CRF in the two studies investigating this association (Gutin et al., 2005, Michels et al., 2013). The HF (ms^2) was significantly and positively associated with CRF in one study (Michels et al., 2013), but not in the other (Gutin et al., 2005). The LF (ms^2) and LF/HF were not significantly

associated with CRF in the only study examining these associations (Michels et al., 2013).

4.4.5.2 Correlational analyses

The only study investigating bivariate relationship is presented in Table 4.3. The RMMSD and HF (ms^2) were significantly and positively associated with CRF. The LF(ms^2) and LF/HF were negatively and significantly associated with CRF (da Silva et al., 2014b).

4.4.5.3 Group analyses

The only study that divided the participants according to CRF levels is presented in Table 4.4. There were no differences in RMSSD, HF, LF (normalized and absolute) and LF/HF between CRF groups (Brunetto et al., 2005).

4.4.6 Sports practice and heart rate variability

4.4.6.1 Regression and correlation analyses

One study investigated the association using regression and correlational analyses (Tables 4.2 and 4.3). Both analysis showed RMSSD to be significantly and positively associated with sports practice, measured in days per week (Cayres et al., 2015).

4.4.6.2 Group analyses

The studies that divided participants according to sports practice are presented in Table 4.4. RMSSD was higher for the group engaged in sports activities in the two studies comparing this index (Cayres et al., 2015, Sharma et al., 2015). The HF and the LF (normalized or absolute) were significantly higher for the sports practice group in four studies (Alom et al., 2011, Nagai and Moritani, 2004, Radtke et al., 2013a, Sharma et al., 2015), but not in one (Vinet et al., 2005). The only study investigating

LF/HF between the groups did not find differences between the groups (Vinet et al., 2005).

Table 4.4: Studies using comparisons between groups of physical activity, cardiorespiratory fitness and sports practice.

		HRV indices				
		RMSSD	HF	LF	HF/LF	Risk of bias
MVPA	Radtke et al. (2013b)	↑	-	-	-	15/24
MPA	Buchheit et al. (2007c)	↔	↔	-	-	17/24
VPA	Buchheit et al. (2007c)	↔	↑	-	-	17/24
	Radtke et al. (2013b)	↔	-	-	-	15/24
CRF	Brunetto et al. (2005)	↔	↔	↔	↔	16/24
Sports practice	Cayres et al. (2015)	↑	-	-	-	15/24
	Radtke et al. (2013a)	-	↑	↑	-	11/24
	Nagai and Moritani (2004)	-	↑	↑	-	7/24
	Nagai and Moritani (2004)	-	↔	↔	-	7/24
	Alom et al. (2011)	-	↑	↑	-	7/24
	Vinet et al. (2005)	↔	↔	↔	↔	7/24
	Sharma et al. (2015)	↑	↑	↑	-	6/24

↑ indicates significant higher values for the groups with higher PA, CRF and sports practice; ↔ indicates no significant differences between the groups based on PA, CRF and sports practice. – not measured. RMSSD: square root of the mean of the sum of the squares of differences between adjacent RR intervals; HF; high frequency; LF: low frequency; CRF: cardiorespiratory fitness; PA: physical activity; LPA: light physical activity; MPA: moderate physical activity; VPA: vigorous physical activity; MVPA moderate-to-vigorous physical activity.

4.4.7 Possible moderating factors

4.4.7.1 Age

From the five studies investigating children below 12years old, only one reported significant associations between RMSSD, LF/HF and MVPA (Michels et al., 2013). The remaining four studies in children aged below 12 years old did not find significant associations between PA and HRV (Buchheit et al., 2007c, Gutin et al., 2000, Iwasa et al., 2005, Krishnan et al., 2009). In contrast, all four studies investigating children above 12 years old found significant associations between PA measures and HRV indices (Farah et al., 2014, Gutin et al., 2005, Henje Blom et al., 2009, Radtke et al., 2013b). No study has formally examined the associations between PA, CRF and HRV across different age groups.

4.4.7.2 Sex

In the two studies comparing PA in boys and girls separately, RMSSD was significantly and positively related to MVPA (Michels et al., 2013) and total PA (Krishnan et al., 2009) among boys but not girls. Similarly, RMMSD and HF (ms^2) were significantly and positively associated with CRF among boys but not girls in the only study dividing participants according to sex (Michels et al., 2013). The only study to separate boys and girls into groups of sports practice found higher RMSSD, HF and LF (normalized and absolute units) for the groups of sports practice compared to the control group for both sexes (Sharma et al., 2015).

4.4.7.3 Body weight status

Two studies investigated the associations of PA and HRV in obese children (Chen et al., 2012, Gutin et al., 2000). Chen et al. (2012) reported significant and positive associations between total PA, HF and LF (ms^2). Gutin et al. (2000) did not find

significant associations between RMSSD, VPA and MPA. One study included obese and healthy children in different groups of sports practice and showed that HF and LF (ms^2) were not significantly different between the groups of active and inactive obese children (Nagai and Moritani, 2004). One study included obese and overweight children and found significant associations between CRF and RMSSD, HF (ms^2), and LF/HF ratio (Table 4.3) (da Silva et al., 2014b).

4.5 Discussion

This is the first review to systematically examine the associations of PA and CRF with HRV derived indices of cardiac autonomic function in children and adolescents. The main findings were: 1) robust evidence shows that RMSSD is significantly and positively related to MVPA; 2) weak and inconclusive evidence was found for the association between PA, CRF and the frequency domain indices; 3) RMSSD was significantly associated with CRF, however, differences in the participants characteristics and CRF assessment might be clouding the evidence; and 4) the evidence for the influence of age, sex and weight status is inconclusive.

4.5.1 Physical activity and heart rate variability

Robust evidence from three out of three low risk of bias studies using regression analysis and controlling for confounders show that the RMSSD is significantly and positively associated with MVPA (Gutin et al., 2005, Michels et al., 2013, Radtke et al., 2013b). The evidence for an association between the HF (normalized and absolute) and PA is weak, with just one out of four studies presenting significant and positive associations. A high dependence of methodological choices to derive frequency indices such as breathing control, mathematical approach and data handling might explain these findings (Task-Force, 1996). In accordance to current PA

guidelines for improvements in traditional CVD risk factors in children and adolescents (Bull, 2010), the present results also show an important role of the combined MVPA on HRV in children and adolescents. However, as the evidence is from cross-sectional observation studies, longitudinal studies are required to confirm this finding. The mechanisms behind the associations between RMSSD and MVPA remains to be elucidated. However, an improved RMSSD might reflect a better vagal control of blood pressure, and therefore a better vagal balance is desirable. Additionally, improvements in RMSSD but not traditional CVD risk factors after exercise training suggest that in adolescents exercise increases vagal modulation independently of traditional risk factors (Bond et al., 2015a), which strengthens the concept that autonomic function might increase our knowledge about cardiovascular risk during childhood.

In contrast to MVPA, in the present review there was no evidence for associations between LPA, MPA and HRV. Recently, VPA has been shown to be significantly associated with traditional CVD risk factors independently of MPA (Fussenich et al., 2016), however, in the present results the evidence for the influences of VPA on HRV is weak. Just one study using group comparison showed significant differences in the HF (nu) (Buchheit et al., 2007c). Discrepancies between the included studies examining VPA exist regarding the age of the participants, confounders and parameters used to measure PA levels. Caution should be taken when interpreting the results of the two studies (Gutin et al., 2000, Iwasa et al., 2005) using subjective measurements (Kavanaugh et al., 2015). Similarly, bias might be present in the studies that objectively measured PA with accelerometers using 60 s epoch acquisition due to the likelihood of underreporting the VPA activities (Sanders et al., 2014). Alternatively, the weak evidence might indicate that VPA per se is not

associated with HRV, as the studies that combined MPA and VPA found positive and significant associations between MVPA and HRV despite using 60 s epoch data (Gutin et al., 2005, Radtke et al., 2013b). Further research is needed to clarify the relationship of PA intensities and HRV outcomes using shorter epochs (< 5s) to better capture VPA in children and adolescents.

Notably, the studies comparing sports practice groups and HRV outcomes presented the highest risk of bias. Bias arising from sample size, statistical approach, exposure and outcome assessments, and dealing with important confounders, hampers the interpretation of the results, meaning any positive relationship between sports practice and HRV in children and adolescents remains controversial. The studies with low risk of bias in this subcategory suggest that RMSSD, HF and LF are improved for the sports practice group compared to the inactive controls (Cayres et al., 2015, Radtke et al., 2013a). However, more robust studies are needed to establish whether these effects can be replicated.

No study has directly explored the effects of biological maturation on the relationship between PA and HRV. In contrast, all studies investigating children above 12 years old found significant associations between PA and HRV. Such findings were not observed for children < 12 years old, with only one out of four studies finding positive and significant associations. Improvements in autonomic modulation and decrements in PA levels across childhood might underpin these findings suggesting an effect of age (Lenard et al., 2004, Reilly, 2016, Silvetti et al., 2001). These results are in accordance with a meta-analysis that showed no significant improvements in HRV of pre-pubertal children after exercise training (da Silva et al., 2014a), and suggest that in young children PA levels do not change HRV indices. However, caution should be

taken when interpreting the results due to the higher risk of bias in the studies including young children. The influence of age and maturation on the relationships between HRV, CRF and PA have yet to be tested within a study using the same methodology and controlling for important confounders.

The evidence for the influence of weight status on the observed associations is weak. The two studies that found positive and significant associations between PA and HRV in overweight children presented high risk of bias (Chen et al., 2012, Nagai and Moritani, 2004), whilst the study with a lower risk of bias did not find significant associations (Gutin et al., 2000). This is worth investigating in futures studies, as weight status is documented to be related to lower HRV and PA levels (Metcalf et al., 2011, Iellamo and Volterrani, 2013). Regarding the effects of sex on the associations, the two studies which analysed boys and girls separately observed significant and positive associations for boys but not for girls. The associations between HRV and sex are clouded by the possible influences of age. HRV has been demonstrated to increase throughout adolescence, reflecting maturation of the autonomic nervous system (Silvetti et al., 2001, Lenard et al., 2004). However, in the studies that analysed boys and girls separately, participants were eight and nine years old which may suggest that in young children, the associations between PA and HRV are sex-dependent. The apparent sex differences in the associations might be explained by the higher amount of MVPA performed by boys compared to girls (Abbott and Davies, 2004). However, this has yet to be tested within a single study with control for biological maturation.

4.5.2 Cardiorespiratory fitness and heart rate variability

Significant associations were observed between RMSSD and CRF. However, one study did not control for confounders (da Silva et al., 2014b), and the CRF assessments varied between the studies which used indirect (e.g. 20 m shuttle test) or submaximal (e.g. $\dot{V}O_2$ at heart rate of 170 bpm) estimates, meaning bias in the measurement of CRF across the studies may be present. The study using direct assessment of CRF failed to find differences in HRV outcomes between the groups (Brunetto et al., 2005), however, confounders were not controlled for and the true effects of CRF might have been clouded. Although the results are unclear, the observed associations are in accordance with adult literature (Buchheit and Gindre, 2006). The most commonly suggested mechanisms of this association is the genetic determinants of CRF and HRV (Singh et al., 1999, Hautala et al., 2009), however relevant studies in paediatric samples are currently lacking.

Inferences about the effects of sex, age and weight status on the observed associations between CRF and HRV is limited due to the number of studies. The only study analysing boys and girls separately found associations for young (6.7 – 9.2years old) boys but not for girls (Michels et al., 2013). Regarding weight status, one study (da Silva et al., 2014b) showed significant associations between CRF and HRV in obese children, and Gutin et al., (Gutin et al., 2005) showed significant associations between HRV and CRF after controlling for body fatness. This suggest that CRF is significantly associated with HRV independently of body fatness. However, caution should be taken due to the limited number of studies exploring the effect of weight status on the relationship between PA, CRF and HRV measures of autonomic function. Similarly, all included studies normalized CRF by body mass (i.e. $mL \cdot kg^{-1} \cdot min^{-1}$) which may not adequately control for body composition and body size

(Welsman and Armstrong, 2000), and therefore might be influencing the analysis. Likewise, PA and CRF are known to independently predict traditional CVD risk factors, suggesting that they operate through separate pathways (Ekelund et al., 2007). The present review, however, does not allow conclusions about independent relationships of CRF and PA on HRV indices. Future studies are needed to clarify the effects of CRF on HRV in children and adolescents, controlling for important confounders in the associations, including PA.

4.5.3 Strengths and limitations

This is the first systematic review to address the relationships between PA, CRF and HRV in children and adolescents. The organization of the studies into categories according to the methodological approach used (e.g. regression analysis) is a strength of the current review and provides unique information for the reader to draw conclusions regarding the strength of the current evidence base. Similarly, we followed the best practice recommendations when elaborating and conducting the review.

Different methodological approaches used to measure the exposure and outcome variables as well as the different source of bias in the included studies are the main limitations. At the outcome level, most studies did not present detail about the conditions preceding HRV assessments. This increases the risk of bias, as factors such as prior exercise and food consumption are known to decrease resting HRV indices (Al Haddad et al., 2009, Bond et al., 2015a). Additionally, discrepancies regarding body position, time and duration of measurement, breathing frequency, error correction and omission of the mathematical parameters for the frequency analysis limit the conclusions that can be drawn (Task-Force, 1996). Finally, selective reporting and publication bias may be present.

At the exposure level, subjective measurements tend to overestimate the amount of PA performed by children and adolescents (Kavanaugh et al., 2015). Likewise, insufficient information about epoch length, minimal amount of days, and hours for data acquisition, were noted in most of the accelerometer studies. These factors are known to influence the PA outcomes (Rich et al., 2013, Sanders et al., 2014). Approaches to measure CRF were different between the studies and confounders were not controlled. Future studies are encouraged to use objective assessments of the exposures, and report the PA measurements in detail, using appropriate methodological approaches for epoch length, days per week, and hours per day.

4.6 Conclusions

This review provides evidence to show that MVPA plays an important role on cardiovascular health via improvements in autonomic function in children and adolescents. However, there is insufficient evidence to conclude a dose-response effect. Furthermore, the evidence for the association of time spent in other PA intensities with HRV is not sufficiently strong and should be investigated in future research. The evidence for the association between CRF and HRV is weak and inconsistent. Overall, heterogeneity in the study samples, exposure and outcome assessments, limits conclusions and highlights the need for further research, especially of a prospective nature, in order to guide policymakers. Although a considerable number of studies have investigated the associations between PA, CRF and HRV, research is still needed to elucidate the possible influences of age, sex, biological maturation, body weight status and the potential physiological mechanisms.

Chapter 5: Cardiac Autonomic Function, Cardiovascular Risk and Physical Activity in Adolescents

5.1 Abstract

This Chapter aimed to investigate in adolescents: 1) the relationships of PA and CRF to traditional CVD risk factors, rest and recovery autonomic function; and 2) whether autonomic function strengthens the associations between PA, CRF and CVD risk. Fifty-four (22 girls) adolescents had traditional CVD risk factors, rest and recovery autonomic function evaluated. CRF was measured using a steep ramp cycle test and PA was assessed with accelerometers. Resting HRV (and $RMSSD_{30}$) and heart rate recovery (T_{30}, HHRt) were used. Clustered traditional ($CVDR_{trad}$) and autonomic ($CVDR_{auto}$) risk scores were created and added to form a composite clustered CVD risk score ($CVDR_{com}$). PA and CRF were significantly and negatively associated to traditional CVD risk factors (stβ ranging from -0.276 to -0.765). Moderate (MPA) and vigorous (VPA) were positively related to resting RMSSD (stβ = 0.402 and 0.453, respectively), and negatively related to T_{30} (stβ = -0.356 and -0.433, respectively) and HHRt (stβ = -0.311 and -0.406, respectively) (all P<0.05). $RMSSD_{30}$ recovered faster in the high compared to low median split for VPA (P< 0.05). Stronger associations for $CVDR_{com}$ compared to $CVDR_{trad}$ were observed for MPA ($CVDR_{com}$: r^2=0.32, P<0.001; $CVDR_{trad}$: r^2=0.17, P=0.002), and VPA ($CVDR_{com}$: r^2=0.18, P=0.001; $CVDR_{trad}$: r^2=0.06, P=0.08). These findings strengthen the proposed additional beneficial effects of PA on autonomic function above traditional CVD risk factors.

5.2 Introduction

The pathobiological process of atherosclerosis starts during childhood and is related to traditional CVDrisk factors such as blood lipids, BP and body composition (Berenson et al., 1998). Physical activity and CRF confer CVD risk reduction during childhood by modifying individual or clustered CVD risk factors (Janssen and Leblanc, 2010).However, in adults the summed improvements in traditional CVD risk factors accounts for ~ 60% of the reduction in CVD risk (Mora et al., 2007), meaning there is a 40% risk factor gap in the explanation of PA benefits (Joyner and Green, 2009). The autonomic and arterial systems have been proposed as components of the risk factor gap and may be considered as novel risk factors (Joyner and Green, 2009). While arterial function has recently been added to a clustered score of traditional CVD risk factors in an attempt to improve the associations between PA and CVD risk in children (Fussenich et al., 2016), the influence of autonomic function above traditional CVD risk factors is unknown.

Assessment of cardiac autonomic function by measuring rest and recovery HRV as well as HRR provides distinct and complementary information (Dewland et al., 2007). While positive relationships between PA and CRF with resting HRV have been demonstrated in youth (Buchheit et al., 2007c, Radtke et al., 2013b, Gutin et al., 2005, Oliveira et al., 2017), further understanding about the potential relationships of PA and CRF to cardiac autonomic function during recovery following exercise is needed. Similarly, the effects of PA intensity on HRV and HRR is not clear. In adolescents, one study reported positive effects for MVPA but not VPA (Radtke et al., 2013b), whereas in another study with pre-adolescents, VPA but not MPA, presented stronger effects (Buchheit et al., 2007c), and a recent systematic review suggests that the associations of PA intensity, CRF and HRV are unclear, as few studies considered PA intensities,

ST and CRF, and bias exist in the assessment of cardiac autonomic function, PA and CRF(Oliveira et al., 2017). Additionally, none of these studies measured cardiac autonomic recovery following exercise nor combined measures of cardiac autonomic function with traditional CVD risk factors.

The aims of this study were: 1) to investigate the relationship of PA intensity and CRF to traditional CVD risk factors, as well as novel CVD risk factors using measurements of autonomic function at rest and recovery; and 2) to investigate whether adding autonomic function measures to a clustered score of traditional CVD risk factors strengthens the associations between PA, CRF and CVD risk.

5.3 Methods

5.3.1 Participants

Participants were recruited from two secondary schools in the South West of England. The volunteers were informed about the study via an assembly and study information sheets were distributed. A flow diagram of the recruitment process with the final number of participants included in the study is presented in Figure 5.1. Participant descriptive data are presented in Tables 5.1 and 5.2. Exclusion criteria included an existing musculoskeletal injury, presence of cardiometabolic disease, taking medications, and showing any contraindications to exercise. Before the study commenced, all participants and their parents/guardians provided written assent and consent, respectively. The study received ethics approval from the institutional Ethics Committee (Ref No: 141022/B/07), and all procedures performed meet the ethical standards of the International Journal of Sports Medicine (Harriss and Atkinson, 2015).

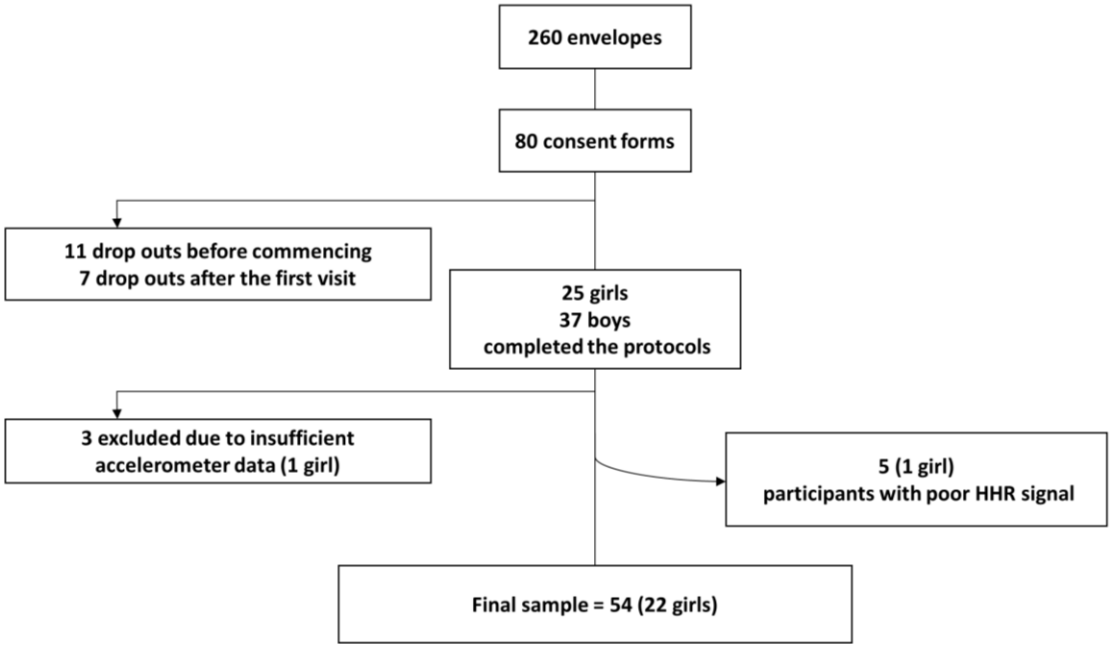

Figure 5.1: Flow chart of recruitment and sample size in the final analysis.

5.3.2 Study design

This is a cross-sectional study where participants completed three visits to a school-based laboratory over a one-week period as follows:

Visit-1: Participants had stature, body mass, sitting height, and waist circumference (WC) measured followed by triceps and subscapular skinfolds. Peak height velocity was used as an indicator of somatic maturity according to equations 3.1 and 3.2 (Mirwald et al., 2002) and participants were classified as pre (-1 year), circa (-1 to +1 year), or post (+1 year) PHV. Body fat percentage was obtained using equations 3.3 – 3.7 (Slaughter et al., 1988).

Visit-2: Participants reported to the laboratory in a fasted state (>10 h) and lay supine for 10min. Resting heart rate was recorded followed by measurements of BP. Next, a

fingertip capillary blood sample was collected to measure lipid profile and glucose concentration. In the 48-h preceding Visit-2 participants were instructed to refrain from performing organised sport and vigorous exercise.

Visit-3: A cycle test to exhaustion (see Section 3.5) was performed to determine CRF. Following exhaustion, participants sat for 10min for assessment of HRR. At the end of this session, participants were given an accelerometer and instructed to wear the device for seven consecutive days for PA measurements.

5.3.3 Traditional cardiovascular disease risk factors

Blood pressure was measured as described in Section 3.8.1.Systolic and DBP values were retained for analysis and MAP calculated. The observed CV between the measurements of SBD and DBP were all <4%. Capillary blood samples were used to determine total cholesterol (TC), HDL, TAG and GLU (CardioChek® PA, PTS Diagnostics, USA) as described in section 3.7.1.

5.3.4 Autonomic function

A 10-min resting period of heart rate measurements (Polar Team2, Kempele, Finland) obtained during the second visit was used to calculate HRV. Participants were asked to pace their breathing frequency at 12 cycles per min using a metronome. Resting heart rate variability was measured using RMSSD, HF, LF as well as the LF/HF as described in Section 3.8.2. HRV measurements performed by our group have been demonstrated to be reliable (CV = 17.6%) (Bond et al., 2017b).

Heart rate recovery was obtained as described in Section 3.8.2 after the steep ramp test described in Section 3.5.

Table 5.1: Characteristics of the participants according to sex.

	All (n=53)	Girls (n=23)	Boys (n=31)
Demographic characteristics			
Age (y)	13.1±0.8	12.9±0.8	13.1±0.9
Stature (cm)	156.6±9.6	153.8±7.8	158.6±10.5
Body mass (kg)	49.2±12.1	50.1±13.8	48.5±11.0
Pubertal status			
Pre (n (%))	18(33.3)	1(4)	17(56)
Circum (n (%))	27(50)	15(65)	12(39)
Post (n (%))	9(16.7)	7(31)	2(5)
Traditional CVD risk factors			
BMI ($kg \cdot m^{-2}$)	19.9±3.5	21.0±4.3	19.1±2.6
Body fat (%)	20±7.6	23.2±6.9	17.8±7.4
WC (cm)	68.5±9.5	68.3±10	68.5±8.9
SPB (mmHg)	113±9	113±9	113±9
DPB (mmHg)	68±7	70±7	67±7
TC ($mmol \cdot L^{-1}$)	3.4±0.5	3.4±0.6	3.4±0.5
HDL ($mmol \cdot L^{-1}$)	1.4±0.3	1.3±0.4	1.4±0.3
TAG ($mmol \cdot L^{-1}$)	0.7±0.2	0.8±0.3	0.8±0.5
Glucose ($mmol \cdot L^{-1}$)	4.3±0.4	4.2±0.5	4.3±0.4
Non-traditional CVD risk factors			
RMSSD (ms)	77.8±40.4	64.7±32.4	87.6±43.4
HF (ln)	7.8±1.1	7.5±1.1	8.0±1.0
LF (ln)	7.1±0.9	6.8±0.9	7.4±0.9
HF (nu)	64.9±16.2	65.2±15.4	64.7±17.0
LF (nu)	34.8±16.2	34.6±15.4	34.9±17.0
LF/HF	0.7±0.7	0.6±0.5	0.7±0.9
T_{30} (s)	192±88.8	193.3±57.5	191.1±107.4
HRR τ(s)	70.8±29.6	66.1±15.8	74.4±36.5

BMI, body mass index; WC, waist circumference; SPB, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL, high-density lipoprotein; TAG, triglycerides; RMSSD, square root of the mean of the sum of the squares of differences between adjacent RR intervals; HF, High-frequency; LF, Low-frequency.

5.3.5 Cardiorespiratory fitness

Peak oxygen uptake (peak $\dot{V}O_2$) was estimated using a validated steep ramp test described in Section 3.5. CRF was subsequently normalised for body mass using a ratio standard ($mL \cdot kg^{-1} \cdot min^{-1}$) and an allometric method as described in Section 3.5.1.

Table 5.2: Fitness and physical activity characteristics of the participants.

	All (n=54)	Girls (n=22)	Boys (n=31)
CRF ($mL \cdot kg^{-1} \cdot min^{-1}$)	46.3±8.1	42.5±9.2	49.2±5.9
CRF ($mL \cdot kg^{-0.58} \cdot min^{-1}$)	235.1±36.1	215.9±35.9	249.4±29.3
ST ($min \cdot day^{-1}$)	329.6±110.4	330.7±105.5	328.9±115.7
LPA ($min \cdot day^{-1}$)	278.7±112.2	305.2±104.7	259.1±115.2
MPA ($min \cdot day^{-1}$)	104.6±33.4	92.0±36.0	114.0±28.5
VPA ($min \cdot day^{-1}$)	11.2±10.4	5.5±6.3	15.4±11.0
MVPA ($min \cdot day^{-1}$)	115.8±41.1	97.6±39.8	129.4±37.2

CRF, cardiorespiratory fitness; ST, sedentary time; LPA, light physical activity; MPA, moderate physical activity; VPA, vigorous physical activity; MVPA, moderate to vigorous physical activity.

5.3.6 Physical activity

Habitual PA was measured using a wrist-worn accelerometer as described in Section 3.6.

5.3.7 Statistical analyses

All data are presented as mean and SD unless otherwise stated. Normality of distribution was checked using Shapiro Wilk's test and skewed data were transformed prior to analysis. A clustered traditional CVD risk score ($CVDR_{trad}$) was calculated as the sum of the following sex-specific standardized z-scores: fasted [GLU], [TAG], [HDL], %BF and BP ([SBP+DBP]/2) (Ekelund et al., 2012). A clustered autonomic risk score ($CVDR_{auto}$) was created by adding the sex-specific standardized z-scores of resting RMSSD, T_{30} and HRRt. Z-scores were inverted when appropriate. In order to explore the effects of PA, ST and CRF beyond the traditional CVD risk factors, the $CVDR_{trad}$ was combined to the $CVDR_{auto}$ to produce a composite CVD risk score ($CVDR_{com}$). This is in accordance with a recent study that has included novel CVD risk factors into a composite CVD risk score (Fussenich et al., 2016).

Separate linear regressions were performed for PA intensities, ST and CRF as the predictor variables. For this purpose, a base model adjusting for sex and maturation was created and the predictors inserted separately. The outcome variables included were: the traditional CVD risk-factors (BMI, %BF, WC, MAP, TC, HDL, TAG and GLU), non-traditional CVD risk-factors (HRV and HHR) and the clustered CVD risk scores ($CVDR_{trad}$, $CVDR_{auto}$ and $CVDR_{com}$). The following variables were log transformed prior to entry into the model: VPA, RMSSD, T_{30}, HRRt, HDL, WC, BMI and TAG. %BF did not present a significant relationship to the outcome variables after adjusting for PA/CRF and was not included as a covariate.

In order to test the effects of PA intensities and CRF on the time course of parasympathetic reactivation as measured by the $RMSSD_{30}$, participants were divided into groups below and above the sex-specific median split for PA intensities and CRF. Median splits were chosen aiming to create equal sample sizes between the CRF groups as well as no thresholds are available in the literature for the allometric scaled CRF used in the present investigation. Repeated measures ANCOVA controlling for sex and maturation were used to examine a time (0 to 600 s) by group (above or below median split) interaction effect for $RMSSD_{30}$. The alpha level was set at 0.05 for all analyses which were performed using SPSS version 22.

5.4 Results

5.4.1 Traditional cardiovascular disease risk factors

Regression coefficients for PA intensities, ST, CRF and traditional CVD risk factors after adjusting for sex and maturation are presented in Table 5.3. ST and LPA were not significantly associated to any of the traditional CVD risk factors. By contrast, MPA was significantly and negatively associated to BMI, %BF, WC and MAP. VPA was

significantly and negatively associated to %BF. Both ratio and allometric scaled CRF were significantly and negatively associated to BMI, %BF and WC. However, the associations between allometric scaled CRF and the body composition variables were weaker compared to the ratio scaled CRF.

5.4.2 Non-traditional cardiovascular disease risk factors

Regression coefficients for the PA intensities, CRF and non-traditional risk factors after adjusting for sex and maturation are presented in Table 5.3. ST, LPA and CRF (ratio standard and allometrically scaled) were not significantly related to any of the rest or recovery autonomic indices. Both MPA and VPA were significantly and positively related to RMSSD and significantly and negatively related to HRRt and T_{30}. The time course of parasympathetic reactivation using the $RMSSD_{30}$ is presented in Figure 5.2. There was no group (below vs. above) by time interaction for ST, LPA, MPA, or CRF for $RMSSD_{30}$ (all $P>0.05$). In contrast, there was a group by time interaction ($P=0.01$) for VPA, with the above the median group presenting a higher $RMSSD_{30}$ after the first 60 s of recovery after exercise (all $P<0.05$).

5.4.3 Clustered cardiovascular disease risk scores

Regression coefficients for the PA intensities, CRF and the CVD risk scores after adjusting for sex and maturation are presented in Table 5.3. ST time and LPA were not significantly related to any of the clustered CVD risk scores. MPA was significantly and negatively associated to $CVDR_{trad}$, $CVDR_{auto}$ and $CVDR_{com}$. VPA was significantly and negatively associated to $CVDR_{auto}$ and $CVDR_{com}$, but not with $CVDR_{trad}$ ($P=0.08$). While ratio scaled CRF was negatively related to $CVDR_{trad}$, allometrically-scaled CRF was not associated to any of the clustered CVD risk scores. After combining $CVDR_{trad}$

and $CVDR_{auto}$ to form $CVDR_{com}$ the coefficient of determination increased from 17 to 32 % and 6 to 18 % for MPA and VPA, respectively.

5.5 Discussion

The key findings of the present investigation were: 1) MPA and VPA were related to traditional CVD risk factors, as well as rest and recovery autonomic function, 2) Girls and boys preforming VPA above the median split presented faster $RMSSD_{30}$;3) MPA and VPA were strongly and negatively related to CVD risk when rest and recovery autonomic indices were added to the traditional CVDR score; and 4) CRF was only significantly and negatively related to traditional CVD risk score, however, when allometrically scaled the significant relationship disappeared.

Table 5.3: Standardised regression coefficients.

	ST (min·day^{-1})		LPA (min·day^{-1})		MPA (min·day^{-1})		VPA (min·day^{-1})		CRF (ml·kg·min^{-1})		CRF (ml·kg$^{-0.58}$·min^{-1})	
Traditional CVD risk factors	β (*P*)	r^2	β (*P*)	r^2	β (*P*)	r^2	β (*P*)	r^2	β (*P*)	r^2	β (*P*)	r^2
BMI (kg·m^2)	-0.017 (0.90)	0.01	0.029 (0.83)	0.01	**-0.276 (0.04)**	**0.07**	-0.229 (0.15)	0.03	**-0.706 (<0.001)**	**0.41**	**-0.430 (0.004)**	**0.13**
%BF (%)	0.022 (0.86)	0.01	0.024 (0.85)	0.01	**-0.310 (0.02)**	**0.09**	**-0.384 (0.01)**	**0.10**	**-0.701 (<0.001)**	**0.40**	**-0.602 (<0.001)**	**0.25**
WC (cm)	-0.050 (0.72)	0.01	0.091 (0.52)	0.01	**-0.316 (0.03)**	**0.09**	-0.300 (0.07)	0.06	**-0.765 (<0.001)**	**0.48**	**-0.496 (0.001)**	**0.17**
MAP (mmHg)	-0.023 (0.87)	0.01	0.142 (0.30)	0.02	**-0.326 (0.02)**	**0.09**	0.182 (0.27)	0.02	-0.191 (0.20)	0.03	-0.170 (0.29)	0.02
TC (mmol·L^{-1})	0.062 (0.66)	0.01	0.062 (0.66)	0.03	0.041 (0.78)	0.01	-0.162 (0.34)	0.02	-0.09 (0.56)	0.01	-0.236 (0.15)	0.04
HDL (mmol·L^{-1})	0.177 (0.20)	0.03	-0.169 (0.23)	0.02	0.209 (0.15)	0.04	0.037 (0.83)	0.01	0.138 (0.37)	0.02	-0.026 (0.87)	0.01
TAG (mmol·L^{-1})	-0.145 (0.28)	0.02	-0.024 (0.86)	0.01	-0.158 (0.26)	0.02	-0.204 (0.22)	0.03	-0.135 (0.37)	0.01	-0.08 (0.63)	0.01
Glucose (mmol·L^{-1})	-0.106 (0.45)	0.01	-0.046 (0.75)	0.01	-0.184 (0.21)	0.03	-0.165 (0.34)	0.02	-0.189 (0.22)	0.03	-0.097 (0.57)	0.01
Non-traditional CVD risk factors												
RMSSD (ms)	-0.005 (0.97)	0.01	0.006 (0.96)	0.01	**0.402 (0.003)**	**0.14**	**0.453 (0.005)**	**0.13**	0.237 (0.11)	0.05	0.299 (0.06)	0.06
HRRT (s)	-0.139 (0.33)	0.02	0.149 (0.30)	0.02	**-0.311 (0.03)**	**0.08**	**-0.406 (0.02)**	**0.11**	-0.034 (0.83)	0.01	0.109 (0.22)	0.01
T_{30} (s)	-0.111 (0.42)	0.02	0.100 (0.49)	0.01	**-0.356 (0.02)**	**0.11**	**-0.433 (0.01)**	**0.12**	0.081 (0.61)	0.01	0.207 (0.22)	0.03
Clustered CVD risk scores												
$CVDR_{trad}$	-0.152 (0.28)	0.02	0.041 (0.78)	0.01	**-0.447 (0.002)**	**0.17**	-0.340 (0.08)	0.06	**-0.438 (0.004)**	**0.15**	-0.277 (0.11)	0.05
$CVDR_{auto}$	-0.115 (0.42)	0.01	0.102 (0.48)	0.01	**-0.483 (0.001)**	**0.20**	**-0.563 (0.001)**	**0.20**	-0.070 (0.67)	0.01	0.020 (0.91)	0.01
$CVDR_{com}$	-0.142 (0.32)	0.02	0.041 (0.78)	0.01	**-0.601 (<0.001)**	**0.32**	**-0.540 (0.001)**	**0.18**	-0.313 (0.05)	0.08	-0.153 (0.38)	0.02

Standardised regression coefficients are adjusted for maturation and sex. ST, sedentary time; LPA, light physical activity; MPA, moderate physical activity; VPA, vigorous physical activity; CRF, cardiorespiratory fitness; BMI, body mass index; BF, body fat; WC, waist circumference; MAP, mean arterial pressure; TC, total cholesterol; HDL, high-density lipoprotein; TAG, triglycerides; RMSSD, square root of the mean of the sum of the squares of differences between adjacent RR intervals.

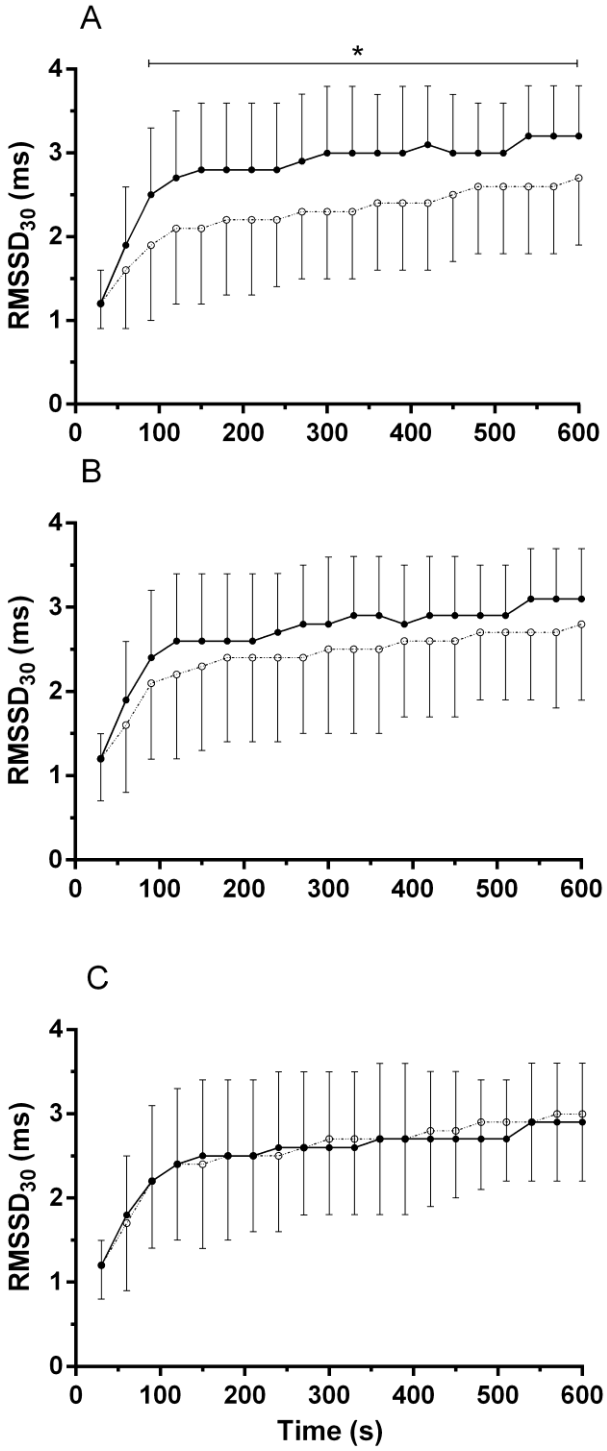

Figure 5.2: Parasympathetic reactivation for the groups above (solid circles) and below (open circles) the sex specific median split of: A) VPA (min·day^{-1}); B) MPA (min·day^{-1}); and C) CRF (mL·kg$^{-0.58}$·min^{-1}). *P<0.05 for the comparison between groups from 60 to 600s (except at 510 s, P=0.053). Values are mean and SD.

In the current investigation, ST and LPA were not significantly related to the health outcomes which corroborates with previous literature (Ekelund et al., 2012), and supports the focus of current PA guidelines on time spent performing MVPA to promote CVD health in adolescents. Both MPA and VPA were negatively associated to %BF highlighting the benefits of PA on CVD risk via lower body composition scores. Although these observations are in accordance with the literature (Janssen and Leblanc, 2010), causality cannot be inferred as increased %BF may lead to reduced MPA and VPA (Metcalf et al., 2011). The lack of significant associations between PA intensities and blood lipids is in accordance with the literature (Ekelund et al., 2007). Importantly, in adolescents with favourable lipoprotein profile, body composition have been associated to CVD risk (McGill et al., 2001). Altogether, our present results highlight the important role of PA on cardiovascular health, via negative associations with body composition in this population, independent of blood lipid profile.

In addition to the traditional CVD risk factors, MPA and VPA were significantly related to resting HRV. This is reflected in other studies reporting the associations of combined MVPA and resting HRV in youth (Gutin et al., 2005, Radtke et al., 2013b). In contrast to the present results, Radtke et al. (2013b) did not find associations between VPA and resting HRV in 15 years old adolescents. Discrepancies between the results might lie in the different levels of total MVPA performed by the participants, and different cut-off points used to define PA intensities, as this has been shown to affect the interpretation of PA results (Banda et al., 2016). Significant associations between CRF and resting autonomic function were not observed in the present study which is in line with the literature showing no consensus for this relationship (Brunetto et al., 2005, Gutin et al., 2005). Interestingly, when CRF was allometrically scaled the positive association between CRF and RMSSD approached significance (P=0.06). As

allometric models are superior in controlling for the effect of body size on CRF, this indicates that body size has a confounding effect on the CRF-HRV associations when using the ratio standard method and needs further investigation.

A novel finding in the current study is the significant and negative associations of HRRt and T_{30} to MPA and VPA. These observations show that MPA and VPA are related to neural control of vagal modulation (measured by resting HRV), as well as vagal tonus in adolescents (Dewland et al., 2007). The group above the median split of VPA, which equated to two and 12 min per day for girls and boys respectively, presented faster $RMSSD_{30}$ with no observed differences between the MPA and CRF groups (see Figure 5.2). This is the first study to investigate the effects of PA intensity and CRF on parasympathetic reactivation throughout 10 min of recovery. In contrast to MPA and VPA, CRF, LPA and ST had no significant associations with HRR and parasympathetic reactivation. As parasympathetic reactivation provides CVD prognostic information (Dewey et al., 2007, Lahiri et al., 2012, Pecanha et al., 2017), the present results suggest an important role for VPA and novel mechanisms by which this intensity is associated with cardiovascular health in adolescents. Possible explanations for the observed associations are the hemodynamic and hormonal alterations occurring during and after VPA. It may be speculated that these alterations are related to an increased catecholamine response, increased cardiac output, redistribution of blood flow to skin and muscles, and the higher demand on the respiratory muscles amongst others, posing important stresses on the autonomic and cardiovascular systems. The current cross-sectional study and measures, however, impede us of testing these mechanisms.

Further to the traditional and non-traditional CVD risk factors, a traditional CVD risk score ($CVDR_{trad}$) as it is known to differentiate CVD risk in children was used (Andersen et al., 2011a). The observed MPA but not VPA relationship possibly reflects the observed association of MPA to BP which was not found for VPA. Similarly, CRF was negatively and significantly related to $CVDR_{trad}$, which is in line with evidence of associations between CVD profile and CRF in youth (Ruiz et al., 2014). However, when CFR was expressed using an allometric model, the observed significance of the relationship disappeared. This observation shows that scaling for body size has an important influence on the CRF-$CVDR_{trad}$ association, which is not typically accounted for in the literature (Ruiz et al., 2014). Future studies should account for the confounding effects of body size in youth because CRF normalized by body mass, using a ratio standard approach, might reflect differences in body size occurring during adolescence rather than the 'true' CRF score.

A recent study has included endothelial function into a CVD risk score in children and examined the associations between MPA, VPA and this new composite CVD score (Fussenich et al., 2016). The authors did not present the changes in the PA relationships to the risk score after the inclusion of endothelial function to the composite risk score so additional explanation could not be inferred. In contrast, our current data show that after combining RMSSD, T_{30} and HRRt to the $CVDR_{trad}$ score, providing a composite risk score ($CVDR_{com}$), the coefficient of determination rose from 17 to 32% for MPA and 6 to 18% for VPA. This increase in the strength of the association shows that in adolescents, MPA and VPA have important influences on autonomic function. This is in accordance with a recent study showing improvements in HRV but not traditional CVD risk factors in this population following an exercise intervention (Bond et al., 2015a). Altogether, these results contribute to the possible

role of PA on CVD risk in adolescents via autonomic function beyond changes in traditional CVD risk factors.

Some limitations must be considered when interpreting the current findings. For instance, the possible influence of the menstrual cycle in females was not possible to be taken into consideration. Specific types of sedentary behaviours (e.g. TV viewing) were not examined in the current study and the 60 s epoch used for the PA analyses may have underestimated the PA status of the participants. Additionally, the participants were recruited using a convenience sample and therefore sample bias might be present and the maturation measure applied might have misclassified some of the participants. Finally, the cross-sectional design limits causality between the observed associations.

5.6 Conclusions

This is the first study to examine the associations of PA intensities and CRF to cardiac autonomic function together with traditional CVD risk factors in this population. Complementary to the observed relationships between PA and traditional CVD risk factors, the current study highlights the strong associations of MPA and VPA to autonomic function at rest and recovery in adolescents, but not CRF, ST and LPA. Additionally, this is the first study to demonstrate the associations between VPA and a more rapid 10 min parasympathetic reactivation. A two and a threefold increase in the association between MPA, VPA and the composite CVD risk score after adding autonomic function to the traditional CVD risk score was observed. These are novel findings and suggest using of cardiac autonomic function within a composite CVD risk score in studies examining associations with PA and CRF. However, in contrary to adult literature, the clinical significance of cardiac autonomic measures in this

population is still unclear. Nevertheless, the present results provide information for health policy advocating the importance of MPA and VPA during adolescence. Finally, although our study provides original data, longitudinal studies are warranted to clarify the causality of the relationships.

Chapter 6: Postprandial Lipaemia, Arterial Stiffness and Heart Rate Variability in Adolescents: Associations with Physical Activity and Cardiorespiratory Fitness

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6.1 Abstract

The aim of this Chapter was to examine whether daily PA and CRF are associated with [TAG], HRV and arterial stiffness following a HFM. Fifty-one adolescents (22 girls) aged 12 – 15 years volunteered to participate, completing three visits to a school-based laboratory over one-week. In the first visit, anthropometric measures were performed. In the second visit, in the following order, HR (for HRV measurements), BP, PWV, and a capillary blood sample were collected pre and 2-h post the ingestion of a HFM. In the third visit, CRF was measured using a cycling test to exhaustion and accelerometers distributed for PA measurement over 7 days. HRV was calculated using time and frequency domains and PWV assessed at the carotid and radial arteries. The HFM led to increases (P<0.001) in [TAG] (pre-HFM =0.80±0.27; post-HFM =1.39±0.55for girls; and pre-HFM = 0.66 ±0.14; post-HFM = 1.15 ±0.49 for boys), decrease(P<0.001) in RMSSD (pre-HFM = 62.5 ± 31.4; post-HFM = 52.1±26.4 for girls; and pre-HFM = 93.9±40.2; post-HFM = 67.9±29.1 for boys) but no change(P=0.96) in PWV(pre-HFM = 7.7 ± 1.1; post-HFM = 7.8 ± 0.9 for girls; and pre-HFM = 9.0 ± 1.7; post-HFM = 9.0 ± 2.0 for boys) . Fasting [TAG] was positively

associated with postprandial [TAG] in boys (stβ=0.628; *P*<0.001) and girls (stβ=0.741; *P*<0.001). PA intensities and CRF were not significantly associated with postprandial [TAG], HRV or PWV (stβ ranging from 0.094 to -0.629; *P*>0.05). However, for boys MPA (stβ=-0.539) and VPA (stβ=-0.498) were negatively associated (*P*<0.001) with the delta changes in RMSSD. PA and CRF are not associated with postprandial cardiovascular risk factors in adolescents, however, MPA and VPA are associated with a higher parasympathetic withdrawal at the postprandial state.

6.2 Introduction

The pathophysiological process of atherosclerosis is known to start during childhood (Berenson et al., 1998), and fasted [TAG] measured in schoolchildren predicts CVD events in the fourth and fifth decades of life (Morrison et al., 2009). Although fasted [TAG] is a strong predictor of CVD, non-fasting [TAG] has been associated with pre-clinical markers of atherosclerosis independently of fasted [TAG] (Pirillo et al., 2014). As most of the day is spent in the postprandial state (Nakamura et al., 2016), strategies to lower postprandial [TAG] in youth are warranted to decrease future CVD burden.

In adolescents, exercise is an important modulator of lipid metabolism, with a single bout of moderate and high-intensity exercise performed before the ingestion of a HFM lowering postprandial [TAG] in boys and girls (Tolfrey et al., 2014). The effect of exercise on postprandial [TAG] is normally attributed to the last bout. For instance, when adults are asked to refrain from exercise in the 48 – 60 h preceding HFM ingestion, no effect of training status is observed (Tsetsonis et al., 1997, Herd et al., 2000, Maraki and Sidossis, 2013). In contrast, PA is associated with a lower postprandial [TAG] in the elderly, even when participants refrained from exercise in the days preceding the ingestion of the HFM (Miyashita et al., 2011). The difference

between ages in the literature suggests that [TAG] responses in youth may be dependent on PA levels. In youth, fasted [TAG] and lower [HDL] are the key predictors of increases in postprandial [TAG] (Couch et al., 2000), and one possible mechanism that PA alters postprandial [TAG] is via improvements in fasted lipid [HDL] and [TAG] in youth with higher levels of PA, specially vigorous intensity VPA (Barker et al., 2018). Likewise, higher levels of CRF is also positively associated with [HDL] and negatively associated with [TAG] (Ekelund et al., 2007), suggesting an association between CRF and postprandial lipemia may also exist, however this has yet to be explored. Furthermore, exercise also alter postprandial lipaemia via other mechanisms than a lowered fasted [HDL] and [TAG], such as hepatic fatty acid oxidation an up-regulation of the lipoprotein lipase. Although these mechanisms are related to the last bout effect, differences between boys and girls have been observed (Bond et al., 2014, Thackray et al., 2018)which may indicate that effects of habitual PA on postprandial lipaemia may be sex dependent.

In addition to postprandial [TAG], elevated postprandial BP is considered a novel CVD risk factor (Uetani et al., 2012). Because PA and CRF are inversely related to resting BP (Janssen and Leblanc, 2010), it is plausible to hypothesize that postprandial BP and the changes in BP after a HFM will be inversely related to PA and CRF levels. Similarly, novel CVD risk factors such as vascular function and stiffness, as well as heart rate variability (HRV), are transiently impaired after a HFM (Bond et al., 2015a, Augustine et al., 2014). The mechanism underpinning the HFM effects on the vasculature is an elevation of oxidative stress and a decreased nitric oxide dilatory capacity at the postprandial state (Bae et al., 2001). This observation occurs concomitantly with decreases in HRV (Bond et al., 2015a), reflecting a lower vagal modulation to the heart (Task-Force, 1996). On the contrary, high, but not moderate,

intensity exercise performed one hour before the ingestion of the HFM has been shown to lead to augmented postprandial arterial function via improvements in nitric oxide-dependent vasodilation (Bond et al., 2015b). This finding shows that habitual PA, and possibly CRF, might confer protection against the deleterious effects of the HFM on arterial stiffness, HRV and BP in youth.

Therefore, the aim of this study is to examine the associations in boys and girls between time spent in moderate and vigorous habitual PA and CRF, and postprandial changes in [TAG], HRV and arterial stiffness. It was hypothesized that habitual moderate and vigorous PA, as well as CRF would be positively associated to postprandial changes in HRV, arterial stiffness, and [TAG].

6.3 Methods

6.3.1 Participants

Participants were recruited from two secondary schools in the South West of England. Participants were contacted in an assembly at the schools and a flow diagram of the recruitment process with the final number of participants included in the analysis is presented in Figure 6.1. Exclusion criteria included an existing musculoskeletal injury, presence of cardiometabolic disease, taking medications, and showing any contraindications to exercise. All participants and their parents/guardians provided written assent and consent, respectively, to participate. The study received ethics approval from the institutional Ethics Committee (Ref No: 141022/B/07).

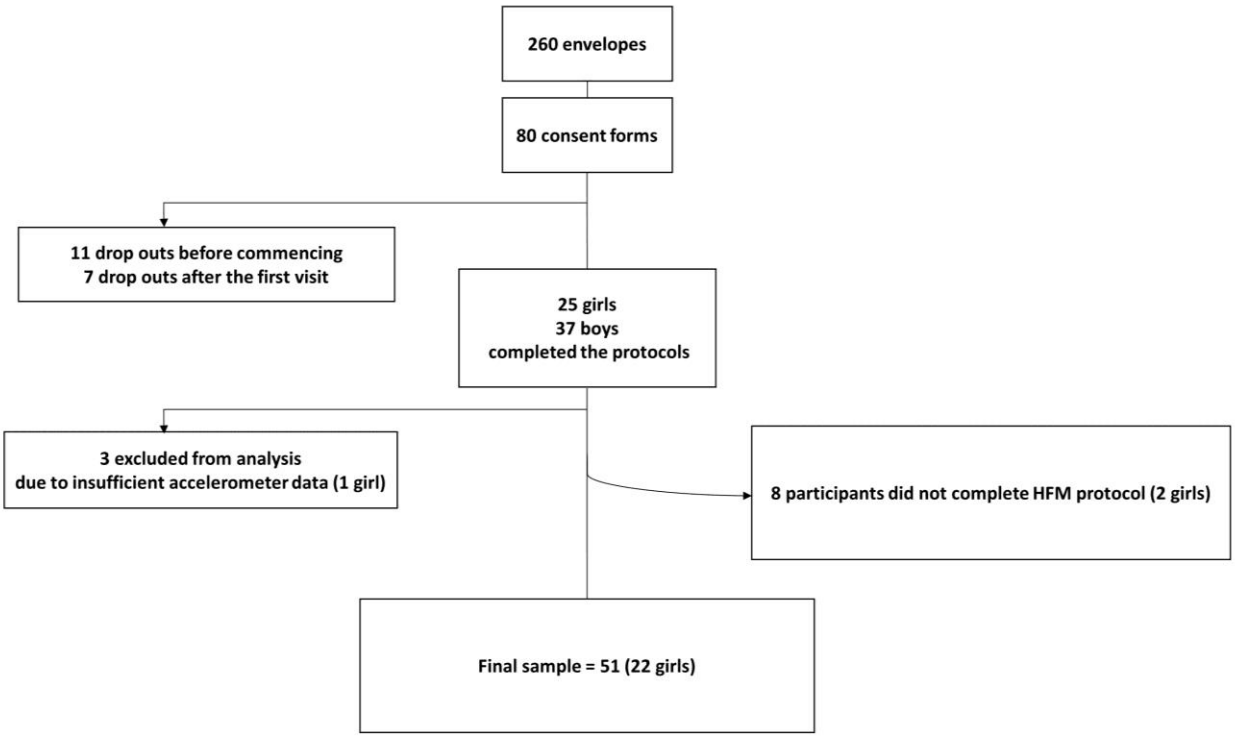

Figure 6.1: Flow chart of recruitment and sample size in the final analysis.

6.3.2 Study design

This is a cross-sectional study where participants completed three visits to a school-based laboratory over a one-week period.

In the first visit participants had stature, body mass, sitting height, and waist circumference (WC) measured followed by triceps and subscapular skinfolds. Peak height velocity was used as an indicator of somatic maturity according to equations 3.1 and 3.2 (Mirwald et al., 2002) and participants were classified as pre (-1 year), circa (-1 to +1 year), or post (+1 year) PHV. Body fat percentage was obtained using equations 3.3 – 3.7 (Slaughter et al., 1988). At the end of this visit, participants were given information of the procedures to follow on the 48h preceding the second visit. Specifically, participants were instructed to avoid organised exercise activities in the

48h before the second visit and to follow a >10 h overnight fast. Parents were contacted and reminded to reinforce the participants to follow the procedures before the second laboratory visit.

Before the start of the second visit, participants were asked if they followed the instructions in the 48h preceding the second visit. All participants reported compliance with the instructions. Volunteers then lay supine for 10min. Resting heart rate was recorded followed by measurements of BP and PWV. Next, a fingertip capillary blood sample was collected. Participants then consumed, within 15min, a HFM and walked to their classes before returning to the laboratory two hours later for repeat measurements of heart rate, BP, PWV and blood sampling. During the two hours break, participants completed their schoolwork whilst sitting. No exercise activities or additional food consumption was permitted during this period. The two hours post-HFM was used because feasibility in a school setting, which limited the hours between the measurements. Additionally, measurements obtained two hours following the HFM can be used as a surrogate of the area under the curve response. In house data show a strong correlation (r=0.94 and r=0.98 for boys and girls) between two hours post [TAG] and the four-hours area under the curve response following the ingestion of a HFM [taken from (Bond et al., 2015b, Bond et al., 2014)].

In the third and final visit a cycle test to exhaustion was performed to determine CRF. At the end of this session, participants were given an accelerometer and instructed to wear the device for seven consecutive days for measurement of their habitual PA.

6.3.3 Autonomic function

Resting heart rate variability was measured using the RMSSD, HF, LF as well as the LF/HF as described in Section 3.8.2. HRV measurements performed by our group have been demonstrated to be reliable (CV = 17.6%) (Bond et al., 2017b).

6.3.4 High-fat meal

The HFM protocol was performed as described in Section 3.7.1 according to previous work. Previous work has demonstrated that the ingestion of this HFM increases peak [TAG] in two hours and also impairs vascular and autonomic functions (Bond et al., 2014, Bond et al., 2015b, Bond et al., 2015d).

6.3.5 Blood pressure and pulse wave velocity

Blood pressure was measured three times as described in Section 3.8.1 and PWV obtained as described in Section 3.8.5.

6.3.6 Blood outcomes

Capillary blood samples were used to determine total cholesterol (TC), HDL, TAG and GLU (CardioChek® PA, PTS Diagnostics, USA) as described in section 3.7.1.. The observed inter-assay CV was 5.1, 5.7, 7.4 and 4.3 % for [TC], [HDL], [TAG] and [GLU], respectively.

6.3.7 Cardiorespiratory fitness

Peak oxygen uptake (peak $\dot{V}O_2$) was estimated using a validated steep ramp test described in Section 3.5. CRF was subsequently normalised for body mass using a ratio standard ($mL \cdot kg^{-1} \cdot min^{-1}$) and an allometric method as described in Section 3.5.1.

6.3.8 Physical activity

Habitual PA was measured using a wrist-worn accelerometer as described in Section 3.6.

6.3.9 Statistical analyses

All data are presented as mean and standard deviation unless otherwise stated. Normality of distribution was checked using Shapiro Wilk's test and skewed data were transformed prior to analysis. Sex differences between variables were examined using independent samples t-tests. Effect sizes were calculated for the sex comparisons and interpreted as <0.2 trivial, ≥0.2 small, ≥0.5 moderate, and ≥0.80 large (Cohen, 1977). Pre- and post-HFM changes and time by sex interactions were assessed using repeated measures ANOVA.

As differences between sexes exist for levels of PA and CRF (Reilly, 2016), and that the HFM responses of girls and boys are differently altered by acute exercise (Bond et al., 2014), multiple linear regression models for boys and girls were performed separately. PA intensities, CRF, fasting [TAG] and [HDL], were used as the independent predictors and inserted separately into the models. Post-HFM [TAG], BP, PWV and HRV were inserted into the models as dependent variables. In addition, delta changes were calculated as the post- minus the pre-HFM values and included as dependent variables. All regression models were controlled for maturity status and BF%. This was because preliminary analyses showed differences between the maturity groups for the main predictors and body fatness has been shown to affect the postprandial [TAG] of adolescents (Moreno et al., 2001). The following variables were log transformed prior to entry into the models: VPA, BF%, RMSSD, PWV, and [TAG].

The alpha level was set at 0.05 for all analyses. All analyses were performed using SPSS version 22.

6.4 Results

6.4.1 Participants

From the initial 62 volunteers, three did not meet the criteria for the PA analysis and four declined to complete the HFM ingestion and four did not adhere to the remainder of the protocol. Thus, 51 (22 girls) participants were included in the final analysis (Figure 6.1). Participants' characteristics are presented in Table 6.1. No significant differences were observed between sexes for age and BMI; however, the percentage of girls post PHV was higher than boys (girls=32% vs. boys=7%; *P*<0.001). Girls presented significantly higher %BF, performed significantly less VPA, MPA, and had a significant lower CRF compared to boys. The average HFM caloric content was 949.7±228.8 kcal. Overall, the meal was well tolerated but four participants declined to consume the HFM.

Table 6.1: Participants' characteristics pre and post high-fat meal.

	Girls (n = 22)	Boys (n = 29)	*P*	Effect size
Age (yeas)	12.9±0.8	13.2±0.8	0.24	-0.38
BMI ($kg \cdot m^2$)	21.1±4.3	19.1±2.0	0.051	0.62
Body mass (kg)	50.5±14.0	48.8±10.0	0.62	0.14
BF (%)	23.2±7.1	17.0±6.2	**<0.001**	0.93
VPA ($min \cdot day^{-1}$)	5.7±6.4	16.3±11.0	**<0.001**	-1.13
MPA ($min \cdot day^{-1}$)	91.9±36.8	117.1±28.1	**0.011**	-0.78
Accelerometer wear time (min)	812.4±90.4	837.8±112.3	0.38	-0.25
CRF ($mL \cdot kg^{-0.58} \cdot min^{-1}$)	217.2±36.2	254.7±27.1	**0.002**	-1.19
CRF ($mL \cdot kg \cdot min^{-1}$)	42.6±9.4	49.9±4.7	**0.002**	-1.02

BMI: body mass index. BF: body fatness. VPA: vigorous physical activity. MPA: moderate physical activity. CRF: cardiorespiratory fitness

Pre- and Post-HFM values for [TAG], [HDL], RMSSD, PWV and BP are presented in Table 6.2. There were no significant sex differences for [HDL] and SBP pre- and post-HFM. Pre-, but not post-HFM, girls presented significantly higher [TAG] and lower RMSSD compared to boys. No significant differences were observed for HF (nu) and LF/HF ratio between sexes. Girls had significantly lower PWV compared to boys both pre- and post-HFM.

6.4.2 Postprandial outcomes

The HFM increased [TAG] and decreased RMSSD in both sexes (P<0.001 for time effect). In contrast, PWV (P=0.96) and SBP (P=0.12) did not change after the HFM (Table 6.2). The standard regression coefficients are presented in Table 6.3. In girls and boys, fasting [TAG] was positively related to postprandial [TAG] (P<0.001) after controlling for BF% and maturity. No associations were observed for fasting [TAG] and [HDL] with the postprandial RMSSD and PWV for both sexes (all P>0.05). MPA, VPA, and CRF were not significantly associated with any of the postprandial outcomes in both sexes (P>0.05).

Table 6.2: Mean and standard deviation of traditional and novel cardiovascular disease risk factors pre and post the high-fat meal.

	Girls (n = 22)	Boys (n = 29)	*P*	Effect size
TAG ($mmol \cdot L^{-1}$)				
Fasting	0.80±0.27	0.66±0.14	**0.02**	0.68
2 h	1.39±0.55*	1.15±0.41*	0.08	0.51
∆2h	0.59±0.35	0.49±0.33	0.29	0.31
HDL ($mmol \cdot L^{-1}$)				
Fasting	1.31±0.39	1.44±0.28	0.15	-0.40
2 h	1.30±0.39	1.46±0.28	0.10	-0.46
∆2h	0.01±0.01	0.02±0.09	0.46	-0.20
SBP (mmHg)				
Fasting	114±9	113±9	0.71	0.10
2 h	113±8	112±9	0.63	0.14
∆2h	-0.9±5.1	-1.1±4.0	0.90	0.03
PWV ($m \cdot s^{-1}$)				
Fasting	7.7±1.1	9.0±1.7	**0.01**	-0.88
2 h	7.8±0.9	9.0±2.0	**0.02**	-0.68
∆2h	0.1±0.7	-0.1±1.4	0.57	0.16
RMSSD (ms)				
Fasting	62.5±31.4	93.9±40.2	**0.01**	-0.85
2 h	52.1±26.4*	67.9±29.1*	0.05	-0.57
∆2h	-10.4±10.1	-26.0±26.3	**0.01**	0.82
HF (nu)				
Fasting	66.1±15.1	66.0±15.1	0.98	0.09
2 h	65.3±15.9	65.8±13.9	0.90	-0.04
∆2h	-0.2±0.5	-0.6±1.1	0.86	-0.05
LF/HF				
Fasting	0.6±0.5	0.6±0.5	0.96	-0.01
2 h	0.6±0.5	0.6±0.6	0.90	0.03
∆2h	0.0±0.3	0.0±0.7	0.85	0.05

*$P<0.05$ compared to fasting values. Probability (P) and effect sizes (ES) are for comparisons between sexes. TAG: triacylglycerol. HDL: high-density lipoprotein. SBP: systolic blood pressure. PWV: pulse wave velocity. RMSSD: square root of the mean of the sum of the squares of differences between adjacent RR intervals. HF: high frequency.

Table 6.3: Associations of physical activity, cardiorespiratory fitness, fasting [TAG], and [HDL] to the delta to postprandial outcomes in adolescent boys and girls.

	Girls					
	MPA ($min \cdot day^{-1}$)	VPA ($min \cdot day^{-1}$)	CRF ($mL \cdot kg^{-0.58} \cdot min^{-1}$)	CRF ($mL \cdot kg^{-1} \cdot min^{-1}$)	Fasting TAG ($mmol \cdot L^{-1}$)	Fasting HDL ($mmol \cdot L^{-1}$)
2-h TAG ($mmol \cdot L^{-1}$)	-0.134	0.094	-0.380	-0.629	**0.741***	-0.355
2-h RMSSD (ms)	0.353	0.305	0.279	0.371	-0.277	0.083
2-h HF (nu)	0.106	-0.023	0.360	0.441	0.350	0.182
2-h LF/HF	-0.238	-0.025	-0.334	-0.387	0.238	-0.126
2-h PWV ($m \cdot s^{-1}$)	-0.265	-0.174	-0.034	-0.122	0.336	0.145
2-h SBP (mmHg)	-0.362	-0.334	-0.413	-0.606	-0.394	-0.032
	Boys					
	MPA ($min \cdot day^{-1}$)	VPA ($min \cdot day^{-1}$)	CRF ($mL \cdot kg^{-0.58} \cdot min^{-1}$)	CRF ($mL \cdot kg^{-1} \cdot min^{-1}$)	Fasting TAG ($mmol \cdot L^{-1}$)	Fasting HDL ($mmol \cdot L^{-1}$)
2-h TAG ($mmol \cdot L^{-1}$)	0.181	-0.125	0.280	0.090	**0.628***	-0.104
2-h RMSSD (ms)	0.258	0.240	0.265	0.329	0.059	0.136
2-h HF (nu)	-0.229	-0.122	0.131	0.274	0.255	0.007
2-h LF/HF	0.301	0.185	-0.073	-0.258	0.132	0.022
2-h PWV ($m \cdot s^{-1}$)	0.182	0.690	-0.366	-0.104	-0.044	0.377
2-h SBP (mmHg)	-0.078	0.125	0.465	0.144	0.283	-0.415

Values are standard beta controlled for maturation and body fat%. *P<0.05. VPA: vigorous physical activity. MPA: moderate physical activity. CRF: cardiorespiratory fitness. TAG: triacylglycerol. HDL: high-density lipoprotein. SBP: systolic blood pressure. PWV: pulse wave velocity. RMSSD: square root of the mean of the sum of the squares of differences between adjacent RR intervals. HF: high frequency.

Table 6.4: Associations of physical activity, cardiorespiratory fitness, fasting [TAG], and [HDL] to the delta changes after the HFM in adolescent boys and girls.

	Girls					
	MPA ($min \cdot day^{-1}$)	VPA ($min \cdot day^{-1}$)	CRF ($mL \cdot kg^{-0.58} \cdot min^{-1}$)	CRF ($mL \cdot kg^{-1} \cdot min^{-1}$)	Fasting TAG ($mmol \cdot L^{-1}$)	Fasting HDL ($mmol \cdot L^{-1}$)
ΔTAG	-0.47	-0.094	-0.517	-0.509	0.380	0.318
ΔRMSSD	0.070	-0.195	-0.163	-0.136	0.010	-0.398
ΔHF	-0.398	-0.430	0.097	0.125	-0.238	-0.050
ΔRatio	0.349	0.410	-0.183	-0.297	0.267	0.010
ΔPWV	-0.216	0.005	**-0.733***	**-0.865***	0.252	-0.069
ΔSBP	0.324	0.054	0.141	0.065	-0.222	0.117
	Boys					
	MPA ($min \cdot day^{-1}$)	VPA ($min \cdot day^{-1}$)	CRF ($mL \cdot kg^{-0.58} \cdot min^{-1}$)	CRF ($mL \cdot kg^{-1} \cdot min^{-1}$)	Fasting TAG ($mmol \cdot L^{-1}$)	Fasting HDL ($mmol \cdot L^{-1}$)
ΔTAG	-0.199	-0.194	0.165	-0.034	0.134	-0.063
ΔRMSSD	**-0.539***	**-0.498***	-0.037	-0.017	-0.017	0.082
ΔHF	-0.330	-0.064	-0.113	0.081	0.115	0.200
ΔRatio	0.324	0.183	0.169	-0.063	0.059	-0.055
ΔPWV	0.109	0.231	-0.015	0.131	-0.109	0.317
ΔSBP	0.260	-0.065	0.127	0.093	-0.443	-0.251

Values are standard beta controlled for maturation and body fat%. *P<0.05. VPA: vigorous physical activity. MPA: moderate physical activity. CRF: cardiorespiratory fitness. TAG: triacylglycerol. HDL: high-density lipoprotein. SBP: systolic blood pressure. PWV: pulse wave velocity. RMSSD: square root of the mean of the sum of the squares of differences between adjacent RR intervals. HF: high frequency.

Standard regression coefficients for the delta changes pre- and post-HFM are presented in the Table 6.4. Fasting [TAG] and [HDL] were not associated with the ∆[TAG], ∆SPB, ∆RMSSD, and ∆PWV in both sexes (all *P*>0.05). In girls, both allometric and ratio scaled CRF were significantly and negatively associated with ∆PWV (*P*=0.018 and *P*=0.023, respectively) and in boys, MPA (*P*=0.005) and VPA (*P*=0.009) were negatively associated with ∆RMSSD.

6.5 Discussion

This study measured novel and traditional CVD risk factors in the fasted and postprandial state and investigated the associations of these markers with PA and CRF levels in adolescent boys and girls. The key findings were: 1) Fasting [TAG] was positively associated with postprandial [TAG]; 2) No significant associations were observed for MPA, VPA and CRF to postprandial [TAG], RMSSD, PWV and BP; 3) MPA and VPA were inversely related to the delta change in RMSSD after the HFM in boys but not girls; and 4) CRF was inversely associated to the delta changes in PWV after the HFM in girls but not boys.

In adolescents, meals varying in fat and carbohydrate content are normally used to measure postprandial outcomes. It is important to standardise the meal because fat content, as well as carbohydrate load, is implicated in vascular and [TAG] changes after the meal (Peddie et al., 2012). A lipid load between 30 g and 50 g of fat leads to a dose dependent change in the rise in [TAG], with doses higher than 80 g causing an exacerbated [TAG] response (Peddie et al., 2012). In the present study, the HFM was delivered with a milk-shake known to provide lipid content higher than 50 g per participant (Bond et al., 2014, Bond et al., 2015d), which is known to increase [TAG] and decrease vascular function and HRV (Bond et al., 2015a, Bond et al., 2014, Bond

et al., 2015d). However, the HFM did not change peripheral PWV in the current study, which is in accordance with postprandial findings in adults and indicates that central PWV might be more sensitive to the HFM challenge (Baynard et al., 2009). Alternatively, PWV might not be altered in the postprandial state, and other local measures of stiffness are desirable (e.g. carotid to femoral PWV) (Murray et al., 2015).

In the present investigation, no significant differences were found between sexes in the postprandial [TAG] concentration, despite girls presenting higher fasted [TAG], as typically observed in the literature (Ekelund et al., 2012). While adult women present different postprandial responses compared to men (Herd et al., 2000), the present investigation corroborates youth data showing no differences between boys and girls for postprandial [TAG] (Bond et al., 2014). In children, a combination of a higher fasted [TAG] and lower [HDL] has been shown to cause a pronounced postprandial [TAG] excursion, even when the confounding effects of body composition are controlled for (Couch et al., 2000). In the present study, none of the participants presented elevated [TAG] alongside lower [HDL], and the main predictor of the rise in [TAG] was the fasted [TAG] (Table 6.2). This is in accordance with the literature (Couch et al., 2000), and alludes to a possible mechanism by which PA and/or CRF may alter postprandial [TAG], via reductions in fasted [TAG] (Ekelund et al., 2012, Ekelund et al., 2007).

However, in the present study PA and CRF were not significantly associated with postprandial outcomes. This is contrary to the well documented reduction in boys and girls postprandial [TAG] when exercise is performed 12 – 16 h before the HFM (Tolfrey et al., 2014). Our results are therefore in accordance with investigations in adults showing that when participants refrain from an exercise bout ~ 60h preceding the HFM, no protective effects of the training status on the responses to the meal is

observed (Tsetsonis et al., 1997, Peddie et al., 2012, Herd et al., 2000). Collectively, these results show that habitual PA has no significant associations with postprandial [TAG] when the acute effects of the activity are eliminated by asking the participants to refrain from moderate to vigorous activities. To the contrary, in elderly participants PA is inversely related with postprandial outcomes even when participants refrain from exercise in the days preceding the HFM (Miyashita et al., 2011). These results indicate that the mechanism that PA alters postprandial responses varies between youth and the elderly.

In addition to postprandial lipemia, we investigated the associations of PA and CRF with postprandial novel risk factors using measures of HRV, BP and PWV. Despite no significant alterations in PWV after the HFM, in girls the delta changes in PWV were inversely related to CRF. While CRF might have a protective effect on PWV after the meal, the lack of significant changes in PWV post-HFM indicates that the inverse association observed between CRF and changes in PWV may have minimum physiological or clinical relevance. Our present study and other investigations indicate that the commonly observed exercise protection against the postprandial decreases in arterial endothelial function and stiffness (Mc Clean et al., 2007, Bond et al., 2015b) are likely mediated by the acute effects of the exercise and not habitual PA or CRF.

Similarly, the lack of association between PA, CRF and changes in HRV pre- and post-HFM is in accordance with training data (Bond et al., 2015a), and shows that the meal challenge will lower autonomic activity regardless of amount of PA or CRF. In boys, however, both MPA and VPA were inversely related to changes in HRV, indicating a higher delta change for the most active adolescents. This finding corroborates with training data showing that the increases in HRV after exercise training led to larger

delta changes in HRV after the ingestion of a HFM compared to pre-training values (Bond et al., 2015a). Therefore, decreases in the cardiac parasympathetic control are necessary in the postprandial state, as previously shown in adults (Lu et al., 1999). The dense HFM caloric load (80 $kJ \cdot kg^{-1}$) delivered in the present study might explain the reductions in HRV, as increases in heart rate after the meal are positively associated with the energy content of the meal (Lu et al., 1999). This indicates that HRV will decrease to a certain point required to achieve the hemodynamic changes needed in the digestive processes and transport of the lipid load.

A limitation of the present study was the lack of control of the activities that participants performed in the two days before the ingestion of the HFM. Although all participants reported they refrained from exercise in the 48h preceding data collection, it is possible that some participants performed bouts of PA physical activity. However, this is unlikely to influence the results, because when adolescents performed 45 $min \cdot day^{-1}$ in the 48h preceding a similar HFM protocol (Bond et al., 2015d) no differences were observed in TAG excursion compared to the present study. Likewise, we were not able to rigidly control the two hours postprandial period when participants returned to normal school-based lessons, although only light intensity PA was performed (i.e. walking from and to the lessons). Whilst this increased the external validity of the investigation compared to laboratorial controlled settings, this may have also introduced errors in the analyses, due the lack of control of the activities during the break, a period when sedentary activities are usually performed. In addition, the effects of the HFM on traditional and novel CVD risk factors were measured two hours after the meal. The time of the post-HFM measurement was decided because in healthy adolescents (Sahade et al., 2013, Bond et al., 2014), [TAG] has been shown to peak two hours after the ingestion of a HFM. Indeed, the postprandial hypertriglyceridemia

observed in the present study (1.25 mmol·L^{-1}) was similar to measures obtained 2 – 4 h after the ingestion of a similar meal in other laboratory based investigations (Bond et al., 2015d, Bond et al., 2014). The measurement of PWV is not able to discriminate endothelial function and the measurement of flow mediated dilation would provide greater mechanistic information but requires equipment not ideally located within a school setting. Additionally, the participants were recruited using a convenience sample and therefore sample bias might be present. Finally, the cross-sectional design limits causality between the observed associations.

6.6 Conclusions

Contrary to the hypothesis that habitual PA or CFR would be associated with postprandial outcomes, neither PA nor CRF were significantly related to HRV, PWV, and [TAG] after a HFM. The main predictor of postprandial [TAG] in youth is fasted [TAG]. Strategies to lower fasted [TAG] may be desirable to reduce postprandial [TAG] excursions. Finally, in boys a higher delta changes in HRV pre and post meal were negatively associated with MPA and VPA levels which might reflect the higher pre-meal HRV of active boys.

Chapter 7: Reliability of Autonomic and Vascular Components of Baroreflex Sensitivity in Adolescents

7.1 Abstract

This Chapter aimed to investigate between- and within-day reliability of BRS and its autonomic and vascular determinants in adolescents. Thirteen male adolescents (14.1 ±0.5 years) participated in this study. For between-day reliability, participants completed four experimental visits separated by a minimum of 48-h. For within-day reliability, participants repeated BRS assessments three times in the morning with one hour between the measures. BRS was evaluated using the cross-spectral gain (LFgain) between blood pressure and heart rate interval. BRS was further divided into: 1) vascular component using AC; and 2) autonomic component measured as LFgain divided by AC (LFgain/AC). LFgain, AC, and LFgain/AC presented between-day CV of 20, 17, and 20%, respectively. Similarly, variables associated with BP such as PP (14.4%), $\dot{Q}$ (11.6%), MAP (7.4%), HR (5.7%) and TPR (14.4%) presented CVs ranging from 6 to 15%. Within-day reliability was poorer compared to between-day for LFgain (25%), AC (27%), and LFgain/AC (34%), as well as all hemodynamic variables (CVs from 11-22%, except heart rate with presented CV of 6%). The present study indicates suitable between- and within-reliability of BRS and its autonomic and vascular determinants, as well as hemodynamic variables associated with BRS, in adolescents.

7.2 Introduction

Atherosclerosis starts in childhood and traditional CVD risk factors in this age group are associated with atherosclerotic progression in adolescence (Berenson et al., 1998) and adulthood (Raitakari et al., 2003). Improvements in traditional CVD risk factors following an intervention such as exercise, however, only partially explains CVD risk reduction with the existence of ~40% risk factor gap (Joyner and Green, 2009). The American Heart Association recognizes that exploring novel CVD risk factors in youth will further contribute to the pathophysiological understanding and CVD management in this population (Balagopal et al., 2011). As improvements in autonomic and vascular functions were found following an exercise intervention with no changes in traditional CVD risk factors in adolescents (Bond et al., 2015a), this highlights the importance of these systems as a target for interventions designed to modify CVD risk.

The interplay between the vascular and autonomic systems can be assessed by measuring BRS. Baroreflex sensitivity is the ability to regulate BP and can be non-invasively assessed using spectral methods (Persson et al., 2001). Specifically, oscillations in BP at a low frequency (0.05 – 0.15 Hz) are known to cause oscillations in inter-beat intervals (i.e. RR intervals) in the same frequency (Robbe et al., 1987). In this scenario, BRS is the gain of the cross-spectrum (LFgain) between BP and RR intervals expressed in $ms \cdot mmHg^{-1}$. Using CCA ultrasound images, BRS gain can be further divided into its vascular and autonomic components (Taylor et al., 2014, Tzeng, 2012). The underlying theory is that carotid distensibility is a surrogate of arterial wall stretching and baroreceptors stimuli (Hunt et al., 2001a, Bonyhay et al., 1996). It is then possible to quantify and express changes in CCA diameter per unit of pressure (i.e. vascular determinant in $\mu m \cdot mmHg^{-1}$), and changes in RR per unit of CCA diameter (i.e. autonomic determinant in $ms \cdot \mu m^{-1}$).

Separating the determinants of BRS can provide non-invasive mechanistic insight of physiological changes in the vascular and autonomic systems in children and adolescents. For instance, it has been suggested that throughout adolescence, the LFgain is maintained via improvements in the autonomic branch (Lenard et al., 2004). While this study provided valuable insights on the maturation of vascular and autonomic systems, there is a dearth of information about test-retest reliability of BRS and its autonomic and vascular determinants. This lack of information is problematic, as reliability is necessary in informing sample size calculations and in the interpretation of results of interventions designed to modify CVD risk. In children, LFgain has been shown to have substantial absolute (i.e. CV <20%) and relative (i.e. intraclass coefficient of correlation (ICC) between 0.6 – 0.8) between-day reliability (Dietrich et al., 2010). Less is known about within-day reliability, with one study including participants with a large age range (7 – 27 years old) showing a CV of 21.1% (Rudiger and Bald, 2001). However, mixing adults and children in the same sample can limit the findings due to the known differences in BRS components between the groups (Lenard et al., 2004). Additionally, no study has investigated the relative and absolute reliability of the autonomic and vascular BRS components in youth.

The aim of this study was to assess between- and within-day reliability of BRS and its autonomic and vascular determinants in adolescents. In addition, as BRS ultimately regulates BP via changes in cardiac output ($\dot{Q}$; the product of HR and stroke volume (SV)), MAPand total peripheral resistance (TPR), the within- and between-day reliability of these hemodynamic outcomes will also be investigated.

7.3 Methods

7.3.1 Participants

Thirteen male adolescents (14.0±0.5 years old) volunteered to take part in this study Participants, with assistance from their parents/guardians, completed a health questionnaire before participation and were free of conditions, such as asthma, congenital heart disease, hypertension, amongst others that could alter autonomic and vascular functions. All procedures conducted in the present investigation were approved by institutional Ethics Committee and assent and consent forms were obtained from adolescents and their parents/guardians, respectively. Two weeks before starting the experimental visits, participants were then familiarized to the BRS protocol. In this same visit, participants had their stature, body mass, skinfolds (to estimate body composition) and maximum oxygen uptake ($\dot{V}O_2max$) measured. $\dot{V}O_2max$ was obtained and verified breath-by-breath (Cortex Metalyzer III B, Leipzig, Germany) using a combined incremental-supramaximal treadmill protocol (Barker et al., 2014). Pubertal status for the sample was determined by self-assessment of secondary sexual characteristics (Morris and Udry, 1980).

7.3.2 Experimental design

To establish between-day reliability, participants completed four experimental visits separated by a minimum of 48h, and with no longer than two weeks in between. For each visit, participants were driven to the laboratory following 12h overnight fast, and all measurements were performed between 8 – 9 am. For within-day reliability, in one of the four visits participants were randomly asked to complete the BRS protocol (see Section 3.8.3) three times with a one-hour interval in each measurement. Participants were instructed to avoid extraneous exercise and to wear accelerometers (GENEActiv, UK) in the 48-h preceding testing. Accelerometer data were treated as

described in Section 3.6. Additionally, in the 48-h preceding Visits 2 – 4 participants were instructed under parental supervision to keep a similar diet to the 48-h preceding Visit-1.

7.3.3 Baroreflex sensitivity protocol

A finger pressure device (Finometer PRO, Netherlands) and a three-led ECG were fitted and the BRS protocol started after a 10-min supine rest in a temperature (21 – 24°C) and light controlled room. The BRS protocol is fully described in Section 3.8.3 and procedures of standardisation were followed by the participants as described in Section 3.8.1.Briefly, the BRS protocol consisted of: 1) measurement of brachial BP to calibrate Finometer BP assessment (Guelen et al., 2008), which has been validated in paediatric groups (Tanaka et al., 1994); 2) after BP calibration, CCA ultrasound images were recorded for 15 cardiac cycles; and 3) following CCA images, participants were instructed to pace breathing frequency at 12 cycles per min for five min. This breathing frequency is known to increase autonomic modulation of heart rate in adolescents (Williams and Lopes, 2002), and also shifts breathing frequency above the LF range, as suggested when examining spontaneous BRS (Bothova et al., 2010, Tzeng et al., 2009)..

7.3.4 Baroreflex sensitivity analysis

Baroreflex sensitivity was obtained using the cross-spectral transfer function described in Section 3.8.3 using previous validated methods (Lenard et al., 2004, Chirico et al., 2015, Robbe et al., 1987, Saul et al., 1991).

7.3.5 Vascular and autonomic determinants

CCA images were recorded ~ 2 cm distal from the carotid bulb using a high-resolution (~ 13 MHz) linear array transducer (Apogee, 1000, SIUI, China) as described in

Section 3.8.4. The vascular components of BRS were determined using equations 3.10 and 3.11 according to published guidelines (Laurent et al., 2006).

During the BRS protocol, beat-to-beat $\dot{Q}$ was obtained from the Finometer and SV was calculated as $\dot{Q}$ divided by the HR from the ECG trace. Total peripheral resistance was calculated as MAP divided by $\dot{Q}$. Hemodynamic variables ($\dot{Q}$, HR, SV, MAP and TPR) were averaged over the same 15 cardiac cycles used for analysis of the CCA outcomes and saved for later analysis.

The autonomic and vascular determinants of BRS were determined according to previous study (Lenard et al., 2004). Briefly, AC was considered as the vascular component of the BRS and expressed as $\mu m \cdot mmHg^{-1}$. To calculate the autonomic determinant, LFgain was divided by the AC and expressed as LFgain/AC in $ms \cdot \mu m^{-1}$.

7.3.6 Statistical analyses

Data are presented as mean and standard deviation unless otherwise stated. Differences between MVPA and food diary outcomes were compared using ANOVA with repeated measures. Sphericity was tested using Mauchly's test and when violated Greenhouse-Geisser correction was applied. SPSS version 22 was used for analyses, and an alpha level of 0.05 was considered significant.

Following recommendations by Hopkins (2000), between- and within-day reliability were calculated as: 1) systematic error as changes in mean and tested using repeated measures ANOVA with least significance differences post hoc comparisons; 2) absolute reliability assessed as random error calculated as the within-subject variation expressed in absolute (typical error (TE)) and normalised as CV; and 3) relative reliability calculated as test-retest correlation using Pearson's correlation. Data were

log transformed and analysed using freely available spreadsheets (http://sportsci.org/resource/stats/).

7.4 Results

7.4.1 Between-day reliability

Participant characteristics are presented in Table 7.1. From the 13 initial participants, two were not included in the CCA analysis due to technical issues with the ultrasound, and another did not complete one of the visits, for reasons unrelated to the study. The final number of participants included was 10. For the BRS measures, in addition to the participant excluded for not completing the visit, another was excluded due to errors being >3% in the ECG data. The number of participants included in the BRS between-day reliability was therefore 11.

Table 7.1: Participants' characteristics.

	Between-day reliability		
	All (n=13)	CCA (n=10)	BRS (n=11)
Age (y)	14.0±0.5	14.1±0.3	14.1±0.4
Stature (cm)	162.2±10.5	163.6±10.8	162.4±10.9
Body Mass (kg)	46.6±13.2	52.1±14.2	49.9±14.1
Body Fat (%)	12±4.7	12.7±4.8	12±4.8
$\dot{V}O_2max$ (mL·kg·min^{-1})	50.1±5.2	52.1±3	50.1±5.3
Stage of maturation	2=3	2=2	2=3
	3=1	3=1	3=0
	4=8	4=6	4=7
	5=1	5=1	5=1
	Within-day reliability		
	All (n=13)	CCA (n=12)	BRS (n=12)
Age (y)	14.0±0.5	14±0.4	14±0.5
Stature (cm)	162.2±10.5	161.7±10.8	161.7±10.7
Body Mass (kg)	46.6±13.2	50.4±13.5	49.2±13.7
Body Fat (%)	12±4.7	12.5±4.6	11.7±4.7
$\dot{V}O_2max$ (mL·kg·min^{-1})	50.1±5.2	50.9±5.3	50.6±5.3
Stage of maturation	2=3	2=3	2=3
	3=1	3=1	3=0
	4=8	4=7	4=8
	5=1	5=1	5=1

CCA: Common carotid artery. BRS: Baroreflex sensitivity.

Physical activity and diet records are presented in Table 7.2. There were no differences for MVPA in the 48h preceding the experimental visits. For this analysis, however, just seven participants had repeated data in the four visits. Similarly, energy intake and the proportion of the energy derived from carbohydrate, lipid and protein were not different between visits (all P>0.05).

Table 7.2: Average physical activity and food consumption in the 48h preceding the experimental visits.

		Day 1	Day 2	Day 3	Day 4	*P*
n=7	MVPA (min·day^{-1})	116.1±56.1	99.8±51.3	126.1±29.7	132.2±75.1	0.46
n=12	Total kcal (kcal·day^{-1})	2025±177	2150±178	1944±134	1975±114	0.68
n =12	Carbohydrate (%)	51±2	50±2	50±2	51±2	0.72
n =12	Lipids (%)	32±2	34±2	32±2	31±1	0.34
n =12	Protein (%)	16±1	15±1	17±1	17±1	0.67

MVPA: moderate-to-vigorous physical activity

Between-day reliability data are described in Table 7.3. There was no significant mean bias between the visits for any of the variables (all $P>0.05$). All variables had an absolute reliability between 2 – 20%, with the most reliable measurement being vessel diameter (DLD = 2.4% and SLD = 2.3%). All variables presented a relative reliability ranging between r=0.50 and $r=0.91$, except for PP (r=0.37).

7.4.2 Within-day reliability

Participant characteristics are presented in Table 7.1. From the 13 participants, one participant was excluded from the CCA analysis due to technical issues with the ultrasound. One participant excluded from BRS due to errors >3% in the ECG trace. The number of participants included in the within-day reliability was 12 (Table 7.1).

Table 7.3: Between-day reliability of BRS gain and its autonomic and vascular determinants.

		Day 1	Day 2	Day 3	Day 4	*P* value ANOVA	*r*	CV	TE
n=10	DLD (µm)	5288.0±278.5	5260.0±300.4	5190.0±313.6	5262.0±420.8	0.39	0.91	2.4	127.0
n = 10	SLD (µm)	6133.0±308.4	6129.0±337.5	6087.0±320.3	6115.0±448.4	0.78	0.90	2.3	143.5
n = 10	Delta diameter (µm)	845.0±126.8	869.0±128.3	897.0±144.6	853.0±154.3	0.32	0.80	7.7	63.0
n = 10	Diastolic CSA (mm)	22.0±2.3	21.8±2.5	21.2±2.6	21.9±3.4	0.40	0.91	4.9	1.1
n = 10	Systolic CSA (mm)	29.6±3.0	29.6±3.3	29.2±3.1	29.5±4.5	0.78	0.89	4.7	1.4
n = 10	Delta CSA (mm)	7.6±1.3	7.8±1.4	7.9±1.4	7.6±1.6	0.62	0.81	8.7	0.64
n = 10	Arterial Strain (%)	16.0±2.6	16.6±2.5	17.4±3.2	16.3±3.4	0.17	0.84	8.0	1.3
n = 10	AC ($\mu m \cdot mmHg^{-1}$)	18.9±4.5	20.0±3.6	19.3±3.9	19.7±5.0	0.85	0.50	16.8	3.1
n = 10	AD (10^{-3}/mmHg)	7.7±1.8	8.3±1.5	8.1±1.9	8.2±2.7	0.80	0.60	17.2	1.3
n = 11	LFgain ($ms \cdot mmHg^{-1}$)	23.6±5.7	21.4±5.9	21.0±5.4	21.1±6.8	0.34	0.63	20.4	3.9
n = 9	LFgain/AC ($ms \cdot \mu m^{-1}$)	1.32±0.49	1.13±0.35	0.96±0.52	1.21±0.45	0.11	0.87	19.8	0.2
n = 11	HR ($beats \cdot min^{-1}$)	66±9	66±5	66±8	67±6	0.84	0.83	5.7	4
n = 11	$\dot{Q}$ ($L \cdot min^{-1}$)	3.0±0.8	3.2±0.7	3.0±0.6	3.0±0.7	0.41	0.82	11.6	0.3
n = 11	SV (mL)	46.6±13.8	48.1±11.6	45.3±9.0	44.8±11.8	0.27	0.87	10.2	4.2
n = 11	PP (mmHg)	46.0±8.2	43.9±6.3	47.0±4.0	44.2±7.3	0.45	0.37	14.7	5.9
n = 11	MAP (mmHg)	78.9±5.4	79.6±6.6	80.8±9.9	77.5±7.8	0.55	0.50	7.4	5.6
n = 11	TPR (units)	27.8±7.4	26.3±5.8	28.0±5.1	26.9±4.4	0.64	0.63	14.4	3.6

LDD: lumen diastolic diameter; LSD: lumen systolic diameter; PP: pulse pressure; AC: arterial compliance; AD: arterial distensibility.

Within-day reliability statistics are presented in Table 7.4. Systematic error was identified for DLD (*P*=0.02) and LSD (*P*=0.04) at 120min post compared to baseline. Similarly, LFgain was higher at 120mincompared to 60min (*P*=0.03). All variables had an absolute reliability between 2 – 34%, with the most reliable measures being vessel diameters (DLD = 2.3% and SLD = 2.2%) and HR (CV = 6%). All variables presented relative reliability ranging between r=0.50 and r=0.89, except for MAP (r=0.42).

7.5 Discussion

This is the first study to investigate between- and within-day reliability of BRS assessment and its autonomic and vascular determinants, as well as the reliability of hemodynamic variables associated with BRS, in adolescents. The key findings of the present investigation were: 1) BRS and its autonomic and vascular determinants presented between-day CVs <20%; 2) vessel diameter presented the best between- and within-day reliability; 3) within-day BRS reliability was poorer compared to between-days; and 4) hemodynamic variables presented between- and within-day CVs <20%.

Table 7.4: Within-day reliability of BRS gain and its autonomic and vascular determinants.

		Baseline	60 min	120 min	*P* value ANOVA	*r*	CV	TE
n=12	DLD (µm)	5220.0±329.9	5269.2±332.6	5360.8±373.0*	**0.038**	0.89	2.3	126.7
n = 12	SLD (µm)	6086.7±340.2	6135.0±346.1	6235.0±387.0*	**0.051**	0.87	2.2	134.0
n = 12	Delta diameter (µm)	866.7±126.1	865.8±125.8	874.2±138.1	0.93	0.79	7.3	60.3
n = 12	Diastolic CSA (mm)	21.5±2.7	21.9±2.8	22.7±3.2*	**0.048**	0.89	4.6	1.12
n = 12	Systolic CSA (mm)	29.2±3.3	29.7±3.3	30.7±3.4	0.06	0.88	4.4	1.39
n = 12	Delta CSA (mm)	7.7±1.3	7.8±1.3	8.0±1.4	0.56	0.80	8.1	0.61
n = 12	Arterial Strain (%)	16.7±2.8	16.5±2.7	16.4±3.0	0.85	0.82	7.9	1.2
n = 12	AC ($\mu m \cdot mmHg^{-1}$)	20.0±3.7	20.8±9.5	19.9±4.4	0.82	0.57	25.4	6.0
n = 12	AD (10^{-3}/mmHg)	8.3±1.4	8.5±3.7	8.1±1.8	0.77	0.45	26.1	2.5
n = 12	LFgain ($ms \cdot mmHg^{-1}$)	21.7±5.8	20.3±7.9	24.4±8.2**	**0.051**	0.74	25.1	4.1
n = 11	LFgain/AC ($ms \cdot \mu m^{-1}$)	1.18±0.36	1.26±0.72	1.28±0.53	0.67	0.81	31.4	0.57
n = 12	HR ($beats \cdot min^{-1}$)	66±5	65±8	63±6	0.11	0.79	6.0	4
n = 12	$\dot{Q}$ ($L \cdot min^{-1}$)	2.9±0.8	2.8±0.8	2.7±0.6	0.56	0.67	19.2	0.4
n = 12	SV (mL)	44.1±13.6	43.4±14.8	44.5±12.3	0.90	0.77	17.7	5.8
n = 12	PP (mmHg)	44.4±9.0	46.4±14.0	44.9±9.3	0.77	0.63	22.0	7.4
n = 12	MAP (mmHg)	78.2±6.7	80.1±7.1	78.8±6.8	0.43	0.83	4.4	3.37
n = 12	TPR (units)	28.9±7.5	31.2±8.9	29.6±5.4	0.61	0.53	18.8	6.05

LDD: lumen diastolic diameter; LSD: lumen systolic diameter; PP: pulse pressure; AC: arterial compliance; AD: arterial distensibility.

*P<0.05 compared to baseline. **P<0.05 compared to 60min.

7.5.1 Between-day reliability

No between-days systematic error was observed for BRS and its autonomic and vascular components. In the present study, participants completed a habituation to the protocol in the weeks before the start of the study. This may have precluded a possible learning effect and caused no systematic changes in the BRS and its autonomic and vascular determinants. The present investigation conducted in a sample of healthy adolescents, showed poorer reliability (20% CV and r=0.63) of the LFgain compared to adults (CV = 5.4% and ICC = 0.76) (Maestri et al., 2009, Reynolds et al., 2016). However, our reliability results are similar to that observed in 11 years old of a CV of 13.8% and ICC of 0.49 for the LFgain (Dietrich et al., 2010). This highlights the importance of population-specific studies investigating the reliability of BRS assessment.

The observed CVs <20% contain biological and technical variability which might be augmented if important sources of errors before and during BRS assessment are not controlled. For instance, aiming to decrease biological variability participants were asked to keep a similar diet and physical activity in the days preceding data collection, and report to the laboratory at the same time of the day following an overnight fast. This was done because prior physical activity and diet can alter autonomic and vascular functions (Al Haddad et al., 2009). Similarly, aiming to decrease technical errors, breathing frequency was kept outside LF range to increase reliability of BRS and autonomic modulation (Davies et al., 1999, Pinna et al., 2007), and participants were familiarized to this procedure before the experiment. Additionally, all data trace was free of >3% errors and all analysis performed by the same researches. The present study indicates that BRS assessed with LFgain presents acceptable between-

day reliability in adolescents; however, the above important factors before and during the measurements should be controlled or the error is likely to be larger.

The present investigation is the first to calculate the magnitude of systematic and random error in the measurements of the autonomic and vascular BRS determinants in adolescents. The measures of the vascular determinant used were AC and AD, as previously reported in this population (Lenard et al., 2004). AC and AD measures presented CVs of 16.8 and 17.2%, without any systematic error between visits (Table 6.3). The reliability observed for AC and AD measures reflect small between-day variation in vessel diameters, and the main source of errors in AC and AD calculation derived from PP measures. These results indicate that factors affecting PP should be minimised when designing studies to further improve reliability. For instance, due to hydrostatic pressure Finometer readings of PP at the finger level exacerbate the differences between systolic and diastolic pressure (Imholz et al., 1998). To minimize this, participants were asked to keep their hands at the heart level during BRS protocol. The autonomic determinant measured using LFgain/AC presented an absolute and relative reliability of 19.8% CV and r=0.87 and did not systematically change between-days. Despite being calculated with a series of other measurements, this is the first study to demonstrate that LFgain/AC is a robust index that can be reliably used to investigate autonomic determinant of BRS in adolescents.

7.5.2 Within-day reliability

Notably all parameters (except vessel size) presented poorer within-day compared to between-day reliability. LDD, LSD and LFgain presented systematic changes two hours after the initial measurement suggesting circadian changes are present. To our knowledge the current study is the first to report this observation in healthy

adolescents. This is in accordance with previous adult literature suggesting an increase in BRS and its autonomic and vascular determinants throughout day (Taylor et al., 2011). The mechanisms underlying circadian changes are beyond the scope of the present investigation, but might involve a heightened sympathetic tone and vascular constriction in the early morning compared to late morning (Panza et al., 1991). This might also explain the increased carotid diameter observed 120min post compared to baseline. Similarly, random errors were exacerbated in the within-day protocol for all measures with the BRS autonomic component presenting CV of 34% and r=0.80. This arises from a sum of factors, such as PP, AC, and LFgain, which were altered between the time assessments. These results highlight that a control group is essential when changes throughout day are investigated (i.e. the effects of exercise or diet intervention on acute BRS changes), and that time of the day should be strictly controlled in between-days protocols.

7.5.3 Reliability of hemodynamic outcomes

BRS assessment and interpretation can be influenced by a diversity of factors. Specifically, BRS is the ability to adjust MAP by triggering a series of mechanisms to modulate $\dot{Q}$ and TPR (Persson, 1996). Poor reliability of MAP, $\dot{Q}$ and TPR therefore would hamper BRS interpretation. In the present investigation MAP, $\dot{Q}$, TPR, SV and HR presented CVs <15% between-days and <21% within-days. The main sources of error in these measurements would be technical and biological variations between days which would affect the observed CV. As all variables (except HR) are determined from finger plethysmography, technical errors can derive from positioning of the cuff, cuff size, and movements during the calibration, as well as possible differences in finger temperature between-days (Imholz et al., 1998). In the current study, aiming to decrease technical errors cuff placement were performed by the same researcher,

with adequate cuff size and participants were thoroughly instructed to stay as quite as possible during BRS protocol and Finometer calibration. Additionally, room temperature was maintained in a narrow range between- and within-days.

7.5.4 Limitations

The present sample comprised only boys, and therefore studies involving girls are needed. There are considerable technical skills required to operate the ultrasound, as well as data processing, which might hamper the application of the BRS protocol. The autonomic gain calculated as the ratio between LFgain and AC although theoretically sound and previously used in this population (Lenard et al., 2004), has not been validated. One alternative would be the use of methods with infusion of vasoactive drugs to test the neural component, however such methods raise ethical concerns for use in a pediatric population. Similarly, CCA measures were used with no information about aorta distensibility (Klassen et al., 2016). Finally, we acknowledge that for AC and AD measurements it is desirable to assess PP at the carotid site, however, others have suggested that BP derived from Finometer is a valid measure of intra-arterial pressure (Guelen et al., 2008), and our present results are comparable to methods measuring PP at the carotid site (Lenard et al., 2004).

7.5.5 Practical applications

The current study provides practical information to aid interpretation of interventions, and in sample size calculation for future trials. Sample size can be calculated considering between-subject variation (i.e. pooled standard deviation) and the observed CV for each outcome. Applying the principle of Cohen's effect sizes of 0.2 (small), 0.5 (moderate), and 0.8 (large) (Cohen, 1977), and using Hopkins between and within variation formulas (available at

http://sportsci.org/resource/stats/ssdetermine.html#long), the number of participants needed to achieve statistical power of 0.80 at an alpha level of 0.05 in a randomized controlled trial investigating changes in LFgain with a control and an experimental group will be 423, 63, and 22 per group, respectively. For AC, the number of participants needed is 537, 80, and 29 and for LFgain/AC the number of participants needed will be 191, 27, and 9. Finally, the calculated sample sizes should be inflated by 20% considering possible data loss due to errors in the ECG and BP trace, as well as in images acquisition.

7.6 Conclusion

There was acceptable (i.e. CV<20%) between-day reliability of BRS and its autonomic and vascular determinants in male adolescents. Similarly, all components of the BP equation, namely MAP, $\dot{Q}$, HR, SV and TPR, presented adequate between-day reliability. CCA diameter was the most reliable variable in the present study and the main source of error in the arterial distensibility and compliance coefficients was PP. Within-day reliability was poorer compared to between-days for all BRS and hemodynamic measurements, possibly due to circadian rhythm. The present results will help future research for sample size calculation and clinical interpretation of findings of interventional studies. Our results also highlight that a control group is essential when changes throughout day are investigated due to the observed diurnal variation.

Chapter 8: Mechanisms of Blood Pressure Control Following Acute Exercise in Adolescents: Effects of Exercise Intensity on Hemodynamics and Baroreflex Sensitivity

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8.1 Abstract

This Chapter aimed to investigate the time course of changes in BRS and its vascular and autonomic components after different exercise intensities in adolescents. Thirteen male adolescents (age = 13.9±0.5 years) completed on separate days in a counterbalanced order: 1) HIIE: 8x1-min running at 90% of maximal aerobic speed with 75s of active recovery; 2) MIIE: 10 – 12 bouts of 1-min running at 90% of gas exchange threshold with 75s of active recovery; and 3) CON. Supine heart rate and blood pressure were monitored continuously at baseline, and 5- and 60-min following the conditions. A cross-spectral method (LFgain) was used to determine BRS gain. Arterial compliance was assessed as the BRS vascular component. LFgain divided by AC (LFgain/AC) was used as the autonomic component. LFgain decreased 5-min post the exercise bouts (HIIE: baseline = 24.4 ± 6.1 ms·mmHg^{-1}, 5-min post = 7.7 ± 4.9 ms·mmHg^{-1},*P*<0.001; MIIE: baseline = 21.0 ± 5.1 ms·mmHg^{-1}, 5-min post = 10.2 ± 4.7 ms·mmHg^{-1},*P*=0.002),but returned to baseline at 60-min post (22.4 ± 9.6 ms·mmHg^{-1} and 21.4 ± 7.1 ms·mmHg^{-1} for HIIE and MIIE at 60-min post, respectively). A time effect was observed for AC at 5-min post (*P*=0.048) without a time group interaction

(*P*=0.54), and returned to baseline at 60-min post. LFgain/AC decreased 5-min post exercise bouts (HIIE: baseline = 1.32 ± 0.49 ms·μm^{-1}, 5-min post = 0.31 ± 0.10 ms·μm^{-1}, *P* < 0.001; MIIE: baseline = 1.07 ± 0.40ms·μm^{-1}, 5-min post = 0.44 ± 0.22 ms·μm^{-1}, *P* = 0.004), but returned to baseline at 60-min post (1.16 ± 0.61 ms·μm^{-1} and 0.94 ± 0.30ms·μm^{-1} for HIIE and MIIE at 60-min post, respectively). Mean arterial pressure was lowered by both exercise intensities at 5-min post (HIIE: baseline = 79.6 ± 6.7 mmHg, 5-min post = 74.4 ± 6.0 mmHg, *P* < 0.001; MIIE: baseline = 79.4 ± 9.7 mmHg, 5-min post = 72.1 ± 7.3 mmHg, *P* = 0.004), but remained decreased at 60-min post following HIIE only (75.0 ± 6.1 mmHg and 82.1 ± 7.5 mmHg for HIIE and MIIE at 60-min post, respectively). Conclusion: BRS decreases 5-min following exercise in adolescents independent of exercise intensity and is mainly driven by a lowered autonomic response. At 60-min post exercise, the ability of BRS to regulate BP is restored after MIIE but not after HIIE, indicating exercise-intensity dependent mechanisms.

8.2 Introduction

Following exercise, arterial BP decreases below resting values characterizing a state of post-exercise hypotension (Halliwill et al., 2013). Understanding this phenomenon has implications for the health benefits of the exercise bout, as well as exercise induced syncope (Halliwill et al., 2013). Post-exercise hypotension is well characterized in the adult literature, and is mainly driven by a decreased total TPR despite increases in $\dot{Q}$ (Halliwill, 2001). The contributions of HR and SV to $\dot{Q}$ following exercise also appears to be dependent on exercise intensity, as SV has been shown to be decreased following supramaximal but not moderate-intensity cycling exercise which may be associated with an increased likelihood of syncope (Crisafulli et al.,

2004). limited data also suggests that post-exercise hypotension is present in children and may last up to 40minfollowing exercise (Rauber et al., 2014). Furthermore, post-exercise hypotension in youth is associated with a lowered BP response to stressful situations such as the cold stressor test and the ingestion of a HFM (Bond et al., 2014, Rauber et al., 2014), which may be clinically important as these stressful conditions have been positively associated with hypertension development in adults (Menkes et al., 1989, Uetani et al., 2012). Finally, although exercise intensity may alter the BP response following exercise in youth (Bond et al., 2014), the specific contribution of hemodynamic factors (e.g. SV, $\dot{Q}$ and TPR) have not been reported in this population. Therefore, studies are needed to investigate the duration of exercise-induced hypotension and to characterize the contributions of SV, $\dot{Q}$ and TPR in youth.

The mechanisms of post-exercise hypotension are well documented in adults, involving a diminished sympathetic influence on the vasculature, increases in myogenic vascular function, release of vasodilatory substances, and improved vasodilatory arterial function (Halliwill et al., 2013, Halliwill, 2001, Halliwill et al., 1996a, Persson, 1996). Collectively, these physiological changes indicate that critical mechanisms of BP control are influenced by the exercise bout. For example, BRS which is responsible for the beat-by-beat adjustments in BP fluctuations, may also be influenced by the exercise stimulus (Reynolds et al., 2017, Halliwill et al., 1996b, Niemela et al., 2008). However, because BRS is composed of a vascular (measured as changes in arterial diameter per changes in units of BP) and an autonomic (measured as changes in HR per units of vascular diameter) component (Kornet et al., 2002, Bonyhay et al., 1996, Taylor et al., 2014), their different contribution to the total BRS gain following exercise is still controversial. For example, in adults decreases in BRS following exercise have been attributed to either both components

(Willie et al., 2011), or to the vascular component (Studinger et al., 2003), with both components returning to baseline values 60minpost-exercise (Studinger et al., 2003, Willie et al., 2011). The difference between exercise modalities, intensities and duration makes comparisons between the results challenging, as these exercise characteristics are likely to impact BRS regulation (Halliwill et al., 2013).

To our knowledge, no study has investigated the role of BRS associated mechanisms on BP control following exercise in a youth population. The translation of adult findings to the youth population is questionable, as BRS has been shown to decrease with maturation in males (Chirico et al., 2015), or to be maintained across different age groups via improvements in the autonomic component (Lenard et al., 2004). Furthermore, the time-course of BRS changes post-exercise, and the effect of exercise characteristics (i.e. intensity) that contributes to BRS in the post-exercise period are unclear in youth. Whether there is an exercise intensity dependent effect on time-course of BRS responses in the post-exercise period, as well as the underpinning mechanisms are important questions considering the accentuated hypotension observed following HIIE compared to moderate-intensity exercise (Bond et al., 2014, Rauber et al., 2014), and the higher cardiorespiratory demands during HIIE in adolescents (Malik et al., 2017).

Therefore, the overall purpose of this study was to investigate the mechanisms underlying the recovery of BP following moderate and high-intensity exercise in healthy adolescents. Specifically, the time course of changes in BRS and its autonomic and vascular components, $\dot{Q}$, and TPR were investigated at five and 60min following moderate and high-intensity interval running in adolescents. Based on the described intensity-dependent effects of exercise on vascular and autonomic functions

in youth (Guilkey et al., 2015, Bond et al., 2015c), it was hypothesized that HIIE would augment the vascular and decrease the autonomic BRS determinants in the hour following the exercise bout, resulting in an overall decrease in the baroreflex gain compared to moderate-intensity interval exercise (MIIE).

8.3 Methods

8.3.1 Ethical approval

All adolescents who volunteered to take part in the present investigation and their parents/guardians provided signed assent and consent forms, respectively. All procedures performed in the present investigation were approved by the institutional ethics committee (Ref No: 160217/B/04). The study conformed to the standard set by the Declaration of Helsinki, except for a registration in a database.

8.3.2 Participants

Thirteen male adolescents volunteered to take part in this study. Pubertal status for the sample was: stage 2=3; stage 3=1; stage 4=8; and stage 5=1, as determined by self-assessment of secondary sexual (pubic hair) characteristics (Morris and Udry, 1980). Health questionnaires were completed before participation and all volunteers were free of conditions affecting cardiac autonomic and vascular systems, such as asthma, congenital heart disease, and hypertension.

8.3.3 Experimental overview

Participants completed four experimental visits with a minimum of three days apart and took no longer than four weeks to finish the study. The visits consisted of:

Visit 1: Participants were familiarized to the BRS protocol and treadmill running. Participants characteristics were obtained as described in Section 3.4. At the end of

Visit 1, participants received food diaries and accelerometers, which were used in the 48h preceding Visits 2 – 4.

Visits 2 – 4: Following an overnight fast, participants were transported to the laboratory and completed the BRS protocol as described in Section 3.8.3 between 8 – 9 am.

After the baseline measurement, participants performed, in a counterbalanced order, the following conditions on separate days: 1) HIIE; 2) MIIE; and 3) control (CON) which are described in details in Section 3.7.2. For CON, participants pursued sedentary activities whilst seated in the laboratory, such as computer and board games.

Participants repeated the BRS protocol, including 10min of rest, starting at 5 (5-min post) and 60 min (60-min post) following the conditions. These time points were chosen because in adults it has been reported that the time-course adjustments in the BRS and its vascular and autonomic determinants differ between 5 and 60 min following exercise (Willie et al., 2011, Studinger et al., 2003). However, the 10min rest preceding the BRS protocol resulted in the measurements starting at 15 and 75min post the conditions. In between the post-exercise measures, participants were seated in the upright position and pursued sedentary activities such as playing board and virtual games.

8.3.4 Baroreflex sensitivity analysis

Baroreflex sensitivity was obtained using the cross-spectral transfer function described in Section 3.8.3.

8.3.5 Vascular and autonomic determinants

Common carotid images were recorded as described in Section 3.8.4. The vascular components of BRS were determined using equations 3.10 and 3.11 according to published guidelines (Laurent et al., 2006).

The autonomic and vascular determinants of BRS were determined according to a previous study (Lenard et al., 2004). Briefly, AC was considered as the vascular component of the BRS and expressed as $\mu m \cdot mmHg^{-1}$. To calculate the autonomic determinant, LFgain was divided by the AC and expressed as LFgain/AC in $ms \cdot \mu m^{-1}$.

8.3.6 Hemodynamic and autonomic modulation

During CCA images acquisition, beat-by-beat $\dot{Q}$ was obtained and averaged for later analysis. In adults, $\dot{Q}$ obtained with the Finometer has been validated (Jansen et al., 2001). TPR was calculated as MAP divided by $\dot{Q}$. Heart rate variability was obtained as described in Section 3.8.2 as the area under the low (LF =0.04 – 0.15 Hz), and high frequency (HF =0.15 – 0.50 Hz) bands in absolute (ms^2), normalized (nu), and as the LF/HF ratio.

8.3.7 Physical activity and dietary intake

For standardisation PA and dietary intake were completed as described in Section 3.8.1.

8.3.8 Statistical analyses

Data are presented as mean and standard deviation unless otherwise stated. Mean differences between the physiological responses to the conditions were tested using paired Student's *t*-tests. Mean differences between MVPA and food diary outcomes between days were compared using ANOVA with repeated measures. The main effects of experimental condition and time, as well as their interaction were tested

using repeated measures ANOVA with two within-subject factors (time and condition), and three levels for each factor (time: Pre, 5-min and 60-min Post; and condition: HIIE, MIIE, and CON). When an interaction was observed, a series of repeated ANOVAs were performed to compare the effects of time and condition, followed by pairwise comparisons with least square differences. Mauchly's test was used to test sphericity and when violated Greenhouse-Geisser correction was used. SPSS v.22 was used for these analyses. The sample size was calculated based on detecting a large effect size (ES, Cohen's d > 0.8) for the change in BRS after exercise (Niemela et al., 2008), with an alpha of 0.05 and power of 0.8 (G-power). Finally, ES were calculated to interpret the magnitude of the pairwise comparisons as small <0.2, moderate >0.5, and large >0.8 (Cohen, 1977).

8.4 Results

The mean (SD) age of the group was 13.9±0.5 years, BF: 12.0±4.9 % and $\dot{V}O_2max$ 50.1±5.2 $mL \cdot kg^{-1} \cdot min^{-1}$. One participant was excluded from the BRS assessment due to errors in the ECG signal, and two from the CCA analysis due to technical issues with the ultrasound. For clarity, the final sample size for each analysis is described in the Figures 8.1 – 8.3 and Tables 8.1 and 8.2. In the 48h before the experimental visits, there were no significant mean differences in the amount of MVPA (P=0.91), energy intake (P=0.55) and macronutrient contribution (P>0.39) (see Table 8.1).

Table 8.1: Mean (SD) physical activity and food consumption in the 48 h preceding the experimental visits.

	HIIE	MIIE	CON	*P*
MVPA (min·day^{-1})(n=9)	117±49	117±32	111±45	0.91
Total (kcal·day^{-1}) (n=13)	1987±732	1912±458	2079±643	0.55
Carbohydrate (%) (n=13)	52±7	50±5	51±8	0.72
Lipids (%) (n=13)	32±1	32±1	33±1	0.55
Protein (%) (n=13)	16±3	17±3	16±4	0.39

MVPA: moderate-to-vigorous. HIIE: high-intensity interval exercise. MIIE: moderate-intensity interval exercise. CON: control.

By design, HIIE elicited significantly greater peak $\dot{V}O_2$ [% of max] (2.2±0.2 [89%] vs 1.6±0.1 [66%] L·min^{-1}; *P*<0.001), and average HR (154±3 [78%] vs 128±5 [64%] bpm; *P*<0.001) compared to MIIE. HIIE was significantly shorter in duration (21.8±0 vs 28.0±1.8 min; *P*<0.001), but the total distance was matched for both conditions (2,763±249 m).

8.4.1 Hemodynamic outcomes

Hemodynamic data are presented in Table 8.2 and Figure 8.1. There was a time by condition interaction for MAP (*P*<0.001). At baseline, no differences were observed between conditions (*P*=0.91). MAP decreased 5-min post HIIE (*P*=0.014; ES=0.81) and MIIE (*P*<0.001; ES=0.85) compared to baseline. Consequently, MAP 5-min post HIIE and MIIE were lower compared to CON (*P*=0.003, ES=0.87; and *P*<0.001, ES=1.12, respectively). At 60-min post, MAP remained decreased after HIIE compared to baseline (*P*=0.015; ES=0.71) and was lower compared to MIIE (*P*=0.001; ES=1.04) and CON (*P*=0.016; ES=0.62).

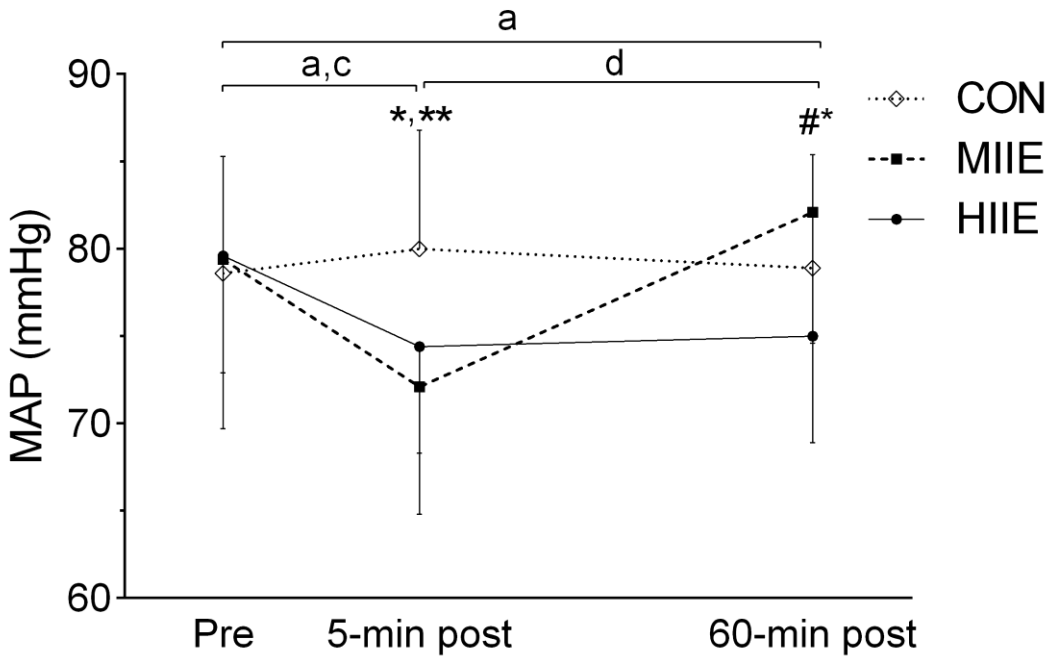

Figure 8.1: Baseline, 5-min and 60-min post the experimental conditions for mean arterial pressure (n = 13). *P< 0.05: HIIE vs CON. $^{\#}P$< 0.05: HIIE vs MIIE. **P< 0.05: MIIE vs CON. a: within HIIE compared to baseline. b: within HIIE compared to 5-min post. c: within MIIE compared to baseline. d: within MIIE compared to 5-min post. Error bars represent SD. For P values and effect sizes refer to text.

Table 8.2: Mean (SD) hemodynamic and autonomic modulation pre and post the experimental conditions.

	HIIE			MIIE			CON		
	Pre	5-min post	60-min post	Pre	5-min post	60-min post	Pre	5-min post	60-min post
HR (bpm) n=12	65±9	85±9$^{a*\#}$	67±8*	65±8	74±9^{c**}	66±9	66±5	65±8	63±6
SV (mL) n=12	45.7±13.5	39.5±10.6^{a}	44.0±10.7	44.4±9.1	36.9±7.2^{c}	42.5±9.8	46.4±12.6	45.8±13.0	44.5±12.3
$\dot{Q}$ (mL·min^{-1}) n=13	3.0±0.8	3.4±0.9$^{a*\#}$	3.0±0.7^{b}	2.9±0.6	2.7±0.6	2.8±0.7	3.0±0.8	2.8±0.8	2.7±0.6
TPR (units) n=13	28.3±7.0	23.3±6.0$^{a*\#}$	26.1±5.2^{b}	28.1±5.3	27.2±5.9	31.2±8.0^{d}	28.4±7.4	30.6±8.7	28.8±6.9
PP (mmHg) n=11	45.6±8.0	35.1±7.1^{a}	44.7±6.4	46.5±4.2	36.7±6.4^{c}	43.5±5.1	42.8±7.1	43.7±11.0	47.9±16.2
HFln (ms^2) n=12	8.4±1.0	6.6±1.5$^{a*\#}$	8.5±1.0^{b}	8.5±0.9	7.5±1.5	8.5±1.0	8.6±0.7	8.2±1.0	8.5±0.7
LFln (ms^2) n=12	7.4±1	5.9±1	7.3±1	7.2±1	6.6±1	7.2±1	7.6±1	7.3±1	7.5±1
HF (nu) n=12	71.1±15	63±16	74.9±9	78.3±8	68.2±19	76±10	71.2±12	69.8±10	69.7±12
LF (nu) n=12	28.7±14	36.2±16	24.9±9	21.5±8	31.6±19	23.4±10	28.6±12	30.1±10	30.0±13
LF/HF n=12	0.48±0.4	0.71±0.6	0.35±0.2	0.29±0.1	0.61±0.7	0.33±0.2	0.44±0.2	0.46±0.2	0.47±0.3

*P<0.05: HIIE vs CON. $^{\#}$P<0.05: HIIE vs MIIE. **P<0.05: MIIE vs CON. a: within HIIE compared to baseline. b: within HIIE compared to 5-min post. c: within MIIE compared to baseline. d: within MIIE compared to 5-min post. For P values and effect sizes refer to text.

There was a time by condition interaction for TPR (*P*=0.035). At baseline, no differences were observed between conditions (*P*=0.99). TPR decreased 5-min post HIIE (*P*=0.003; ES=0.77). Consequently, TPR 5-min post HIIE was lower compared to MIIE (*P*=0.009; ES=1.12) and CON (*P*=0.029; ES=0.98). At 60-min post, TPR returned to baseline after HIIE (*P*=0.09, ES=0.30). Conversely, TPR increased 60-min after MIIE compared to 5-min post (*P*=0.034; ES=0.56).

There was a condition by time interaction for $\dot{Q}$ (*P*=0.023). At baseline, no differences were observed between conditions (*P*=0.86). $\dot{Q}$ increased 5-min post HIIE (*P*=0.036; ES=0.48). Consequently, $\dot{Q}$ 5-min post HIIE was higher compared to MIIE (*P*=0.001; ES=0.87) and CON (*P*=0.035; ES=0.72). At 60-min post, $\dot{Q}$ returned to baseline after HIIE (*P*=0.91, ES=0.02). As there was no time by condition interaction for SV (*P*=0.08), the observed $\dot{Q}$ responses were mediated by a time by condition interaction for HR (*P*<0.001). At baseline, no differences were observed between conditions for HR (*P*=0.55). HR increased 5-min post HIIE (*P*<0.001; ES=2.19) and MIIE (*P*<0.001; ES=1.01). Consequently, HR 5-min post HIIE and MIIE were higher compared to CON (*P*<0.001, ES=2.43; and *P*=0.005, ES=1.07, respectively). HIIE elicited a greater increase in HR 5-min post compared to MIIE (*P*<0.001; ES=1.22). At 60-min post, HR returned to baseline after HIIE (*P*=0.21; ES=0.24) and MIIE (*P*=0.96; ES=0.01) but stayed elevated after HIIE compared to CON (*P*=0.005; ES=0.66). A significant main effect of time (*P*=0.014) was also present for SV, which was decreased at 5-min post compared to baseline (*P*=0.015; ES=1.57) but returned to baseline at 60-min post (*P*=0.20; ES=0.59).

8.4.2 Baroreflex sensitivity outcomes

BRS and its autonomic and vascular components are depicted in Figure 8.2. There was a time by condition interaction for LF_{gain} (P<0.001). At baseline, no differences were observed between conditions (P=0.09). LF_{gain} decreased 5-min post HIIE (P<0.001; ES=3.02) and MIIE (P=0.002; ES=2.18). Consequently, LF_{gain} 5-min post HIIE and MIIE were lower compared to CON (P=0.001, ES=1.91; and P=0.004, ES=1.56, respectively). At 60-min post, LF_{gain} returned to baseline after HIIE (P=0.99, ES=0.09) and MIIE (P=0.49, ES=0.24). LF_{gain} increased 60-min post CON compared to 5-min post (P=0.046, ES=0.50).

There was no time by condition interaction for AC (P=0.63). However, a significant main effect of time (P=0.012), but not condition (P=0.69), was present. At 5-min post, AC increased compared to baseline (P=0.016; ES=0.96) but returned to baseline at 60-min post (P=0.39; ES=0.20). These increases were mainly driven by the exercise conditions as observed by the large effect sizes for HIIE (ES=0.84), MIIE (ES=1.00) but not CON (ES=0.17).

There was a time by condition interaction for LFgain/AC (P<0.001). At baseline, no differences were observed between conditions (P=0.07).LF_{gain}/AC decreased 5-min post HIIE (P<0.001; ES=2.84) and MIIE (P=0.001; ES=2.00). Consequently, LF_{gain}/AC 5-min post HIIE and MIIE were lower compared to CON (P=0.004, ES=1.89; and P=0.008, ES=1.54, respectively). At 60-min post, LFgain/AC returned to baseline after HIIE (P=0.84, ES=0.28) and MIIE (P=0.41, ES=0.38).

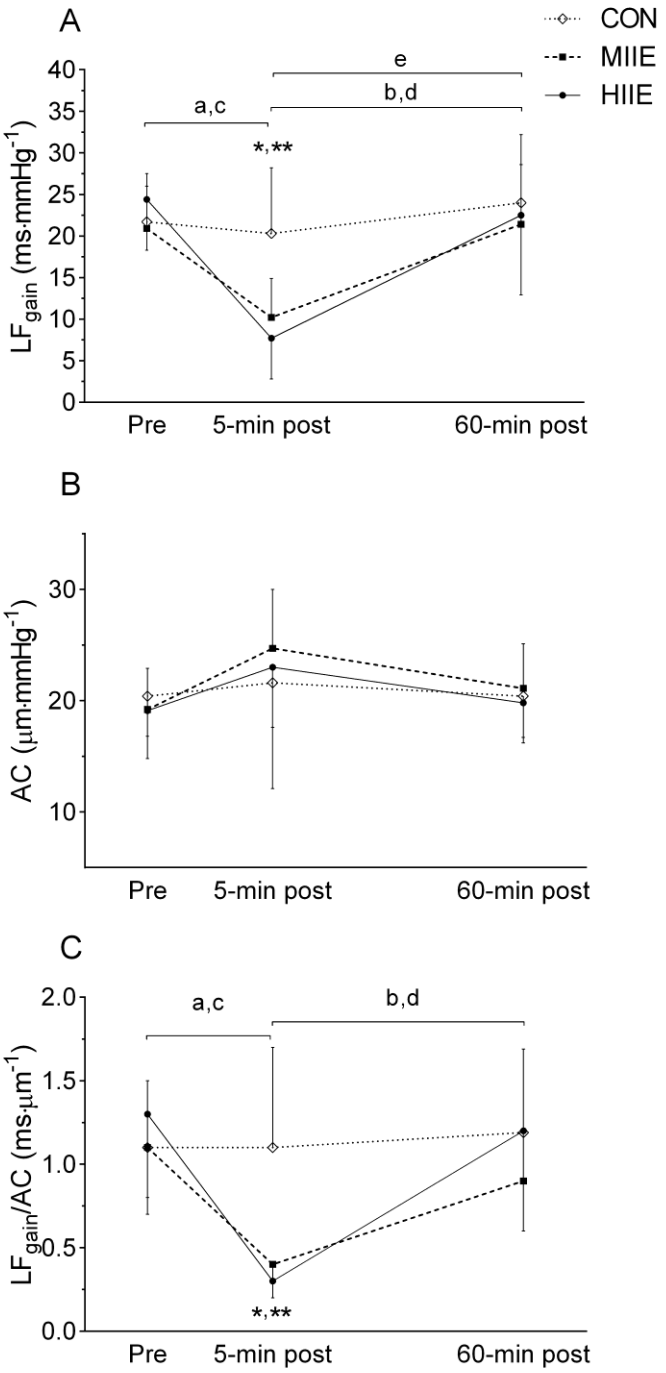

Figure 8.2: Baseline, 5-min and 60-min post the experimental conditions for A) BRS gain (n=12); B) BRS vascular component (n=11); and C) BRS autonomic component (n=10). *P< 0.05: HIIE vs CON. $^{\#}P$< 0.05: HIIE vs MIIE. **P< 0.05: MIIE vs CON. a: within HIIE compared to baseline. b: within HIIE compared to 5-min post. c: within MIIE compared to baseline. d: within MIIE compared to 5-min post. e: within CON compared to 5-min post. Error bars represent SD. For P values and effect sizes refer to text.

8.4.3 Carotid artery outcomes

Common carotid artery properties are depicted in Figure 8.3. There was a time by condition interaction for DLD (P=0.014). At baseline, no differences were observed between conditions (P=0.11). DLD decreased 5-min post HIIE (P=0.022; ES=0.53). Consequently, DLD 5-min post HIIE was smaller compared to MIIE (P=0.025; ES=0.30) and CON (P=0.043; ES=0.51). At 60-min post, DLD increased after HIIE compared to baseline (P=0.047; ES=0.39) and 5-min post (P<0.001; ES=0.86). Likewise, DLD increased 60-min after MIIE compared to baseline (P=0.047; ES=0.67).

There was a time by condition interaction for SLD (P=0.001). At baseline, no differences were observed between conditions (P=0.45). SLD decreased 5-min post HIIE (P=0.005; ES=0.70). Consequently, SLD at 5-min post HIIE was smaller compared to MIIE (P=0.002, ES=0.69) and CON (P=0.013, ES=0.74). At 60-min post, SLD increased for HIIE and MIIE compared to baseline (P=0.016, ES=0.47; and P<0.001, ES=0.74, respectively) and 5-min post (P<0.001, ES=1.13; P=0.001, ES=0.68, respectively).

There was no time by condition interaction for AD (P=0.47). However, a significant main effect of time (P=0.002), but not condition (P=0.50), was present. At 5-min post, AD increased compared to baseline (P=0.010; ES=0.82) but returned to baseline ate 60-min post (P=0.94; ES=0.20).

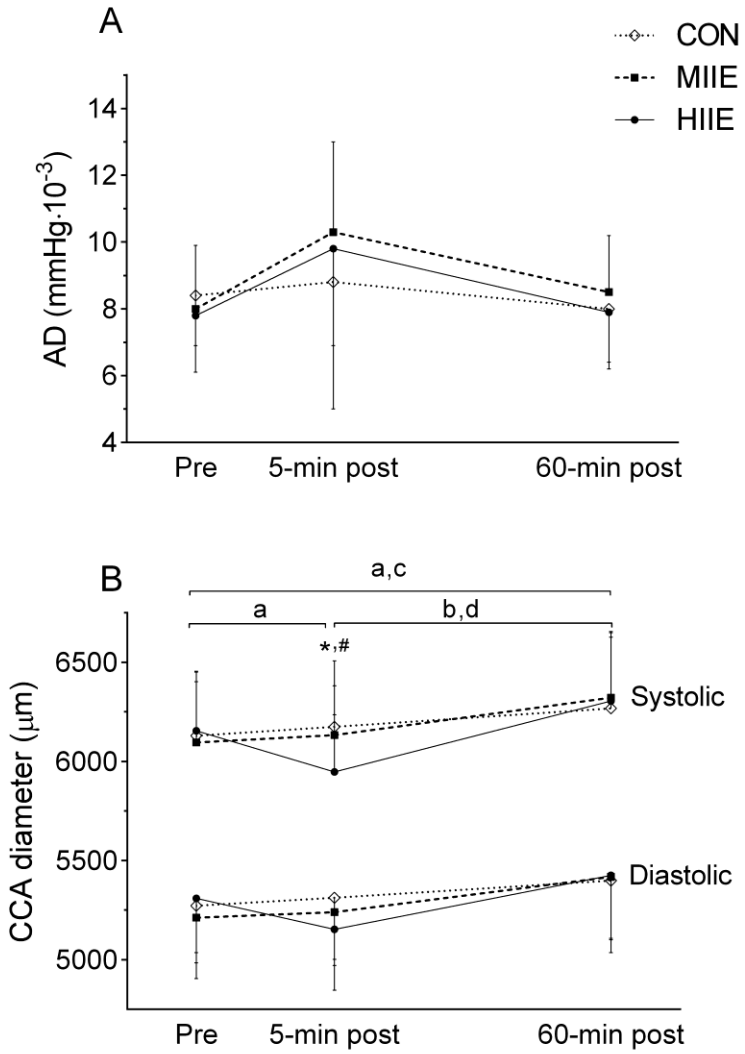

Figure 8.3: Baseline, 5-min and 60-min post the experimental conditions for A) Arterial distensibility (n = 11); (n = 11); B) common carotid artery diameter (n = 11). *P< 0.05: HIIE vs CON. $^{\#}P$ < 0.05: HIIE vs MIIE. a: within HIIE compared to baseline. b: within HIIE compared to 5-min post. c: within MIIE compared to baseline. d: within MIIE compared to 5-min post. Symbols and letters apply to both systolic and diastolic diameter. Error bars represent SD. For P values and effect sizes refer to text.

8.4.4 Heart rate variability

Heart rate variability is presented in Table 8.2. There was a time by condition interaction for HF (P=0.006). At baseline, no differences were observed between conditions (P=0.69). Compared to baseline, HF decreased 5-min post HIIE (P<0.001;

ES=1.43) and MIIE (*P*=0.005; ES=0.84). HIIE elicited a greater decrease in HF 5-min post compared to MIIE (*P*=0.019; ES=0.64). Likewise, HF 5-min post HIIE was lower compared to CON (*P*=0.003; ES=1.25). At 60-min post, HF returned to baseline after HIIE (*P*=0.54, ES=0.11) and MIIE (*P*=0.79, ES=0.03). No time, or time by condition interactions were observed for the other HRV indices (Table 8.2).

8.5 Discussion

This is the first study to investigate the time course of changes in BP following HIIE and MIIE and the associated mechanisms in a sample of healthy adolescents by focussing on BRS and its autonomic and vascular determinants. The novel findings of this study were: 1) MAP was lower 5-min post HIIE and MIIE due to a decreased TPR, as both HR and $\dot{Q}$ were elevated. At 60-min post, MAP remained lower after HIIE only, but $\dot{Q}$ and TPR had returned to baseline; 2) BRS gain was reduced 5-min following HIIE and MIIE, but was restored to baseline 60-min after exercise; 3) the reduction in BRS gain immediately post HIIE and MIIE was mainly driven by changes in the autonomic component, as LFgain/AC was reduced; 4) 5-min after HIIE, but not MIIE, the CCA was constricted but 60-min after exercise CCA was vasodilated for both exercise intensities.

8.5.1 5-min post responses

In the present study, MAP decreased 5-min post HIIE and MIIE. The observed decreases in MAP are in accordance with adult literature (Halliwill et al., 2013), however the observed mechanisms underpinning post-exercise hypotension were different between HIIE and MIIE. Specifically, MAP decreases observed 5-min following HIIE were caused by a lowered TPR despite the observed increased in $\dot{Q}$ (Halliwill, 2001), whereas 5-min post MIIE, a fall in SV (ES=0.90) and a consequent

maintained $\dot{Q}$ explained the decreased MAP. The divergent $\dot{Q}$ responses were caused by a heightened vagal withdrawal following HIIE, which is reflected in the observed reduced HF and a consequent elevated HR. The observed parasympathetic withdraw following HIIE corroborated with the well documented responses to exercise (Pecanha et al., 2017). The different mechanisms for post-exercise hypotension between the exercise intensities is a novel finding of the present investigation and is in accordance with a previous adult investigation showing a decreased pre-load due to a lowered venous return which was matched by an increased HR and $\dot{Q}$ following supramaximal, but not submaximal exercises (Crisafulli et al., 2004).

Our novel findings are the first to show an intensity independent decrease in LFgain in the first five min of recovery following MIIE and HIIE in adolescents. This observation is in accordance with evidence from adult literature showing decreases in BRS up to 60min following both aerobic and resistance exercises (Studinger et al., 2003, Niemela et al., 2008, Reynolds et al., 2017). The increased AC and AD in the first min of recovery following HIIE and MIIE indicates that the baroreceptors stimuli due to arterial stretching did not lead to adjustments in RR intervals. This is reinforced by the observed lowered LFgain/AC at 5-min following HIIE and MIIE indicating an autonomic dependent reduction in BRS gain. Our data is different to adult literature, which showed both autonomic and vascular changes contribute to the BRS decrements after exercise (Studinger et al., 2003, Willie et al., 2011). Some limitations arise from comparing the current findings to the adult literature. For example, differences between exercise mode (i.e. running vs cycling; interval vs continuous) and intensity (i.e. maximum to exhaustion (Studinger et al., 2003), and moderate (Willie et al., 2011)), as well as the methods used to measure BRS and its autonomic and vascular determinants. However, because adolescents present a higher arterial distensibility

and a less mature autonomic function (Lenard et al., 2004), the higher dependence on the autonomic determinant observed in the present investigation may represent developmental characteristics between children and adults. Future studies directly comparing adolescents and adults would be useful to support this claim.

The observed autonomic-dependent decrease in BRS following HIIE and MIIE may reflect a decreased autonomic central processing due to BRS resetting (Hart et al., 2010), and/or a lowered vagal influence on the heart due to local substances released by sympathetic stimulation (Herring and Paterson, 2009). However, this remains speculative as our present findings do not extend to the cross-talk between vagal and sympathetic systems. It also possible that the reduction in the BRS gain in the present study reflects a shift in the overall gain of the reflex curve (Schwartz et al., 2013), but no information about the operation point, saturation and range of baroreflex can be provided with the methods used in the present investigation. Regardless, our present study is the first to demonstrate that the reduction in the BRS gain is dependent on autonomic function in the 5-min following HIIE and MIIE in adolescents. As the recovery period has been suggested as part of the stimuli leading to training adaptation (Luttrell and Halliwill, 2015), the observed drop in the autonomic BRS determinant may provide mechanistic insights into the reported associations between moderate and vigorous intensity physical activity to cardiac autonomic function in adolescents (Oliveira et al., 2017). Future studies are needed to investigate the interdependence of the acute and chronic adaptations of the BRS and its vascular and autonomic components.

In our present investigation AC was increased 5-min post all conditions. Given that AC is calculated as delta diameter divided by PP, the observed increases in AC were

driven by decreases in PP, because delta diameter was kept similar to baseline at 5-min post. Our data is the first to demonstrate that in the 5-min following HIIE and MIIE, AC is improved in adolescents. Although our measurement of PP is limited for AC assessment (Steinback et al., 2005), the present findings of a lowered SV reinforces the observed increased in AC at 5-min post-exercise. For example, in contrast to the observed data in the present study, a smaller SV would lead to smaller AC and AD (Myers et al., 2002). These results indicate that despite the decreased MAP and PP, the difference in vessel distension is similar, and highlights that the drop in BRS observed after exercise was mainly driven by a lowered autonomic response to the baroreflex stimuli, rather than mechanical changes in the vascular BRS determinant.

In the present investigation, CCA was constricted 5-min post HIIE but not MIIE or CON. Our results extend previous adult findings (Studinger et al., 2003), by providing novel data showing that in adolescents the constriction of CCA is intensity-dependent and caused by decreases in both systolic and diastolic diameter. It has been previously stated that during high-intensity exercise the vasodilatory stimuli of shear stress (Atkinson et al., 2015) causes CCA to dilate (Studinger et al., 2003). After exercise cessation, the vasodilatory stimulus of shear stress diminishes, and the smooth muscle constricts stimulated by myogenic vessel activity (Studinger et al., 2003). The current observed constriction of the CCA following HIIE may therefore be explained by an accentuated sympathetic activity during the exercise bout, translated in the observed higher HR during HIIE, and superior hemodynamic stimulus on the CCA smooth musculature. This constriction following HIIE appears to be characteristic of the CCA, because a previous adolescent study have shown a vasodilated brachial artery immediately following HIIE (Bond et al., 2015c). Additionally, in the present study TPR was lowered reflecting dilated vasculature at the muscular site.

8.5.2 60-min post

This is the first study to demonstrate that at 60-min post HIIE, but not MIIE, MAP is decreased characterizing post-exercise hypotension in a sample of healthy adolescents. These results are in accordance with adult literature suggesting that the hypotensive effects of the exercise bout can last up to h following the exercise (Halliwill, 2001), as well as pediatric literature showing a strong hypotensive stimuli of HIIE after a HFM challenge (Bond et al., 2014). The contribution of TPR and $\dot{Q}$ to the observed hypotension following HIIE are not clear as no significant differences were observed for these variables at 60-min post. Scrutiny of the data, however, indicates differences at the individual level, with some participants presenting a lower $\dot{Q}$ mediated by a reduction in HR, while others presented a lower TPR. The observed intensity effects show that the control of BP via alterations in TPR is restored 60-min post MIIE due to the observed higher TPR values. Altogether, the present study provides original data showing a strong hypotensive stimulus of HIIE in healthy adolescents.

In the present study, 60-min post HIIE and MIIE the LFgain returned to baseline. Despite no comparative paediatric studies our data are in accordance with adult literature (Studinger et al., 2003), and indicate a rapid recovery of the cardiovascular BRS gain in healthy adolescents. The mechanisms of a restored LFgain were similar between exercise-intensities as both vascular (measured as AC and AD), and neural (measured as LFgain/AC) components also recovered to baseline 60-min following HIIE and MIIE. Furthermore, vagal modulation measured with the HF index also returned to baseline, showing that input from baroreceptors was translated into cardiac vagal modulation 60-min post-exercise.

Although LFgain is restored to baseline following HIIE and MIIE, MAP was still lower than baseline after HIIE but not after MIIE. Because BRS after exercise has been suggested not to contribute to post-exercise hypotension, but rather acts to restore BP to baseline values (Halliwill et al., 1996b, Kim et al., 2011), the present results indicate important differences between exercise intensities 60-min post-exercise. For instance, the return of MAP to baseline after MIIE indicates that the ability of BRS to adjust BP is restored following this intensity. In fact, our present observations of a higher TPR after MIIE shows a restored sympathetic influence on the vessels via BRS adjustments in TPR (Kim et al., 2011, Ogoh et al., 2002). On the contrary, 60-min post HIIE, the ability of BRS to restore BP to the baseline value is still blunted, and TPR and $\dot{Q}$ are not augmented. These data indicate that 60-min post HIIE, there may exist a decreased sympathetic influence on the vessels, possibly due to vasodilatory substances, such as nitric oxide activity (Bond et al., 2015c) and activation of histaminic receptors (Halliwill et al., 2013). Although the sympathetic branch of BRS was not measured in the present investigation, our results provide important mechanistic insights and highlight the potent role of exercise-intensity on the recovery of post-exercise hypotension in adolescents, which requires further study.

8.5.3 Practical implications

Although the fall in BP in the present investigation in healthy adolescents seems unremarkable compared to adult literature, which normally reports decreases of 8 – 9 mmHg depending on the pre-exercise BP (MacDonald, 2002), the observed five mmHg fall in the present study is similar to previous investigations involving nine year old children (Rauber et al., 2014). The practical importance of these findings is currently unknown and future investigations are needed. However, in the present study the fall in BP of normotensive adolescents may have a clinical importance if translated to hypertensive ones, as previously reported in adults (Kenney and Seals, 1993). Similarly, the hypotensive effects of the exercise can lead to a lowered BP response to stressful situations (e.g. cold pressor test and HFM) in youth (Bond et al., 2014, Rauber et al., 2014). Importantly, our study is the first study to characterise the mechanisms of post-exercise hypotension in youth, and the described vascular and autonomic adjustments may be linked to a better cardiovascular disease risk factor profile in this population (Roemmich et al., 2014). Finally, in youth aiming to avoid syncope following HIIE, an active recovery or a cool down period is encouraged to maintain an adequate pre-load and SV (Crisafulli et al., 2004).

8.5.4 Limitations

Several limitations should be considered when interpreting the present findings. Firstly, all hemodynamic measures were derived from finger plethysmography and have not been validated in the present population. Thus, the present estimates might be different than the real hemodynamic values. However, this limitation does not hamper our interpretation as the direction of changes following the experimental condition is our main outcome. Secondly, PP was not measured at the carotid site when measuring CCA distensibility (Steinback et al., 2005). However, our results are

comparable to an adolescent investigation measuring PP at the CCA site (Lenard et al., 2004). Thirdly, the autonomic determinant of BRS used in the present investigation, although reliable (Oliveira et al., 2018a), has not been validated against methods using vasoactive substances. Finally, the BRS vascular determinant was performed solely on CCA, and no information about aortic distensibility were obtained (Klassen et al., 2016).

8.6 Conclusions

The present study provides unique insight into the interplay between the vascular and autonomic systems in the control of BP following exercise in youth. BRS decreases 5-min following HIIE and MIIE in adolescents, but is restored at 60-min post. The autonomic component is the main determinant of the observed fall in BRS. At 60-min post HIIE, MAP is lowered showing a strong stimulus from exercise and a blunted BRS ability to restore BP. Our findings highlight different mechanisms of BP control following different exercise intensities. Finally, exercise intensity appears an important determinant of carotid artery vasoconstriction following exercise.

Chapter 9: Effects of Exercise Intensity on Vascular and Autonomic Components of The Baroreflex Following Glucose Ingestion in Adolescents

9.1 Abstract

This Chapters aimed to investigate the effects of an OGTT on BRS in a sample of healthy adolescents, and how acute exercise bouts of different intensities alter the effects of the OGTT on BRS. Thirteen male adolescents (14.0 ± 0.5 years) completed three conditions on separate days in a counterbalanced order: 1) HIIE; 2) MIIE; and 3) CON. At ~ 90-min following the conditions participants performed an OGTT. Supine HR and BP were monitored continuously at baseline, 60-min following the conditions, and 60-min following the OGTT. A cross-spectral method (LFgain) was used to determine BRS gain. Arterial compliance was assessed as the BRS vascular component. LFgain divided by AC (LFgain/AC) was used as the autonomic component. Although non-significant, LFgain moderately decreased post-OGTT when no exercise was performed (pre-OGTT = 24.4 ± 8.2ms·mmHg^{-1}; post-OGTT = 19.9 ± 5.6 ms·mmHg^{-1}; ES = 0.64, *P*> 0.05). This was attributed to the decreases in LFgain/AC (pre-OGTT = 1.19 ± 0.5 ms·μm^{-1}; post-OGTT = 0.92 ± 0.24 ms·μm^{-1}; ES = 0.69, *P*>0.05). Compared to CON (Δchange =-4.4 ± 8.7 ms·mmHg^{-1}), there were no differences for the pre- post-OGTT delta changes in LF/gain for HIIE (Δchange = -3.5 ± 8.2 ms·mmHg^{-1}) and MIIE (Δchange = 1.3 ± 9.9 ms·mmHg^{-1})Similarly, compared to CON (Δchange = -0.23±0.40ms·μm^{-1}) there were no differences for the pre- post-

OGTT delta changes in LF/gain for HIIE (Δ = change -0.22±0.49ms·μm^{-1}) and MIIE (Δ = change 0.13±0.36ms·μm^{-1}).The results indicate that BRS decreases in adolescents following a glucose challenge with no apparent effects of exercise.

9.2 Introduction

Atherosclerosis has its origins during childhood with elevated BP contributing to plaque formation independently of other cardiovascular disease (CVD) risk factors in youth (Franks et al., 2010, McGill et al., 2001). A sentinel for hypertension development is decreased BRS. In young adults a lower BRS is present in normotensive children of hypertensive parents (Boutcher et al., 2011), and impaired BRS is associated with high BP in normotensive adolescents (Honzikova and Zavodna, 2016, Fitzgibbon et al., 2012). These studies indicate BRS dysfunction may be associated with CVD burden in youth and is worthy of further research so as to inform preventative health strategies. Baroreflex sensitivity is composed of autonomic and vascular components which contribute towards the beat-to-beat detection and adjustment of BP fluctuations (Hunt et al., 2001a). Using ultrasound (Taylor et al., 2014, Tzeng, 2012), the contribution of the autonomic and vascular determinants of BRS can be non-invasively estimated in a reliable manner (Oliveira et al., 2018a), and are ideally suited for studying BRS in paediatric groups.

In non-diabetic children, glucose intolerance assessed during an OGTT predicts adult premature death (Franks et al., 2010). The metabolic effects of elevated blood [GLU] following an OGTT have implications for the arterial and autonomic systems, as evidenced by decreased autonomic modulation and increased vascular stiffness in diabetic adolescents (Shin et al., 2010), which may contribute to chronic BRS dysfunction in youth with diabetes (Honzikova and Zavodna, 2016). However, a

lowered BRS caused by rises in [GLU] is not only observed in diseased populations. For example, a decreased BRS has been reported in healthy adults during an OGTT, which was attributed to a diminished autonomic determinant (Holwerda et al., 2015). The mechanism by which glucose decreases BRS remains controversial however, as a rise in [GLU] leads to lowered vagal modulation (Holwerda et al., 2015, Cao and Pilowsky, 2014, Lefrandt et al., 2000) (i.e. reduced autonomic component), and increased CCA stiffness due to endothelial dysfunction (Zhu et al., 2007, Wilkinson et al., 2004) (i.e. reduced vascular component). Although growth and maturation are associated with an augmented BRS due to maturation of the autonomic component (Lenard et al., 2004), the influence of a glucose load on the BRS and its associated mechanisms is unknown in youth. As glucose intolerance is associated with poor vascular and autonomic functions in diabetic youth (Shin et al., 2010), it is plausible that elevated [GLU] may reduce BRS in healthy adolescents. A better understanding of the BRS physiology under different challenges, such as during an OGTT, can help inform strategies to target CVD risk reduction in paediatric groups.

Physical activity is an important strategy to improve glucose metabolism (Henderson et al., 2012), and is also positively associated with autonomic and vascular functions in children and adolescents (Fernhall and Agiovlasitis, 2008, Oliveira et al., 2017). While in adults temporally decreasing PA levels does not exacerbate the deleterious effects of an OGTT on BRS (Holwerda et al., 2015), the possible effect of increasing PA via prior exercise on the subsequent BRS responses to an OGTT is currently unknown. In healthy adolescents, a single bout of high and moderate-intensity exercise has been shown to reduce the increase in blood [GLU] during an OGTT (Cockcroft et al., 2015, Cockcroft et al., 2017b), suggesting that acute exercise may alter the BRS responses to an OGTT by lowering blood [GLU]. Additionally, in the

hours following high but not moderate-intensity exercise, improvements in arterial function are observed in healthy adolescents (Bond et al., 2015c, Bond et al., 2015b), showing that exercise may preserve the vascular component of BRS. As the intensity of exercise has recently been proposed to be a determinant of CVD risk reduction in youth (Carson et al., 2014, Barker et al., 2018), elucidating whether exercise of different intensities alters the BRS response to an OGTT will further contribute to our understanding of CVD risk reduction in youth.

The aims of the present study were to investigate in healthy adolescents: 1) the effect of an OGTT on BRS and its vascular and autonomic components; and 2) whether an acute bout of moderate and high-intensity exercise alters the effects of an OGTT on BRS and its associated mechanisms. It was hypothesised that 1) the OGTT would impair BRS via decreases in the autonomic and vascular determinants; 2) a prior bout of moderate and high-intensity exercise would lead to a significant lower [GLU] concentration from OGTT compared to a non-exercise control situation (CON); and 3) that both HIIE and MIIE would maintain BRS at baseline values following the OGTT due to preserved vascular and autonomic components.

9.3 Methods

9.3.1 Participants

Thirteen healthy male adolescents (14.0 ± 0.5 years; body mass index = 18.6 ± 3.0 $m \cdot kg^{-2}$; BF = 12.0 ± 4.7%; $\dot{V}O_2$ max = 50.9 ± 5.3 $mL \cdot kg^{-1} \cdot min^{-1}$) volunteered to take part in this investigation. The status of pubertal development, as measure using five stages of pubic hair development (Morris and Udry, 1980) was: Tanner stage 2 n=3, 3 n=1, 4 n=8, 5 n=1. Before participating in the study, participants and parents completed a health questionnaire and all participants were free of conditions, such as

diabetes, hypertension, asthma, or any disease altering autonomic and vascular functions. Assent and consent were obtained from participants and parents/guardians respectively, and all procedures were approved by the institutional ethics committee (approval number: 160217/B/04).

9.3.2 Experimental design

Participants completed four visits to the laboratory with a minimum of 72-h between each visit and no more than four weeks to finish all visits. The visits were all conducted in the morning following an overnight fast, as detailed below:

Visit 1: Participants were familiarized to the BRS protocol and treadmill running. Participants characteristics were obtained as described in Section 3.4. At the end of Visit 1, participants received food diaries and accelerometers, which were used in the 48 h preceding Visits 2 – 4.

Visits 2 – 4: The outline of this visit is presented in the Figure 9.1. Following an overnight fast, participants were transported to the laboratory and completed the BRS protocol as described in Section 3.8.3 between 8 – 9 am.

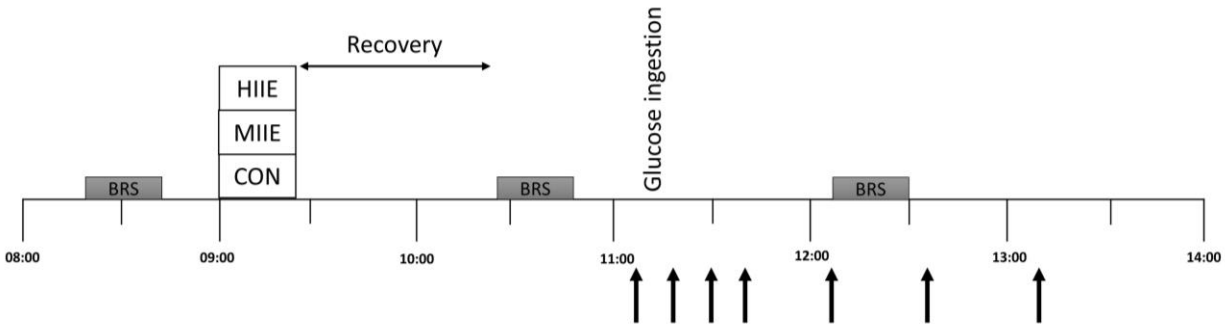

Figure 9.1: Overall scheme of visits 2-4. Black arrows: blood sampling.

Following the baseline assessments, participants completed in a counterbalanced order the following experimental conditions on separated days: 1) HIIE; 2) MIIE; and

3) CON which are described in details in Section 3.7.2.For CON, participants pursued sedentary activities in a seated position, such as computer and board games.

At 60minfollowing the experimental conditions (pre-OGTT), participants repeated the BRS protocol. Following this BRS measurement, an OGTT took place as described in Section 3.7.1. The OGTT has been shown to be reliable in a sample of healthy adolescents (i.e. observed test-retest coefficient of variation of 5 – 7% for glucose derived indices (Cockcroft et al., 2017a)). Participants repeated the BRS protocol 60 min post OGTT (post-OGTT) as it has been shown that BRS is reduced at this time point following an OGTT in adults (Holwerda et al., 2015).

9.3.3 Baroreflex sensitivity analysis

Baroreflex sensitivity was obtained using the cross-spectral transfer function described in Section 3.8.3.

9.3.4 Vascular and autonomic determinants

Images were recorded as described in Section 3.8.4. The vascular components of BRS were determined using equations 3.10 and 3.11 according to published guidelines (Laurent et al., 2006).

The autonomic and vascular determinants of BRS were determined according to a previous study (Lenard et al., 2004). Briefly, AC was considered as the vascular component of the BRS and expressed as $\mu m \cdot mmHg^{-1}$. To calculate the autonomic determinant, LFgain was divided by the AC and expressed as LFgain/AC in $ms \cdot \mu m^{-1}$.

9.3.5 Food and physical activity standardisation

For standardisation proposes PA and dietary intake were completed as described in Section 3.8.1.

9.3.6 Statistical analysis

Data are presented as means and standard deviation. Physiological responses to exercise were investigated using paired t-tests. To test the first aim of this study, the effects of the OGTT on the physiological parameters, paired t tests were performed from CON group pre and post-OGTT. To test possible differences between outcomes at baseline, repeated measures ANOVA with three levels for condition was applied. As no differences were found for any variables at baseline, to test aim 2 of this study, delta changes pre- and post-OGTT were calculated for each condition (HIIE, MIIE and CON) and the differences between conditions were tested using one-way repeated measures ANOVA. Total area under the curve and iAUC analyses quantified the plasma [GLU] responses to the OGTT using the trapezium rule (GraphPad Prism 6.02, USA) and differences between conditions were tested using repeated measure ANOVA. Sphericity was tested using Mauchly's test and when violated corrections were performed using Greenhouse-Geisser. Post-hoc comparisons were applied when adequate using the least square difference procedure. Analyses were performed using SPPS v.22, with the alpha level set at 0.05. Finally, the magnitude of mean differences were interpreted using effect size (ES): ≥0.2 small, ≥0.5 moderate, ≥0.8 large (Cohen, 1977).

9.4 Results

One participant was excluded from the BRS assessment due to errors in the ECG signal, and two from the CCA analysis due to technical issues with the ultrasound. For clarity, the final sample size for each analysis is described in Figures 9.2 and 9.3 and in Tables 9.1. In the 48h before the experimental visits, there were no significant differences in the amount of MVPA (HIIE=117 ± 49, MIIE=117 ± 32, CON=111 ± 45 min·day^{-1}; *P*=0.91), energy intake (HIIE=1,987 ± 732, MIIE=1,912 ± 458, CON=2,079 ± 643 kcal·day^{-1}; *P*=0.55) and relative macronutrient contribution (carbohydrates: HIIE =52 ± 7, MIIE=50 ± 5, CON=51 ± 8%; lipids: HIIE=32 ± 1, MIIE=32 ± 1, CON=33 ± 1%; proteins: HIIE=16 ± 3, MIIE=17 ± 3, CON=16 ± 4%; all *P*>0.05) between the experimental conditions.

HIIE elicited significantly greater peak $\dot{V}O_2$ [%of max] (2.2 ± 0.2 [89%] vs 1.6 ± 0.1 [66%] L·min^{-1}; *P*<0.001), and average HR [%of max] (154 ± 3 [78%] vs 128 ± 5 [64%] bpm; *P*<0.001) compared to MIIE. HIIE was significantly shorter in duration than MIIE (21.8 ± 0 vs 28.0 ± 1.8 min; *P*<0.001),

9.4.1 Oral glucose tolerance test

Oral glucose tolerance test responses are depicted in Figure 9.2. As expected, the OGTT resulted in increases in [GLU] over time (*P*<0.001) but no condition by time interaction (*P*=0.11) was observed. There was no condition main effect for the tAUC (*P*=0.12) and iAUC (*P*=0.15) analysis of the [GLU] response to the OGTT. However, a moderate reduction in iAUC (ES=0.51) and tAUC (ES=0.52) for [GLU] was observed for HIIE vs. MIIE, and HIIE vs. CON respectively.

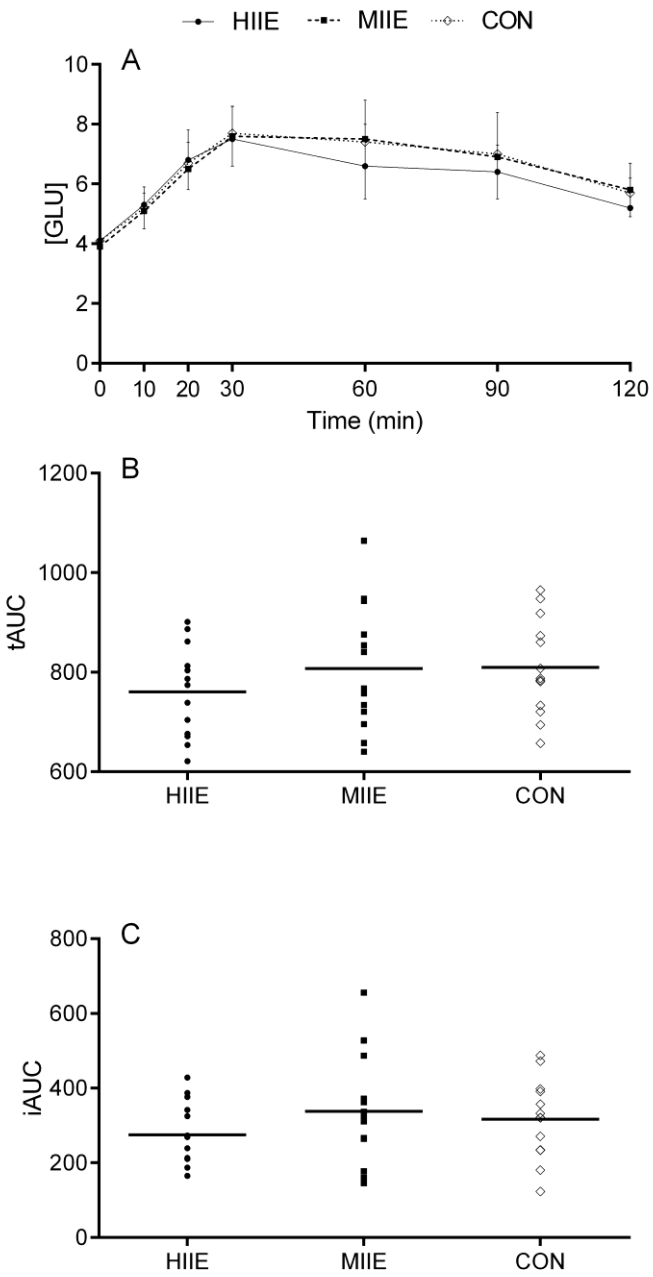

Figure 9.2: Effects of the experimental condition on: A) [GLU] (n=13), B) total area under the curve (n=13) for the different conditions; and C) incremental area under the curve (n=13).

9.4.2 Baroreflex sensitivity

LFgain (pre=24.4 ± 8.2, post-OGTT=19.9 ± 5.6; *P*=0.11; ES=-0.63), AC (pre=20.4 ± 4.2, post-OGTT=22.6 ± 5.8; *P*=0.22; ES=0.55), and LFgain/AC (pre=1.19 ± 0.5, post-OGTT=0.92 ± 0.2; *P*=0.07; ES=-0.63) were not significantly altered by the OGTT during CON, although moderate effects were observed for all comparisons. When the delta changes were compared between CON, HIIE and MIIE no effect of condition was present for LFgain (*P*=0.31), AC (*P*=0.63), and LFgain/AC (*P*=0.10) pre- and post-OGTT (Figure 9.3).

9.4.3 Common carotid artery

Common carotid artery properties are presented in Table 9.1. DLD (*P*=0.004; ES=-0.21) and ΔD (*P*=0.048; ES=0.30) were significantly altered by the OGTT during CON. No significant effects were observed for SLD pre and post-OGTT (*P*=0.30; ES=0.09). When the pre- post-OGTT delta changes were compared between CON, HIIE and MIIE, no effect of condition was observed for DLD (*P*=0.12), SLD (*P*=0.51), and ΔD (*P*=0.40). All effect sizes between conditions were considered small (all ES <0.5).

9.4.4 Blood pressure and heart rate variability

Blood pressure and HRV are presented in Table 9.1. SBP (*P*=0.11; ES=-0.63), DBP (*P*=0.22; ES=0.55), MAP (*P*=0.07; ES=-0.63), and LF (*P*=0.07; ES=-0.63) were not significantly altered by the OGTT but exhibited moderate effects. On the contrary, post-OGTT HF significantly increased moderately (*P*=0.010; ES=0.54). When the pre-post-OGTT delta changes were compared between CON, HIIE and MIIE, there was no effect of condition for the delta changes in SBP (*P*=0.85), DBP (*P*=0.28), MAP (*P*=0.36). Similarly, no effects of condition for the delta changes in LF (*P*=0.63) and

HF (*P*=0.10) pre- and post-OGTT. All effect sizes between conditions were considered small (all ES <0.5).

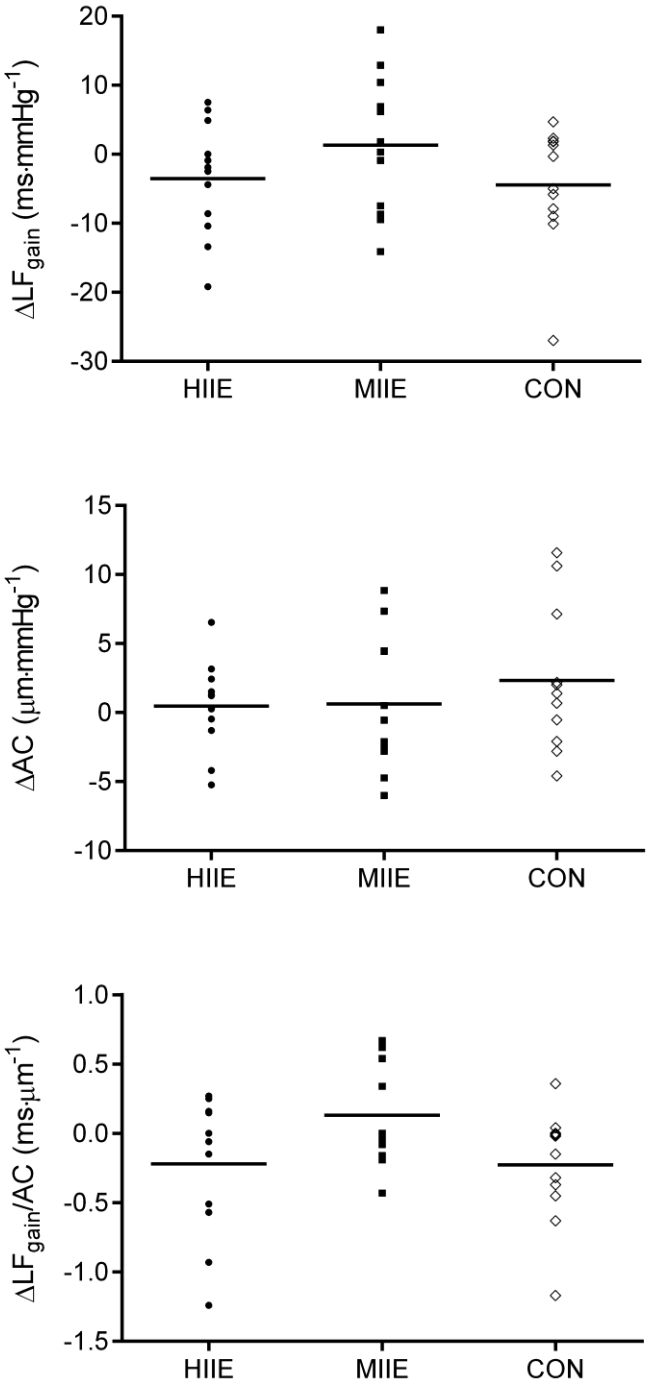

Figure 9.3: Effects of the experimental condition on the delta changes pre- and post-OGTT for A) LFgain (n=12); B) AC (n=11); and C) LFgain/AC (n=10).

Table 9.1: Common carotid, blood pressure and heart rate variability outcomes.

	HIIE			MIIE			CON		
	Pre-OGTT	Post-OGTT	Δ	Pre-OGTT	Post-OGTT	Δ	Pre-OGTT	Post-OGTT	Δ
DLD (μm) n = 11	5425.5 ± 324.7	5465.5 ± 284.0	40.0 ± 162.4	5417.3 ± 309.2	5377.3 ± 387.5	-40.0 ± 148.5	5400.0 ± 364.4	5323.6 ± 370.5	-76.4 ± 67.0
SLD (μm) n = 11	6305.5 ± 344.1	6335.5 ± 229.9	30.0 ± 207.4	6322.7 ± 304.3	6300.0 ± 341.6	-22.7 ± 118.1	6268.2 ± 387.6	6234.5 ± 355.0	-33.6 ± 103.0
ΔD (μm) n = 11	880.0 ± 144.9	870.0 ± 131.8	-10 ± 106.4	905.5 ± 116.2	922.7 ± 139.2	17.3 ± 122.1	868.2 ± 143.2	910.9 ± 137.7	42.7 ± 62.8
SBP (mmHg) n = 13	108 ± 10	110 ± 13	1.5 ± 9.8	113 ± 9	114 ± 15	0.3 ± 10.7	111 ± 11	110 ± 12	-0.6 ± 9.4
DBP (mmHg) n = 13	61 ± 6	65 ± 9	3.9 ± 6.8	69 ± 7	67 ± 11	-1.4 ± 9.6	65 ± 6.7	66 ± 7	0.82 ± 6.4
MAP (mmHg) n = 13	75 ± 6	78 ± 10	3.2 ± 7.0	82 ± 7	81 ± 11	-0.9 ± 8.7	78.9 ± 6.5	79 ± 7	0.14 ± 5.9
HFln (ms^2) n = 12	8.5 ± 0.9	8.3 ± 0.8	-0.23 ± 0.73	8.5 ± 1.0	8.4 ± 0.9	-0.06 ± 0.79	8.4 ± 0.7	8.8 ± 0.7*	0.39 ± 0.43
LFln (ms^2) n = 12	7.3 ± 1.2	7.0 ± 1.0	-0.29 ± 0.61	7.2 ± 0.8	7.3 ± 0.7	0.11 ± 0.60	7.5 ± 0.6	7.4 ± 0.6	-0.15 ± 0.60

HIIE: high-intensity interval exercise. MIIE: moderate-intensity interval exercise. CON: control DLD: diastolic lumen diameter. SLD: systolic lumen diameter. SBP: systolic blood pressure. DBP: diastolic blood pressure. MAP: mean arterial pressure. HF: high-frequency. LF: low-frequency. *P < 0.05 compared Pre-OGTT in the same condition. For P values and effect sizes refer to text.

9.5 Discussion

To our knowledge this is the first study to examine the influence of hyperglycaemia, delivered using an OGTT, on BRS in health adolescents. The OGTT caused a moderate but non-significant decrease in BRS and its autonomic determinant. Another novel feature of the current study is that we examined the role of different exercise intensities performed ~ 90 min prior to the OGTT on the changes in BRS, [GLU], and hemodynamics. The main findings regarding exercise were: 1) HIIE but not MIIE moderately decreased the glucose responses to an OGTT; and 2) exercise performed 90-min had no effect on BRS following the OGTT.

9.5.1 Effects of glucose on baroreflex sensitivity

This is the first study in a sample of healthy adolescents investigating the impact of an acute glucose load on the mechanistic control of blood pressure. To date most research investigating BRS during metabolic challenges have been performed in adults, or in populations with diabetes, obesity, or elevated blood pressure (Malin et al., 2016b, Straznicky et al., 2009, Holwerda et al., 2015). In the present study, the increase in blood [GLU] following the OGTT led to a moderate (i.e. ES = 0.64) yet non-significant decrease in the LFgain in the CON condition. Although our findings failed to reject the null hypothesis, the magnitude of the observed changes are similar to the study by Holwerda et al. (2015) who reported a moderate and significant decrease (pre-OGTT = 20±9; post-OGTT = 14±6; ES = 0.78) in LFgain 60min following the ingestion of a glucose load in healthy adults. The decrease in BRS appears to be moderated by increases in [GLU], because BRS at 60min post a hyperinsulinemic euglycemic clamp did not decrease compared to baseline, suggesting that glucose is responsible for a decreased BRS when [GLU] peaks at around 7.5 mmol·L^{-1} (Holwerda

et al., 2015). We further extend Holwerda et al. (2015) findings by investigating the likely mechanisms by which [GLU] leads to decreases in BRS by estimating the autonomic and the vascular determinants of BRS. Our present observations demonstrated that although non-significant, a moderate effect was observed for the OGTT on the changes in the autonomic marker of BRS, measured as the LFgain/AC. These results are in accordance with adult data showing a lowered autonomic modulation caused by rises in blood [GLU] (Cao and Pilowsky, 2014, Cao et al., 2016), and may provide a mechanism linking cross-sectional findings of a lowered vagal modulation and impaired BRS in children with diabetes (Honzikova and Zavodna, 2016).

We also investigated the effects of [GLU] on the vascular determinant of BRS measured as CCA compliance (Lenard et al., 2004). A decrease in CCA compliance, therefore, would be an indicative of a decreased vascular determinant of the BRS (Oliveira et al., 2018a), and consequently a decrease in the overall BRS. As arterial compliance is partially dependent on endothelial function (Wilkinson et al., 2004), the lack of decreases in CCA compliance in the present study may be explained by previous literature showing no decrease in endothelial function in normal weight children during an OGTT (Dengel et al., 2007). Moreover, different to the present study, adult studies have shown increases in arterial stiffness, assessed as pulse wave velocity, following an OGTT (Baynard et al., 2009, Kobayashi et al., 2018). Differences in the arterial stiffness assessment method (i.e. CCA compliance and distensibility vs central and peripheral pulse wave velocity), or differences in arterial stiffness due to aging (Lenard et al., 2004), may explain discrepancies between the present study and the adult literature. Alternatively, it is possible that the lack of [GLU] effects on CCA compliance in the present study reflect the aerobic fitness of the

participants. For example, Kobayashi et al. (2015), provided data showing increases in central arterial stiffness of participants with lower (38.8 ± 1.9 mL·kg^{-1}·min^{-1}), but not with higher $\dot{V}O_2$max (50.2 ± 2.7 mL·kg^{-1}·min^{-1}) following a glucose challenge. The present sample had a $\dot{V}O_2$max of 50.9 ± 5.3 mL·kg^{-1}·min^{-1}, which may have conferred protection against an increase in arterial stiffness due to hyperglycaemia. Unfortunately, the homogenous nature of the present sample for $\dot{V}O_2$max distribution does not allow this hypothesis to be further investigated. Future studies are needed to test the effects of aerobic fitness on vascular stiffness during OGTT in youth.

9.5.2 Effects of exercise intensity on glucose

In the present investigation, a moderate yet non-significant effect was observed for the reduction in the iAUC and tAUC for [GLU] following HIIE compared to CON and MIIE, respectively (Figure 9.2). These results are different to recent investigations, where moderate to large effects were observed for the reduction in iAUC and tAUC for [GLU] following cycling HIIE and continuous moderate exercise in healthy adolescents (Cockcroft et al., 2015, Cockcroft et al., 2017b). Direct comparison between the studies is complex due to the different mode of exercise (i.e. interval running vs interval and continuous cycling), and participants maturity characteristics. Similarly, the time between the exercise and the OGTT challenge was different between the investigations, which may influence the findings. In the present investigation participants undertook ~ 90min of recovery between the end of the exercise and the ingestion of the glucose load, whereas in Cockcroft et al. (2015) participants ingested the glucose 10min following exercise. The longer recovery period in the present study (i.e. 90min) may favour glucose appearance in the blood due to restoration of blood flow to the splanchnic circulation, contrary to a shorter (i.e. 10min) recovery period, when blood flow is still directed to the muscle and skin and exogenous

glucose appearance is lower. Alternatively, because in the 60min following the exercise there is an increase in peripheral insulin sensitivity but not hepatic (Malin et al., 2016a), the 90min recovery in the present study may have facilitated endogenous glucose uptake by the muscle, and when the OGTT started the exogenous source was cleared slowly by the liver and muscle. Perhaps a longer follow-up after the exercise conditions (i.e. 24h), would provide experimental data on the clearance of blood [GLU], due to a slow phase of glycogen repletion(Price et al., 1999). Although insulin was not measured, it is likely that the exercise bouts increased insulin sensitivity, as recently reported in healthy adolescents (Cockcroft et al., 2015).

9.5.3 Effects of exercise intensity on the determinants of the baroreflex sensitivity

This is the first study to investigate the impact of different exercise intensities on BRS following the ingestion of a glucose load, which limits direct comparisons with previous studies. We reasoned that performing exercise before the ingestion of a glucose load would confer vascular protection by blunting possible increases in vascular stiffness following the OGTT, as recently reported in adults (Kobayashi et al., 2018). Our results do not support this hypothesis as no differences were observed between conditions (HIIE, MIIE and CON) for the delta changes in arterial compliance. Recently the OGTT has been shown to decrease femoral arterial compliance in adults only, when participants' physical activity levels are decreased for five consecutive days (Credeur et al., 2018), suggesting a protective role of physical activity levels on arterial compliance. As such, although participants refrained from exercise in the 48h preceding data collection in our present study, that the amount of physical activity performed in the week protected against the decreases in vascular compliance following the OGTT. Future studies should test the effects of decreases or increases in physical activity on the autonomic and vascular determinants of BRS in adolescents.

We also reasoned that a decrease in [GLU] following HIIE and MIIE would maintain a BRS value similar to pre-OGTT ingestion. This hypothesis has foundations on the mechanistically link between rises in [GLU] and decreases in BRS recently described in adults (Holwerda et al., 2015). However, the moderate decrease in glucose tAUC and iAUC caused by HIIE did not translated in augmented or maintained BRS and its autonomic determinant as evidenced by the lack of differences between CON and HIIE on the delta changes of LFgain and LFgain/AC. It can be speculated that a higher decrease on [GLU] than the observed in our present study is necessary to keep BRS preserved from the [GLU] effects. No studies exist providing dose-response between GLU and autonomic function.

As with all studies there are a few limitations that must be recognised. Firstly, although the present method to measure the autonomic determinant of BRS is reliable (Oliveira et al., 2018a), is has not been validated against drug blockade methods. However, our present results and the literature showing the effects of blood [GLU] on autonomic modulation, reinforces the validity of the present measures (Cao and Pilowsky, 2014, Cao et al., 2016). Secondly, PP was not measured at the carotid site when measuring CCA distensibility (Steinback et al., 2005). However, our results are comparable to an adolescent investigation measuring PP at the CCA site (Lenard et al., 2004). Finally, we did not measure insulin and more mechanistic information about the effects of insulin sensitivity is speculative in the present study.

9.6 Conclusions

In healthy adolescents, increases in blood [GLU] caused moderate decreases in BRS likely via decreases in the autonomic BRS determinant. HIIE and MIIE performed before the ingestion of the glucose load did not have an effect on the observed

decreases in BRS. This study provides unique information in adolescents of a lack of exercise influences on BRS responses to blood [GLU] and future studies are needed either to replicate or not these findings in healthy and diseased adolescents.

Chapter 10: Effects of High-intensity Interval Training on the Vascular and Autonomic Components of the Baroreflex at Rest and Post-Exercise in Adolescents

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10.1 Abstract

This Chapter aimed to: 1) to investigate the effects of HIIE training and detraining on BRS and its vascular and autonomic components at rest; and 2) to investigate the effects of HIIE training on BRS recovery following an acute bout of HIIE. Nineteen volunteers were randomly allocated to: 1) four weeks HIIE training (HIIE-T) performed three times per week; or 2) a CON condition with no intervention for the same duration as HIIE training. PRE, POST and following two weeks of detraining (DET) resting supine heart rate and blood pressure were measured and a cross-spectral method (LFgain) was used to determine BRS gain. Arterial compliance was assessed as the BRS vascular component. LFgain divided by AC (LFgain/AC) was used as the autonomic determinant of BRS. In addition to the resting measures, LFgain, AC and LFgain/AC were measured at30- and 120-min following a HIIE exercise bout at PRE and POST. HIIE-T did not change resting LFgain (adjusted change in means CON=-0.01 ms·mmHg^{-1}, HIIE-T=1.4 ms·mmHg^{-1}; *P*=0.66; ES=0.21), AC(CON=2.4µm·mmHg^{-1}, HIIE-T=0.9µm·mmHg^{-1}; *P*=0.44; ES=0.36)or LFgain/AC (CON=-0.02 ms·µm^{-1}, HIIE-T=0.09 ms·µm^{-1}; *P*=0.68; ES=0.19). Similarly, for both

CON and HIIE-T, LFgain decreased 30-min (all *P*<0.05) compared to baseline at PRE (CON: baseline = 23.2 ± 10.8 ms·mmHg^{-1}, 30-min post = 12.6 ± 7.8 ms·mmHg^{-1}; HIIE-T: baseline = 21.9± 6.4 ms·mmHg^{-1}, 30-min post = 13.4± 6.0ms·mmHg^{-1}) and POST (CON: baseline = 23.0 ± 12.0 ms·mmHg^{-1}, 30-min post = 13.3 ± 8.5 ms·mmHg^{-1}; HIIE-T: baseline = 23.5±5.6 ms·mmHg^{-1}, 30-min post = 11.3± 3.8ms·mmHg^{-1}). The decreases in LFgain for CON and HIIE-T were paralleled by decreases in LFgain/AC (all *P*<0.05) 30-min compared to baseline at PRE (CON: baseline = 1.12±0.50ms·µm^{-1}, 30-min post = 0.71± 0.50ms·µm^{-1}; HIIE-T: baseline = 1.34±0.44ms·µm^{-1}, 30-min post = 0.82± 0.48ms·µm^{-1}) and POST (CON: baseline = 1.10±0.73ms·µm^{-1}, 30-min post = 0.64± 0.53ms·µm^{-1}; HIIE-T: baseline = 1.43±0.69ms·µm^{-1}, 30-min post = 0.54± 0.29ms·µm^{-1}). HIIE-T does not change BRS and its autonomic and vascular determinant in a sample of healthy adolescents at rest and acutely following a HIIE bout.

10.2 Introduction

Elevated BP is positively associated with atherosclerotic progression in youth (McGill et al., 2001). A sentinel in the development of hypertension is impaired cardiac BRS. For example, decreased cardiac BRS at rest has been shown to be a predictor of hypertension over five years in a longitudinal investigation in adults (Ducher et al., 2006), and BRS impairment is already observed in adolescents with elevated BP (Fitzgibbon et al., 2012, Honzikova and Fiser, 2009, Honzikova and Zavodna, 2016). Because cardiac BRS is ultimately measured as the gain between vascular compliance and autonomic adjustments in HR (Taylor et al., 2014, Hunt et al., 2001a, Tzeng, 2012), interventions that improve these systems in youth may act as a preventive strategy of hypertension. Exercise training is a suitable strategy to improve

BRS, with the intensity of exercise being important as increases in BRS obtained after HIIE training (Heydari et al., 2013) but not following moderate-intensity training (Loimaala et al., 2000, Goldberg et al., 2012) in healthy adults. In adolescents HIIE training can also increase BRS due to a reported association between VPA, resting autonomic function and AC(Oliveira et al., 2018b, van de Laar et al., 2010), suggesting that VPA delivered as HIIE has potential to increase BRS via its autonomic and vascular components.

While there are no studies investigating HIIE training and BRS in healthy adolescents, a previous investigation in this population have demonstrated significant increases in cardiac autonomic function measured via HRV after two weeks of HIIE training (Bond et al., 2015a). As such, it can be hypothesised that resting cardiac BRS can also be improved reflecting increases in its autonomic determinant following HIIE training, although this remains unknown in youth. Another possible mechanism underlying increases in resting cardiac BRS is via increases in CCA compliance. For example, Monahan et al. (2001a) demonstrated that 12weeks of aerobic training increased resting cardiac BRS due to increased CCA compliance in old adults (56 years old). Whether these findings can be replicated in youth, a population with a more distensible CCA compared to old adults (Monahan, 2007, Lenard et al., 2004), is unclear.

Similarly, whether possible adaptations to resting BRS and its autonomic and vascular determinants following an exercise intervention, such as HIIE, are maintained after training cessation is unknown. For example, in adults two weeks of detraining completely reversed improvements in resting HRV obtained after aerobic training (Gamelin et al., 2007). A similar pattern seems to exist in adolescents, where improvements in resting HRV at 24 h was almost reversed after just 72 h following

HIIE training cessation (Bond et al., 2015a). Similar to HRV, although not in a detraining study, five days of decreased physical activity has led to increased femoral artery stiffness in adults (Credeur et al., 2018). The possible impact of these results on BP control via BRS is still speculative, as one adult study did not show reductions in cardiac BRS following five days of decreased physical activity (Holwerda et al., 2015). Collectively, these studies indicate that two weeks of detraining following HIIE training may reverse training-induced adaptations to resting cardiac BRS, but the mechanism are poorly understood.

In addition to measurements of BRS under resting conditions, it has been previously reported that cardiac BRS gain is decreased 30min following moderate-intensity exercise (Hart et al., 2010) and the whole BRS curve is improved up to 145min flowing moderate-intensity exercise (Halliwill et al., 1996b) regardless of training status. A cross-sectional investigation has, however, shown that training status may alter BRS following exercise. For example, trained adults experienced a greater decrease in BRS 30min following HIIE compared to untrained pairs (Cote et al., 2015a). Interestingly, a higher post-exercise hypotension was also observed for the training adults, indicating a possible effect of training on post-exercise BRS resetting to a lower BP operating point (Smith et al., 2000), which ultimately exacerbates post-exercise hypotension. The cross-sectional nature of the investigation limits causality and whether the acute changes in BRS after exercise is responsive to training is currently unknown.

The aims of this study were to investigate in a healthy sample of adolescents: 1) the effects of four weeks of HIIE training on resting BRS and its autonomic and vascular determinants; 2) to investigate the effects of two weeks of detraining on resting BRS

and its autonomic and vascular determinants; and 3) to investigate the effects of four weeks of HIIE training on the responses of BRS and its autonomic and vascular components following a single bout of HIIE. It was hypothesized that: 1) four weeks of HIIE training would increase the overall BRS via its autonomic and vascular components at rest; 2) two weeks of detraining would reverse the adaptations observed following HIIE training; and 3) following HIIE training, BRS would decrease acutely after exercise compared to pre HIIE training, allowing an accentuated post-exercise hypotension in adolescents.

10.3 Methods

10.3.1 Participants

Twenty-one male adolescents volunteered to take part in this study. Participants were recruited using a convenient sample from local secondary schools. Assemblies were conducted to explain the risk, benefits and the protocol of the study. At the end of each assembly, envelopes containing the study details were distributed. A total of 70 envelopes were delivered to potential participants from which 21 were returned. All 21 volunteers who returned the envelopes were enrolled in the study and randomly allocated to either a CON or a HIIE-T group.

Health questionnaires were completed before participation, and all volunteers were free of conditions affecting the cardiac autonomic and vascular systems, such as asthma, congenital heart disease, and hypertension. All procedures were approved by the Sport and Health Sciences, University of Exeter Ethics Committee (Ref No: 161207/B/02). Assent and informed consent were obtained from the adolescents and their parents/guardians, respectively.

10.3.2 Study design

A schematic of the study is presented in Figure 10.1. Participants performed four visits to the laboratories consisting of:

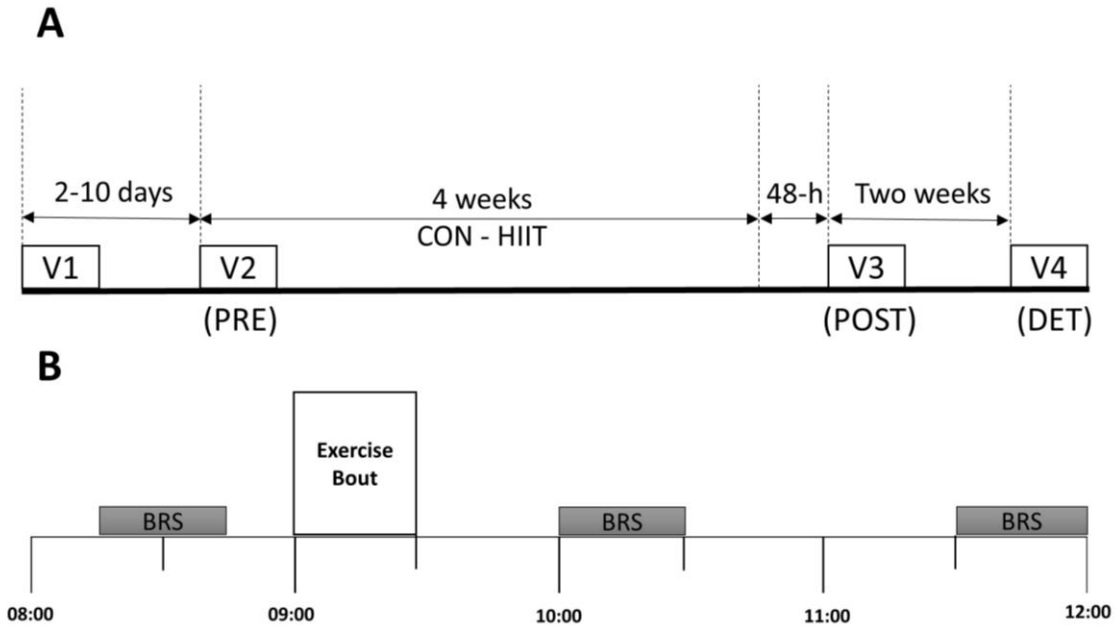

Figure 10.1: Overview of the experimental design. A) overview of the six weeks plan is presented. B) schematic of the protocols performed in visits 2 (PRE) and 3 (POST).

Visit-1 (familiarisation): Participants were familiarised to the procedures of the study and participants' characteristics were obtained as described in Section 3.4. To determine MAS for prescription participants performed a 20 m shuttle run test as described in Section 3.7.2. Heart rate (HR) was monitored (Polar Team2, Polar, Kempele, Finland), and maximum effort was considered when participants achieved a HR within 90% of age predicted maximum (i.e. 220 – age), displayed signs of subjective fatigue, and an unwillingness to continue the test despite strong verbal encouragement. At the end of Visit-1 participants received a package containing

adapted drawings of pubic hair development for self-assessment of maturity status (Morris and Udry, 1980).

Visit-2 (PRE): This visit took place between 2 – 10 days following Visit-1. Following an overnight fast, participants were transported to the laboratory and completed the BRS protocol as described in Section 3.8.3.

Following the baseline measures, participants performed a HIIE session in a sports hall consisting of eight bouts of 1-min at 90% of the MAS, interspersed by 75sof recovery (see training intervention details). During the HIIE session, heart rate (HR) was monitored (Polar Team2, Polar, Kempele, Finland). At 30min (30-min post) and 120min (120-min post) following the exercise session, participants repeated the BRS protocol. These time points were selected because in trained adults BRS is decreased at 30-min compared to untrained pairs (Cote et al., 2015a), and post-exercise hypotension can last up to 120-min post-exercise (Halliwill et al., 2013).

Visit-3 (POST): This visit took place four weeks following Visit-2. The procedures of Visit-3 were identical to Visit-2. To avoid possible effects of detraining, or the acute influences of the last training session, Visit-3 took place 48h following the last training session for the HIIE-T condition. Aiming to match the time elapsed between data collection for the HIIE-T condition, Visit-3 for CON was completed 48h following four weeks after completion of Visit-1.

Visit-4 (detraining – DET): This visit took place two weeks following Visit-3. This visit was identical to Visits 2 and 3, except that participants did not complete an exercise bout. Therefore, only resting data were collected.

10.3.3 Group allocation

Group allocation was conducted by two researchers and participants were not present. The allocation procedures were completed following Visit-1. Participants were randomly allocated to either CON or HIIE-T. For this, a simple randomisation was conducted by drawing 21 identical cards from a closed container. Each card contained either the letter C (10 cards) or T (11 cards). The cards were blindly assigned to each participants' codes that were inside 21 shuffled opaque envelopes. The group assignment was revealed after randomisation took place and participants and parents contacted to arrange Visit-2. Due to the nature of the intervention, participants were not blind to the conditions. Researchers were however, blinded for data handling and statistics for which codes were used.

10.3.4 Training intervention

Participants allocated to the HIIE-T group performed three training sessions per week as described in Section 3.7.2 for four weeks providing a total of 12 HIIE sessions. For the CON group, no intervention was performed. All participants in the present investigation kept their usual exercise routine and HIIE-T was delivered as extra exercise sections.

For all training sessions, HR was monitored (Polar team 2) and internal training load calculated using the Edwards training impulse (TRIMP) method (Borresen and Lambert, 2009). For this, the time spent in five different HR zones was multiplied by 1 – 5, respectively. The zones were calculated as 1=50 – 60%; 2=60 – 70%; 3=70 – 80%; 4=80 – 90%; and 5=90 – 100% of peak HR obtained during the shuttle run test. This was used as a descriptive measurement of the participants' internal training load during the HIIE sessions.

10.3.5 Baroreflex sensitivity analysis

Baroreflex sensitivity was obtained using the cross-spectral transfer function described in Section 3.8.3. In addition, BRS was also calculated using the sequence method described in Section 3.8.3.

10.3.6 Vascular and autonomic determinants

Common carotid images were recorded as described in Section 3.8.4. The vascular components of BRS were determined using equations 3.10 and 3.11 according to published guidelines (Laurent et al., 2006).

The autonomic and vascular determinants of BRS were determined according to a previous study (Lenard et al., 2004). Briefly, AC was considered as the vascular component of the BRS and expressed as $\mu m \cdot mmHg^{-1}$. To calculate the autonomic determinant, LFgain was divided by the AC and expressed as LFgain/AC in $ms \cdot \mu m^{-1}$.

10.3.7 Autonomic modulation

Heart rate variability was obtained as described in Section 3.8.2 as the area under the low (LF = 0.04 – 0.15 Hz), and high frequency (HF = 0.15 – 0.50 Hz) bands in absolute (ms^2), normalized (nu), and as the LF/HF ratio.

10.3.8 Statistical analyses

Data are presented as mean and standard deviation unless otherwise stated. Normal distribution was investigated using Shapiro Wilk's test and log transformation performed when appropriated. To compare the effects of training on the resting (Baseline) measures, a series of univariate analysis was performed. For this, delta changes (POST-PRE) were calculated and inserted in the model as the dependent variable. Group (HIIE or CON) was inserted as fixed factor and the baseline measures (PRE) used as covariate to control for baseline differences between the groups. The

group effect was then obtained, and effect sizes calculated for the between groups comparisons after adjustments for the baseline values. Effect sizes were interpreted as <0.2 (trivial), >0.2 (small), >0.5 (moderate) and >0.8 (large) (Cohen, 1977). To compare the effects of detraining, a similar approach was used only when a training effect was obtained. For this delta changes (DET-POST) were inserted as dependent variable, group as fixed factor, and the POST measures as covariate.

To investigate the effects of HIIE training on the acute exercise responses, three-factor repeated measures ANOVA with two within- and one between-subject factors were performed. The between-subject factor (group) contained two levels (CON and HIIE training). One within-subject (moment) also contained two levels (PRE and POST training); and one within-subject (time) contained three levels (baseline, 30-min and 120-min post the HIIE bout). The effects of time, moment, group*time, moment*time and group*moment*time interactions were tested. When a significant effect was present, post-hoc ANOVAs were performed followed by standard pairwise comparisons.

Pearson's correlation coefficients were used to investigate the association between accumulated internal training loads and possible adaptations to training. To test whether the acute responses to HIIE were associated with possible adaptations to training, delta differences of the measurements obtained at 30-min minus baseline were calculated and Person's correlations performed with the delta POST-PRE training of the corresponding variables. SPSS was used for all analysis, and $P<0.05$ was considered statistically significant.

10.4 Results

From the initial 21 participants, two dropped out after Visit-2 for reasons unrelated to the study, i.e., one participant dropped out due to illness and the other for unknown reasons. The final sample size included in the analysis was 19. Participants' characteristics are presented in Table 10.1. Training compliance was 100% for the HIIE sessions and no adverse effects were reported. The training load of each session over the four weeks is presented in Table 10.2.

Table 10.1: Participants' characteristics.

	Pre		Post		Detraining	
	CON (n = 9)	HIIE-T (n=10)	CON (n=9)	HIIE-T (n=10)	CON (n=9)	HIIE-T (n=10)
Height (cm)	164.1 ± 9.8	159.3 ± 8.6	166.6 ± 10.4*	161.1 ± 8.7*	166.6 ± 10.3*	161.1 ± 8.7*
Body Mass (kg)	50.1 ± 8.8	44.4 ± 6.2	50.2 ± 8.7	45.0 ± 6.2	50.8 ± 8.8	45.2 ± 6.1
Fat mass (%)	21.6 ± 7.5	18.0 ± 7.3	21.9 ± 9.1	18.4 ± 6.2	21.3 ± 7.9	17.6 ± 6.4
BMI ($kg \cdot m^{-2}$)	18.6 ± 2.5	17.4 ± 0.8	18.2 ± 2.3*	17.2 ± 0.9*	18.4 ± 2.4	17.3 ± 0.8
Tanner	1 = 0 2 = 1 3 = 4 4 = 4 5 = 0	1 = 1 2 = 4 3 = 1 4 = 3 5 = 0	–	–	–	–
MAS ($km \cdot h^{-1}$)	11.8 ± 0.9	12.2 ± 0.5	–	–	–	–

BMI: body mass index. MAS: maximal aerobic speed. *P<0.05 compared to PRE.

10.4.1 Effects of high-intensity interval exercise training and detraining on resting measurements

Changes in resting BRS and its autonomic and vascular determinants are presented in Figure 10.2. After adjustments for baseline, there were no effects of training for LFgain (adjusted change in means CON=-0.01, HIIE-T=1.4 $ms \cdot mmHg^{-1}$; P=0.66; ES=0.21), AC (CON=2.4, HIIE-T=0.9 $\mu m \cdot mmHg^{-1}$; P=0.44; ES=0.36), and LFgain/AC (CON=-0.02, HIIE-T=0.09 $ms \cdot \mu m^{-1}$; P=0.68; ES=0.19).

Table 10.2: Mean and standard deviation of the observed training load and heart rate profile during the 12 training sessions.

Training session	Internal training Load (AU)	Average HR (bpm)	Average HR (% of max)	Peak HR (bpm)	Peak HR (% of max)
First	72.3 ± 8.6	157 ± 12	76.9 ± 4.6	197 ± 9	96.8 ± 2.2
Second	75.2 ± 9.2	161 ± 11	79.1 ± 3.3	199 ± 11	97.9 ± 2.5
Third	75.1 ± 8.5	162 ± 10	79.4 ± 3.5	198 ± 6	96.7 ± 2.7
Fourth	75.5 ± 8.9	161 ± 10	79.2 ± 3.7	196 ± 8	96.2 ± 2.8
Fifth	73.7 ± 7.7	159 ± 7	77.9 ± 3.2	196 ± 7	96.1 ± 2.4
Sixth	70.3 ± 8.7	158 ± 9	77.6 ± 3.6	194 ± 7	95.1 ± 2.7
Seventh	87.1 ± 7.8	161 ± 7	78.9 ± 3.0	196 ± 7	96.1 ± 2.2
Eighth	81.4 ± 6.9	158 ± 8	77.5 ± 3.1	193 ± 8	94.9 ± 2.7
Ninth	81.7 ± 9.8	155 ± 11	75.9 ± 3.8	193 ± 9	94.7 ± 3.1
Tenth	101.3 ± 7.7	160 ± 8	78.0 ± 2.8	197 ± 9	95.9 ± 2.8
Eleventh	99.6 ± 11.4	159 ± 10	77.0 ± 3.7	194 ± 7	94.5 ± 2.1
Twelfth	98.8 ± 9.4	159 ± 8	78.0 ± 2.9	194 ± 7	95.3 ± 1.7

AU: arbitrary units. HR: heart rate. bpm: beats-per-minute.

Changes in resting BRS measured as the sequence method and blood pressure are presented in Table 10.3. After adjustments for baseline there were no effects of training for Seq++ (adjusted change in means CON=-2.4, HIIE-T=-4.2 ms·mmHg^{-1}; *P*=0.52; ES=0.30), Seq-- (CON=8.6, HIIE-T=6.2 ms·mmHg^{-1}; *P*=0.43; ES=0.37). Similar results were observed for SPB (CON=-2.3, HIIE-T=-2.4 mmHg; *P*=0.97; ES=0.01), and DBP (CON=1.8, HIIE-T= -3.7 mmHg; *P*=0.20; ES=0.62). However, PP decreased for CON compared to HIIE pre and post training (adjusted change in means CON=-5.1, HIIE-T=2.2 mmHg; *P*=0.027; ES=1.12). At DET, the changes in PP compared to POST were similar between groups (adjusted change in means CON=0.58, HIIE-T=1.58 mmHg; *P*=0.84; ES=0.09).

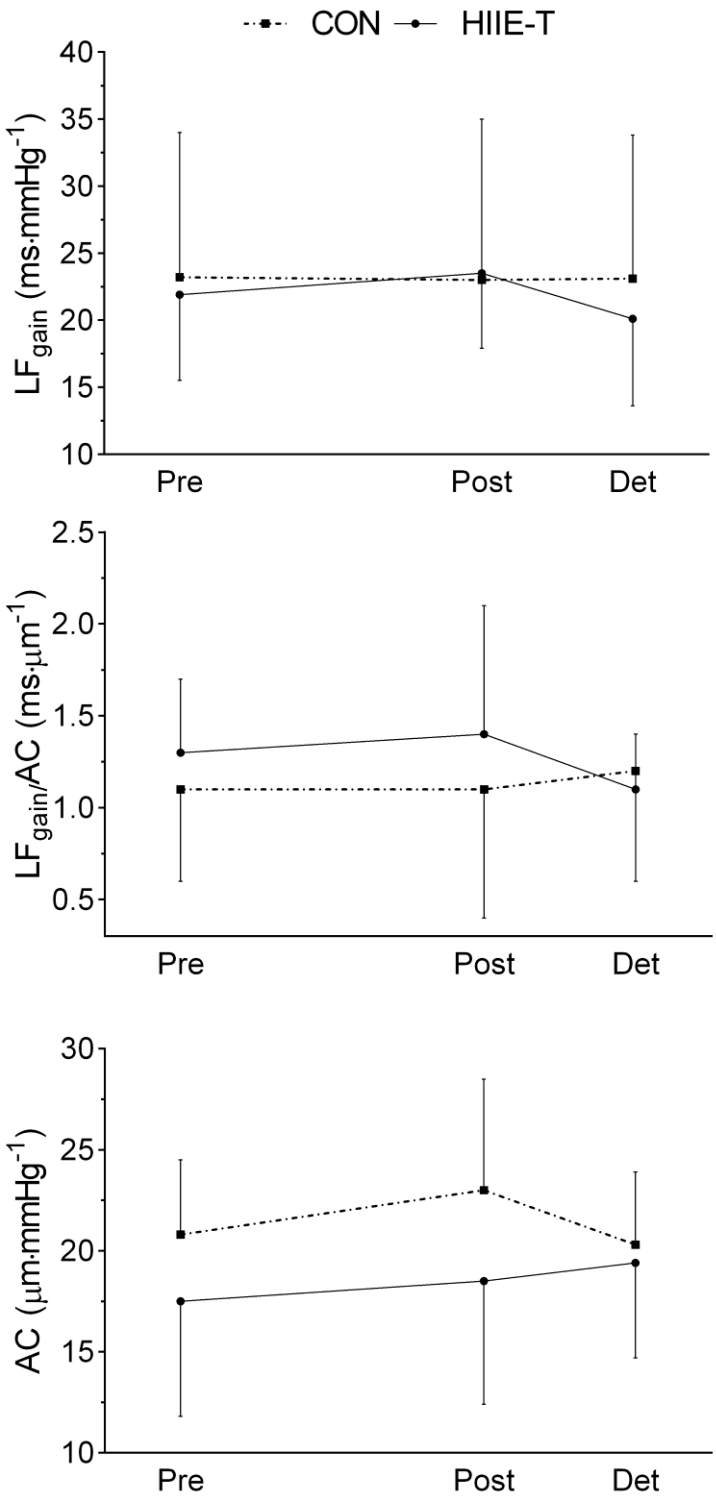

Figure 10.2: Mean and standard deviation of baroreflex sensitivity and its autonomic and vascular determinants.

Table 10.3: Mean and standard deviation of blood pressure, baroreflex sensitivity and arterial properties at pre, post and detraining for both groups.

	Pre		Post		Detraining	
	CON (n = 9)	HIIE-T (n = 10)	CON (n = 9)	HIIE-T (n = 10)	CON (n = 9)	HIIE-T (n = 10)
Seq++ ($ms \cdot mmHg^{-1}$)	35.4 ± 12.4	33.0 ± 7.2	32.2 ± 9.4	29.4 ± 3.9	32.3 ± 13.0	33.5 ± 8.2
Seq-- ($ms \cdot mmHg^{-1}$)	21.5 ± 11.0	20.7 ± 8.0	27.0 ± 12.0	23.7 ± 10.7	17.6 ± 11.1	23.5 ± 11.3
IMT (mm)	0.43 ± 0.03	0.43 ± 0.04	0.43 ± 0.02	0.44 ± 0.03	0.44 ± 0.03	0.44 ± 0.03
ΔD (μm)	950.0 ± 120.1	871.0 ± 197.6	970.0 ± 154.6	938.0 ± 234.4	986.7 ± 118.5	907.0 ± 142.8
AD ($mmHg \cdot 10^{-3}$)	7.5 ± 1.6	6.2 ± 2.0	8.2 ± 2.4	6.6 ± 2.0	7.1 ± 1.2	6.9 ± 1.7
IEM ($mmHg:10^{-3}$)	713.4 ± 125.9	910.8 ± 263.7	692.8 ± 208.8	854.3 ± 261.8	747.5 ± 123.4	785.8 ± 176.4
SBP (mmHg)	111.1 ± 6.3	111.8 ± 7.1	108.9 ± 11.0	109.3 ± 7.5	110.2 ± 12.3	110.2 ± 11.0
DBP (mmHg)	64.9 ± 8.2	60.1 ± 7.3	65.9 ± 11.9	57.2 ± 7.1	60.7 ± 7.9	61.8 ± 8.1
PP (mmHg)	46.2 ± 5.6	51.6 ± 8.7	43.1 ± 5.7**	52.1 ± 6.8	49.5 ± 7.2	48.4 ± 9.8

CON: Control group. HIIE-T: Training group. HR: heart rate. HF: high frequency. SBP: systolic blood pressure. DBP: diastolic blood pressure. PP: pulse pressure. IMT: intra-media thickness. ΔD: changes in lumen diameter from diastole to systole. AD: arterial distensibility. IEM: incremental elastic modulus. ***P*<0.05 for the adjusted PRE-POST changes in mean for CON compared to HIIE-T. For *P* values and effect sizes refer to text.

Changes in CCA properties are presented in Table 10.3. After adjustments for baseline, there were no effects of training for cIMT (adjusted change in means CON=-0.003, HIIE-T=0.005 mm; *P*=0.38; ES=0.41), ΔD (CON=18.4, HIIE-T=68.8 μm; *P*=0.32; ES=0.47), AD (CON=0.7, HIIE-T=0.3 mmHg·10^{-3}; *P*=0.59; ES=0.25), and IEM (CON=-33.5, HIIE-T=-44.9 mmHg:10^{-3}; *P*=0.88; ES=0.06).

Resting cardiac autonomic modulation is presented in Table 10.4. After adjustments for baseline values, there were no effects of training for HR (adjusted change in means CON=1, HIIE-T=-4 bpm; *P*=0.10; ES=0.80), HF (CON=-0.01, HIIE=0.13 ms^2; *P*=0.58; ES=0.26), and HF adjusted to HR (CON=0.001, HIIE-T=0.011 $ms^2 \cdot bpm^{-1}$; *P*=0.20; ES=0.61), total power (CON=0.41, HIIE-T=0.57 ms^2; *P*=0.75; ES=0.14), LF (CON=0.44, HIIE-T=0.41 ms^2; *P*=0.94; ES=0.04), LF nu (CON=7.7, HIIE-T=4.3 au; *P*=0.52; ES=0.30), HF nu (CON=-7.7, HIIE-T=-4.4 au; *P*=0.52; ES=0.30), and LF/HF ratio (CON=0.14, HIIE-T=0.09 au; *P*=0.61; ES=0.24).

No significant associations were observed between accumulated training loads and changes in the outcomes (all r <0.5; *P*>0.05).

Table 10.4: Mean and standard deviation of autonomic modulation at pre, post and detraining for both groups.

	Pre		Post		Detraining	
	CON (n=9)	HIIE-T (n=10)	CON (n=9)	HIIE-T (n=10)	CON (n=9)	HIIE-T (n=10)
HR (bpm)	63±6	60±8	62±8	57±5	60±7	59±7
HF (ln)	8.7±0.9	8.6±0.8	8.7±0.9	8.7±0.8	8.7±0.8	8.7±0.8
HF adjusted HR (au)	0.14±0.03	0.14±0.02	0.14±0.03	0.16±0.02	0.15±0.03	0.15±0.02
LF (ln)	7.3±0.5	7.0±0.7	7.7±1.03	7.4±0.7	7.8±0.6	7.4±0.8
Total power (ln)	16.0±1.3	15.6±1.4	16.4±1.9	16.2±1.2	16.5±1.3	16.1±1.4
HF (nu)	79.6±7.2	81.6±10.7	72.7±9.7	76.5±11.8	70.3±9.6	76.2±11.3
LF (nu)	20.3±7.3	18.1±10.7	27.2±9.8	23.4±11.9	29.6±9.7	23.5±11.4
LF/HF (au)	0.26±0.10	0.25±0.19	0.40±0.19	0.34±0.24	0.44±0.18	0.34±0.23

CON: Control group. HIIE-T: Training group. HR: heart rate. HF: high frequency. LF: low frequency.

10.4.2 Effects of high-intensity interval exercise training on acute post-exercise responses

The observed internal training load for the exercises bouts were 72.3 ± 8.6 and 77.2 ± 10.6 au at PRE, and 61.4 ± 8.9 and 73.0 ± 5.3 au at POST for HIIE-T and CON, respectively. After adjustments for baseline values, an effect of training was present on the changes in the internal training load between the groups (adjusted change in means CON=-3.3, HIIE-T=-12.0 au; P=0.042; ES=1.16).

The effects of training on post-exercise measurements are presented in Figure 10.3. For LFgain and LFgain/AC, an effect of time was present (P<0.001; P<0.001 for LFgain and LFgain/AC, respectively). On the contrary, there was no group by time (P=0.96; P=0.36), group by moment (P=0.96; P=0.95), time by moment (P=0.74; P=0.15), or group by moment by time interactions (P=0.47; P=0.22). Follow-up

analysis revealed that at both PRE and POST, LFgain decreased 30-min post HIIE compared to baseline for both groups (P<0.001) and returned to baseline values 120-min post-HIIE (P=0.07 compared to baseline). LFgain/AC mirrored the observed LFgain findings. At PRE and POST LFgain/AC decreased 30-min post HIIE compared to baseline for both groups (P<0.001) and returned to baseline values 120-min post-HIIE (P=0.11).

For AC, there was no effect of time (P = 0.61), group by time (P = 0.18), group by moment (P = 0.61), or group by moment by time (P = 0.91) interactions. On the contrary, a time by moment interaction was present (P = 0.041). Follow-up analysis revealed that at PRE, no differences were observed between baseline, 30-min and 120-min post for both groups (P = 0.61). However, at POST a time effect was present with an elevated AC at 30-min post compared to baseline (P = 0.006), which returned to baseline at 120-min post (P = 0.29) for both groups.

The effects of training on post-exercise autonomic modulation are presented in Table 10.5. For HR and HF, an effect of time was present (P < 0.001; P < 0.001 for HR and HF, respectively). On the contrary, there was no group by time (P = 0.96; P = 0.19), group by moment (P = 0.96; P = 0.40), time by moment (P = 0.74; P = 0.22), or group by moment by time interactions (P = 0.47; P = 0.31). Follow-up analysis revealed that at PRE and POST, HR increased 30-min post HIIE compared to baseline for both groups (P < 0.001), and returned to baseline values 120-min post-HIIE (P = 0.07 compared to baseline). HF (ln) mirrored the observed HR findings. At PRE and POST, HF decreased 30-min post HIIE compared to baseline (P < 0.001), and returned to baseline 120-min post (P = 0.11). For MAP there was no effect of time (P = 0.81),

group by time (P = 0.47), group by moment (P = 0.56), time by moment (P = 0.21), or group by moment by time interactions (P = 0.12).

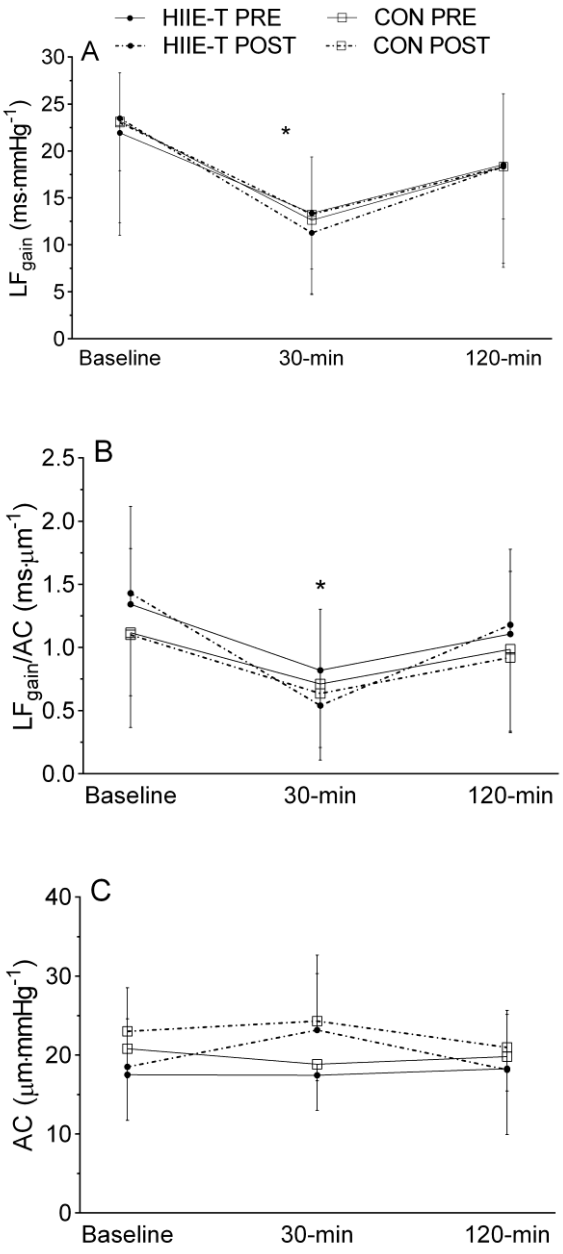

Figure 10.3: Mean and standard deviation of baroreflex sensitivity and its autonomic and vascular determinants at baseline, 30-min and 120-min following HIIE. *P<0.05 compared to baseline for both groups.

Table 10.5: Mean and standard deviation of the baseline and post-exercise arterial properties, blood pressure and autonomic modulation pre- and post-training for both groups.

		CON			HIIE-T		
		Baseline	30-min post	120-min post	Baseline	30-min post	120-min post
PP (mmHg)	Post	970.0±154.6	932.2±183.6*	917.8±152.5*	938.0±234.4	846.0±181.8*	828.0±214.7*
	Pre	46.2±5.6	46.1±4.7	46.1±6.2	51.6±8.7	47.9±4.8	49.2±9.1
MAP (mmHg)	Post	43.1±5.7	39.9±7.0*	44.5±5.6	52.1±6.8	38.5±9.4*	47.7±7.9
	Pre	78.8±7.3	80.1±3.9	79.5±6.7	75.6±6.0	76.1±4.0	76.4±5.0
HR (bpm)	Post	78.8±11.3	72.7±7.0	75.3±5.3	72.8±6.8	74.0±3.9	73.6±6.0
	Pre	63±6	78±9a	71±9	60±8	75±10b	64±7
HF(ln)	Post	62±8	74±9a	67±11	57±2	67±7b	61±4
	Pre	8.7±0.9	7.2±1.3a	8.2±1.4	8.6±0.8	7.8±1.4b	8.2±1.0
	Post	8.7±0.9	7.5±1.4a	8.1±1.5	8.7±0.8	8.1±1.1b	8.5±1.0

CON: Control group. HIIE-T: Training group. PP: pulse pressure. MAP: mean arterial pressure. HR: heart rate. HF: high frequency. *= effect of time course compared to baseline independent of group.

10.4.3 Associations between post-exercise responses and changes in baseline measurements after training

The observed Δ change in LFgain 30-min post-exercise was positively associated to the Δ training changes in the LFgain (r=0.55; *P*=0.014). Similar results were observed for Seq++ (r=0.60; *P*=0.007) and Seq-- (r=0.65; *P*=0.003). No other significant associations between post-exercise responses and changes with training were observed.

10.5 Discussion

This is the first randomised controlled trial to investigate the effect of HIIE training on resting and following acute exercise BRS and its autonomic and vascular determinants in healthy adolescents. The main findings of the present study were: 1) there was no effect of HIIE training on resting cardiac BRS and its autonomic and vascular determinants in healthy adolescents; 2) because no effect of training was observed, detaining did not influence any of the outcomes; 3) BRS was decreased at 30-min following HIIE due to a lowered autonomic determinant, which was not influenced by HIIE training; and 4) significant negative associations were observed between baseline decreases in BRS 30-min following HIIE, and the delta changes in BRS after 4-weeks of HIIE-T or CON. However, the lack of a training effect on BRS shows that the observed associations reflect lower variability in the BRS measurements for the adolescents with a sustained BRS function 30-min following HIIE.

10.5.1 Effects of training on baroreflex sensitivity and its autonomic and vascular determinants

Our present findings showed that resting BRS does not change after four weeks of HIIE training in healthy adolescents. The present observations are in accordance with previous results in adults showing that following five months of aerobic training at 75% of maximum aerobic capacity no changes in BRS were observed (Loimaala et al., 2000). However, one investigation including old adults (56 years old) have shown increases in BRS following 12 weeks of aerobic training at 65% of maximal aerobic capacity (Monahan et al., 2001a). Although the intensity (i.e. moderate vs high-intensity) and duration (12-weeks vs 4-weeks) of the interventions were different, the discrepant results indicate that age may be an important characteristic to consider when BRS is the outcome. This possible age effect may be related to differences in arterial stiffness between old and young subjects as evidenced by a decrease in CCA distensibility from childhood to adulthood (Lenard et al., 2004, Lenard et al., 2000). In fact, the observed improvements in BRS in old adults are driven by increases in CCA distensibility (Monahan et al., 2001a, Monahan et al., 2001b), which suggests that increases in CCA distensibility may be necessary for overall BRS improvement. This has been shown in one study with adults where concomitant improvements existed in BRS and arterial distensibility following 12 weeks of HIIE training (Heydari et al., 2013). The lack of increases in CCA distensibility in the current study showed that exercise training does not alter this parameter at rest, and corroborates recent findings suggesting the existence of a ‘ceiling effect’ as healthy arteries may already present an optimal CCA distensibility (Montero et al., 2017).

If such a ceiling effect exists for the vascular determinant of BRS at rest, a possible influence of HIIE training towards an improved beat-by-beat control of BP in healthy

adolescents may occur via increases in the autonomic determinant of BRS. This is the first investigation to estimate the contribution of the autonomic and vascular components of cardiac BRS following a HIIE intervention. However, no significant improvements were observed in LFgain/AC. Although no investigation in adolescents exists to contrast our findings, our results are in accordance with a cross-sectional investigation involving old adults in which non-significant effects of training status exist on the autonomic determinant of BRS (Monahan et al., 2001b). Conversely, other cross-sectional data suggests that the autonomic determinant of BRS is higher in participants with a higher training status (Komine et al., 2009). Several caveats are worthy of mentioning when contrasting to the current study including: the cross-sectional design of the cited studies, the study population (i.e. adults, old adults), and the methods used to measure the autonomic component of BRS (i.e. vasoactive drugs, Valsalva manoeuvre, and spontaneous indices).

Corroborating with the lack of improvements on the autonomic determinant of BRS, in the present study no effects of HIIE intervention was noted on resting autonomic modulation measured via HRV. This is a surprising finding, as in a sample of similar healthy adolescents, two weeks of HIIE caused significant increases in HRV (Bond et al., 2015a). Although a lack of a control group impedes conclusions about the training effect, a possible explanation for the differences between our present findings and Bond et al. (2015a), may lie in the likely presence of saturation of vagal modulation in the present study. A saturation represents a HR point at which no more improvements in HRV can be observed (Kiviniemi et al., 2004). In our present investigation, a trend was observed for a decreased in resting HR with a large effect size between CON and HIIE-T (P=0.10; ES=0.80). We further normalised HF according to HR to decrease the saturation effects and a moderate effect size, although not significant, was then

observed between HIIE-T and CON (ES=0.61). It is recognised that 24 h HRV analysis is required to obtain a measurement of saturation (Kiviniemi et al., 2006); however the lack of improvements in HRV in the present investigation should be interpreted with caution. Finally, it is currently unknown whether increases in vagal modulation would reflect a better cardiac BRS.

In the present investigation, HIIE-T did not alter BP at rest. The lack of change in BP is in accordance with a meta-analysis comparing the effects of training on resting BP in healthy children (Kelley et al., 2003). It has been demonstrated a positive association between BMI (r=0.61) and consequent BP reductions with exercise training (Kelley et al., 2003). This can partially explain our findings as all the participants were below the 85th percentile for BMI. Similarly, no changes were observed in the present investigation on resting arterial properties. Our results are in accordance with a meta-analysis in adults, in which four randomised controlled trials did not alter carotid IMT (Huang et al., 2016). On the contrary, aerobic exercise training has been shown to decrease carotid IMT in obese and overweight children, and baseline BMI and training in min per week were significant predictors of the exercise effects (Garcia-Hermoso et al., 2017). In addition, although differences such as duration (12 – 24 weeks) and exercise intensities (50 – 70% of maximum heart rate) exist between the studies included in the meta-analysis and the current investigation, a key factor in the discrepant results may have been the weight status. Therefore, it is likely that our short intervention (4 weeks of HIIE-T) was not enough to induce vessel remodelling at the CCA in a sample of healthy normal weight adolescents. Future studies are necessary to investigate the effects of longer interventions, different intensities, and children at elevated CVD risk, such as hypertensive and overweight.

10.5.2 Effects of high-intensity interval exercise intervention on the acute responses to exercise

This is the first study to investigate the effects of HIIE-T on BRS and its autonomic and vascular components to a bout of acute HIIE. Our results show that HIIE training significantly changed the internal training load observed for a similar external stimulus. The decreased training load reflect a lowered sympathetic activation during the HIIE bouts following training, and a consequent lowered HR response during the HIIE session (Buchheit, 2014). Although no measures of sympathetic activity, such as catecholamine spill over, were conducted in the present study, a lowered TRIMP may reflect a diminished sympathetic response to exercise in healthy adolescents as an adaptation of the autonomic system to training.

However, despite a diminished sympathetic activity during the HIIE bout, similar post-exercise responses in BRS and its autonomic and vascular determinants were observed following HIIE-T. Our results are in accordance with the adult literature that has shown a decreased BRS and its autonomic and vascular components up to 60-min following the exercise bout (Studinger et al., 2003) and is comparable to baseline 145-min following exercise (Halliwill et al., 1996b). Contrary to our hypothesis, the present findings demonstrated that training did not changes the magnitude of the observed decrease in BRS flowing HIIE. Similarly, no significant effects of training were observed for the post-exercise hypotension. This may reflect the baseline BP of the participants, which may preclude decreases in BP following the HIIE bout, as previously suggested for adults (MacDonald, 2002). Furthermore, it is likely that lack of BP changes following HIIE in the present study, blunted possible BRS adaptation to HIIE-T. For example, trained adults experienced a greater decrease in BRS 30-min following HIIE which was paralleled with a higher post-exercise hypotension (Cote et

al., 2015a) indicating BRS resetting to a lower BP operating point (Smith et al., 2000). These findings may reflect a physiological adaptation of the baroreflex to support a lowered BP following exercise in the trained participants. Our results may indicate that post-exercise hypotension is a necessary adjustment that drives training adaptation (Devereux et al., 2015), or post-exercise BRS does not change with exercise training in healthy adolescents.

In the present study, BRS was decreased due to a lowered autonomic determinant at both PRE and POST-HIIE-T. Whether the decreases in autonomic determinant would serve as a mechanism to training adaptation as previously suggested (Buchheit et al., 2008) was not confirmed in the present investigation. The acute decreases in LFgain/AC observed following the first HIIE session was not associated with the changes in resting LFgain/AC and autonomic modulation following training. These results indicate that other exercise characteristics but not the recovery period (Luttrell and Halliwill, 2015), contributes to improvements in BRS with training; however, in the present study the lack of improvements with training and lack of other measurements during the training session do not allow this hypothesis to be investigated. Similarly, the HIIE intervention did not alter HRV responses acutely following exercise. These results are different compared to Buchheit et al. (2008), who found that 8 weeks of HIIE led to a significant faster parasympathetic reactivation in adolescents. The difference between the exercise intensity from which vagal reactivation was obtained in the present study (i.e. HIIE) compared to Buchheit et al. (2008) (i.e. running at 60% of MAS) may explain the differences. It is well stablished that HIIE causes a pronounced vagal withdrawal compared to lower exercise intensities (Stanley et al., 2013), that can take up to 90-min to return to baseline values (Stanley et al., 2013). Therefore, it is likely that the HIIE stimulus in the present study caused a pronounced

vagal withdrawal for both HIIE-T and CON groups and if a submaximal bout was used a faster vagal reactivation would be noted for the HIIE-T group following training.

Due to the possible ceiling effect which limits improvements on resting CCA distensibility (Montero et al., 2017), it can be hypothesised that a possible health-related benefit of exercise on arterial stiffness is via increases in CCA distensibility acutely in the hours following the exercise (Kingwell et al., 1997). In the present investigation, however, no influence of HIIE-T was noted on CCA distensibility in the two hours following HIIE. A possible mechanism leading to an improved CCA distensibility acutely following exercise at the post HIIE intervention would be an increased smooth muscle relaxation caused by nitric oxide (Wilkinson et al., 2004), or an improved myogenic response to subtle changes in pulsatile pressure occurring with the weekly repetition of the HIIE bouts (Davis, 2012). The lack of improvements observed in the present investigation, however, suggest no trainability of the acute responses to HIIE. Whether CCA distensibility in the post-exercise period responds to training, and the mechanisms associated, are worth future investigation.

10.5.3 Strengths and limitations

One strength of the present investigation was the randomised controlled trial design. Similarly, compliance with the exercise training was excellent at 100% for the HIIE-T participants. We also performed a comprehensive analyses of the autonomic and vascular determinants of the BRS using reliable methods (Oliveira et al., 2018a). Another strength of the present investigation was the timing between the end of HIIE-T and the post-training measurements. To avoid possible detraining or acute effects of the last bout on the autonomic and arterial systems, participants were tested 48-h after the last HIIE-T session.

A few limitations are worth noting. For example, the convenience sampling approach limits the findings to a specific sample of adolescents. Similarly, we could not control for the exercise activities undertaken by the participants outside the CON and HIIE-T interventions. It is likely that participants were involved in other exercise routines which increased the overall training load during the twelve weeks and a ceiling effect was present for the adaptations of the autonomic system, as previously described (Iwasaki et al., 2003). Another limitation is that for the measures of arterial compliance BP was not obtained at the CCA. Finally, the present study might be underpowered, however, according to a recent investigation a sample size of nine participants would be enough to detect a large effect on the LFgain/AC in healthy adolescents (Oliveira et al., 2018a).

10.6 Conclusions

A HIIE intervention of four weeks does not change resting BRS and its autonomic and vascular determinants in a sample of healthy adolescents. Additionally, with or without HIIE training, BRS decreases 30-min following exercise in adolescents, but is restored at 120-min post. At both PRE and POST training, the autonomic component is the main determinant of the observed fall in BRS. Our findings highlight that in healthy adolescents HIIE training does not change the mechanisms of beat-to-beat control of BP both at rest and following acute exercise.

Chapter 11: Implications and Future Directions

To further contribute to the discussions presented within Chapters 4 – 10, this chapter aims to address how the collective work presented in the thesis contributes to the literature, and to discuss potential practical applications. Central themes discussed in Chapters 4 – 10 will be revisited and extended, and avenues for future research will be highlighted. This chapter also addresses the strengths and limitations of the work presented in the thesis before finishing with some concluding remarks.

11.1 Contributions to the literature

11.1.1 Risk factor gap

Around 40% of CVD risk reduction accrued by PA has been hypothesised to occur via changes in the autonomic and vascular systems (Joyner and Green, 2009), and a novel feature of this thesis was to expand this concept to healthy adolescents. Chapter 4 aimed to systematic review the literature to investigate associations between PA, CRF and cardiac autonomic function to contribute to the risk factor gap in youth. Chapter 4 evidenced that the literature was not clear regarding possible associations between resting HRV, PA and CRF in youth. This was because the high level of bias between the studies, the variety of methodological approaches to assess HRV, PA and CRF, participants age, and sample bias. Despite these limitations, MVPA showed a positive association with resting HRV in three of the studies with low bias, suggesting health benefits may be obtained via PA through the autonomic pathway in youth. To further investigate the role of PA on the risk factor gap in adolescents, Chapter 5 aimed

to investigate the associations between habitual PA, PA intensities, CRF, HRV and HRR in health adolescents. In addition, Chapter 5 also examined whether adding indices of autonomic function to a clustered CVD risk factor score based on traditional risk factors improved the strength of associations between CVD risk PA and CRF. A threefold increase in the magnitude of the negative association between MPA and VPA to the CVD risk was observed, suggesting that the association between PA and autonomic function expands beyond the associations between PA and clustered traditional CVD risk. These findings are in line with a short two-week HIIE training intervention where improvements in autonomic function were observed without improvements in traditional CVD risk factors (Bond et al., 2015a), contributing to the risk factor gap concept.

The exact mechanism behind these adaptation in humans are yet unclear, but animal models showing improvements in HRV following aerobic training indicate adaptations in the central autonomic centres in the brainstem dependent on the NO pathways in the paraventricular nucleus (Mastelari et al., 2011). Extrapolation of these findings to humans is speculative, but central adaptations are likely consequences of exercise. Another likely mechanism is an increased cholinergic signalling at the sinoatrial node. This mechanism may explain the findings of Chapter 5, in which both resting HRV and HRR, as well as a faster parasympathetic reactivation were positively associated with MPA and VPA levels. Giving support to this hypothesis, adult literature found a dissociation between HRR and resting HRV, with the first reflecting the cholinergic signalling at the heart, and the later a central modulation of HR (Dewland et al., 2007).

At first glance these results appear to corroborate the current PA guidelines. However, further scrutiny of the data reveal that the amount of MVPA performed by the

participants of Chapter 5 was 115 min·day^{-1}, which nearly doubles the minimum guideline of 60 min·day^{-1}. These findings are in accordance with Andersen et al. (2006) who also showed a decrease in cluster of traditional CVD risk factors for adolescents performing more than 90 min·day^{-1} of MVPA. However, data presented in Chapter 5 is cross-sectional which impedes causality and future investigations are needed to investigate participants with lower PA levels and to investigate cause-effect of PA on autonomic function beyond traditional CVD risk factors. Despite the limitations, the work within this thesis provides a foundation about the role of autonomic function to the risk factor gap. Future studies, employing either prospective of randomised controlled trial designs, should aim to address a possible dose-response to further contribute to the knowledge presented in this thesis.

Previous research in adolescents aged 14 years old has demonstrated that improvements in autonomic function following HIIE training are not significantly associated with improvements in arterial function (Bond et al., 2015a), which may indicate that exercise alters these systems in an independent manner. A novelty of the present thesis was to investigate a possible interaction between the vascular and autonomic systems by assessing cardiac BRS and its autonomic and vascular determinants. In youth, using this approach it has been previously demonstrated that from childhood to adulthood BRS gain is maintained via an increase in the autonomic determinant, as the vascular determinant is progressively lowered as age increases (Lenard et al., 2004). The work in Chapter 7 aimed to determine the between- and within-day reliability of the autonomic and vascular determinants of baroreflex sensitivity in adolescents. This is a novelty of this thesis and adds to the literature by demonstrating that separating BRS into the autonomic and vascular components is a reliable approach in male adolescents. This is important for future research to identify

appropriate study sample sizes as previously suggested (Hopkins, 2000), and to facilitate interpretation of the magnitude of change in BRS due to exercise or disease in adolescents.

Before the completion of this thesis, no studies had investigated how exercise changes the determinants of BRS in adolescents. For this purpose, Chapter 8 aimed to address how moderate and high-intensity interval running alters the post-exercise recovery of BP, BRS and its autonomic and vascular determinants; and Chapter 10 aimed to investigate the autonomic and vascular adaptations of BRS in response to four weeks of HIIE training and two weeks of detraining. In addition, Chapter 10 also investigated the acute changes in BRS following exercise and its autonomic and vascular determinants were also examined at before and after four weeks of HIIE training. The work completed in Chapter 8 and 10, in addition to the reliability data, contribute to this gap in knowledge about BRS function in youth by providing evidence that: 1) following HIIE (Chapters 8 and 10) and MIIE (Chapter 8) the BRS gain is decreased due to a lowered autonomic determinant which reflects on the control of arterial BP; and 2) four weeks of HIIE training does not change the autonomic and vascular determinant of BRS in a sample of healthy adolescents. Future studies are needed to investigate longer training durations in healthy adolescents and to investigate the effects of HIIE training in adolescents with elevated risk. Nevertheless, the findings of this thesis adds to an existing body of research that was previously limited to investigations in adults showing that the vascular determinant is important for BRS maintenance with ageing following an exercise intervention (Monahan et al., 2001a), that the BRS autonomic component is elevated in adults who engage in exercise (Komine et al., 2009), and that up to 30 min following moderate continuous exercise

both the autonomic and vascular determinants are lowered in adults (Willie et al., 2011).

11.1.2 Physical activity intensities

Current PA guidelines to youth combine MPA and VPA into MVPA, with only an emerging discussion about the independent effects of VPA and MPA intensities. The guidelines only suggest that youth should perform VPA at least three times per week without considering duration (WHO, 2010), with a main focus on bone and muscular strength developments. However, contemporary research indicates that MPA and VPA should be considered separately in terms of modifying CVD risk (Fussenich et al., 2016, Carson et al., 2014, Barker et al., 2018). This rationale underpinned the work presented in Chapters 4 – 10, and the present findings in this thesis further contributes to the evidence base by presenting the separate associations for MPA and VPA with cardiac autonomic function. Chapter 4 showed that the associations between VPA and cardiac autonomic function were controversial, which a lack of significant association between VPA and resting autonomic function. The level of evidence for associations between VPA and cardiac autonomic function was weak due to high bias in the studies, the limited number of high quality investigations comparing VPA levels, different exposure (i.e. questionnaires and accelerometers) outcome assessment (i.e. different HRV indices), and different age groups. Considering the limitations presented in Chapter 4, Chapter 5 showed an important positive association between objectively measured VPA and cardiac autonomic function in youth. Specifically, CVD risk calculated without the inclusion of autonomic function and only with traditional CVD risk factors was not significantly associated with VPA (stβ = -0.340; r^2 = 0.06). On the contrary, when autonomic function was added to the CVD risk score, a negative association between VPA and CVD risk score (stβ = -0.540; r^2 = 0.18) was present

indicating that VPA is associated with autonomic function but not traditional CVD risk factors in Chapter 5. With these findings, and others (Barker et al., 2018, Fussenich et al., 2016, Carson et al., 2014), an initial argument can be raised for guidelines to focus on VPA separately to MPA.

It can be speculated that one of the mechanisms underpinning the observed associations are the on and off kinetics of the autonomic activity to the vigorous bouts which triggered central (i.e. autonomic modulation measured as HRV) and local (i.e. cholinergic receptors at the heart level) adaptations. This mechanism is founded on the well described parasympathetic withdrawal during exercise, which is dependent on the exercise intensity (Pecanha et al., 2017). A pronounced parasympathetic withdrawal during exercise causes a delayed parasympathetic recovery and in the long term this pattern can improve central and peripheral mechanisms of autonomic function in youth.

These are initial steps, as the current VPA evidence for youth is not strong compared to the evidence gathered from prospective observational and experimental studies in adults, which have permitted the guidelines to recommend that time spent performing VPA (i.e. 75 min·week^{-1}) can be half of the time spent performing MPA (Bull, 2010). Furthermore, in this thesis only cross-sectional associations were investigated, and lack of cause-effect is still evident. Future studies are needed focusing on longitudinal associations between VPA and autonomic function, as previously observed for VPA and arterial compliance(van de Laar et al., 2010), as well as traditional CVD risk factors (Carson et al., 2014).

The results in favour of VPA in the present thesis are promising due to the amount of this activity performed by the participants compared to MPA in Chapter 5 (12 vs 104

min·day^{-1} of VPA and MPA, respectively). Indeed, time spend in different intensities appears to be an important aspect in the literature, with an existent negative association between 7 – 25 min·day^{-1} of VPA, but not ~ 50 min·day^{-1} of MPA, linked with improvements in traditional CVD risk factors in youth (Fussenich et al., 2016, Barker et al., 2018). Similarly, promising results of improvements in arterial and autonomic function in apparently health youth have been obtained from studies using HIIE interventions as a form to deliver VPA (Costigan et al., 2015, Logan et al., 2014, Bond et al., 2015a, Buchheit et al., 2008), suggesting HIIE training is an attractive strategy for future research. However, caution should still be taken when making recommendations to improve cardiac autonomic function of youth based on the limited body of evidence.

11.1.3 Cardiorespiratory fitness

The systematic review (Chapter 4) and the observational work (Chapters 5) within this thesis failed to present any significant associations between CRF and cardiac autonomic function. The evidence presented in the systematic review in Chapter 4 showed that diverse factors such as different methods of assessing CRF, quality of the studies included, and the confounders accounted for, clouded possible conclusions about the associations between CRF and autonomic function. With the limitations obtained in the systematic review in Chapter 4, Chapter 5 included CRF as an exposure and also failed to identify a significant association between CRF and cardiac autonomic function, after adjusting for key confounders. This is on the contrary to the inverse association obtained between CRF and traditional CVD risk in Chapter 5 (stβ = −0.438), in accordance with an existent body of evidence showing significant associations between indirectly measured CRF and traditional CVD risk factors in youth (Barker et al., 2018, Ekelund et al., 2007). Interestingly, when CRF was

allometrically scaled in Chapter 5 the positive association between CRF and autonomic outcomes approached significance (stβ = 0.299; *P* = 0.06). This highlights that body size may be an important confounder of the associations likely due to the negative association between body fat content and HRV as previously demonstrated in youth (Eyre et al., 2014). Finally, Chapters 4 and 5 provide initial steps into the associations between CRF and autonomic function, and future studies are encouraged to investigate longitudinal associations, similar to data that is available for CRF and arterial compliance (Ferreira et al., 2003).

11.1.4 Acute responses to exercise

11.1.4.1 Arterial responses to exercise

The acute physiological adjustments following exercise have been implicated in the health related befits of the last exercise bout (Bond et al., 2017a) and suggested to provide a foundation for future training adaptations (Romero et al., 2017, Luttrell and Halliwill, 2015, Devereux et al., 2015). This rationale drove the investigation of how exercise acutely alters the autonomic and vascular systems in youth. Following HIIE and MIIE in Chapter 8, AC and AD were elevated compared to baseline at five min but returned to baseline at 60 min. In Chapter 10, AC and AD were not altered by the exercise bout. It is likely that the observed drop in BP in Chapter 8, but not 10, explains the observed findings. The reasons why a divergent BP response were observed between chapters is unclear, but the literature suggest the presence of responders and non-responders for post-exercise hypotension (Costa et al., 2016), which may be a factor. To provide mechanistic data about these findings is beyond the scope of this thesis, however the pattern is consistent with an improved NO dependent dilation observed in adolescents in the brachial artery (Bond et al., 2015c), suggesting potential similar mechanisms. Another potential explanation is the presence of

vasodilatory substances, such as histamine (Halliwill et al., 2014). It is important to note that both NO and histamine production are dependent on shear stress (Ando and Kamiya, 1993), which may be a key component of short high intensity exercise bouts due to an elevated blood pressure during the exercise session (Studinger et al., 2003).

Due to described decreases in arterial compliance with ageing (Lenard et al., 2004), a central theme within this thesis was that adult findings have limited application to youth. A recent investigation has demonstrated divergent AC and AD responses to exercise between children and adults, also suggesting that maturation alters AC and AD following exercise (Melo et al., 2016). As such, in Figure 11.1 participants from Chapter 8 were further divided into groups of less (n = 4; Tanner stages 1 – 3) and more mature (n = 8; Tanner stages 4 – 5). Although caution should be taken when interpreting these findings due to the small sample size, it can be observed that less mature adolescents increase CCA compliance and distensibility following exercise compared to the more mature participants. Although future research is warranted to confirm these findings, these results highlight different AC and AD responses following exercise between maturity groups. The mechanisms underpinning these findings are unclear and future investigations are needed to explore the effects of changes in sex hormones, vessel characteristics such as elasticity (Lenard et al., 2004), increases in stroke volume which is also associated with AC and AD (Myers et al., 2002), and the likely effect of decreases in PA and CRF levels (Reilly, 2016).

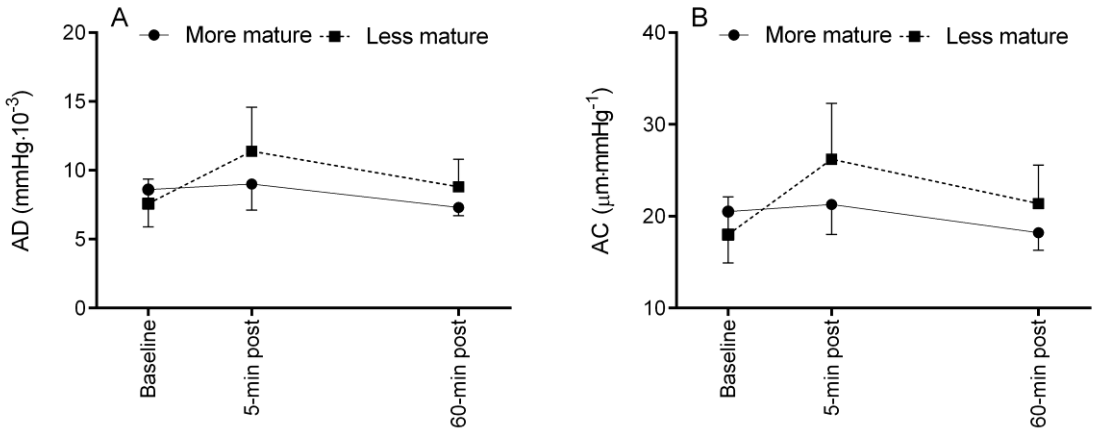

Figure 11.1: Possible effects of maturation on the dynamic responses to exercise. Participants are divided into less (n = 4) (squares) and more (n = 8) (circles) mature. Data are from Chapter 8.

11.1.4.2 Baroreflex responses to exercise

Combining the findings of Chapters 8 and 10, this thesis also expands adult findings, describing how exercise acutely changes BRS, to a paediatric population. For example, in accordance with the adult literature (Halliwill et al., 1996b, Niemela et al., 2008), BRS of healthy adolescents was lowered up to 30 min following interval exercise performed at a moderate and high-intensity. This is a novel finding and the present thesis provides a foundation for future work investigating mechanisms of BP control following exercise in youth. This is important from a physiological perspective as to date it is not clear whether adult findings translate to this population who may have more elastic vessels and a less mature autonomic function (Lenard et al., 2004). Additionally, BRS adjustments following exercise is attributed to the maintenance of a lowered BP (Halliwill et al., 2013), and future work is needed to expand the current findings to adolescents with hypertension who should benefit more of improvements on beat-to-beat control of BP.

The underpinning physiological mechanisms of the observed drop in BRS following exercise are unclear. It is likely that BRS gain following exercise resets due to an operating point reflecting a lower BP, normally observed following exercise (Halliwill et al., 2013), which results in a decrease of the overall BRS gain (Hart et al., 2010). Contributing to this mechanism, Chapter 8 is the first investigation to show a concomitant fall in BP and BRS gain in healthy adolescents following exercise, and a maintained lower BP one hour following HIIE but not MIIE. These results highlight an intensity dependent effect on the control of BP following exercise, strengthening the case for HIIE. Future work is still needed to follow-up these initial findings, but a drop in BP following exercise may have clinical importance in youth as suggested for adults (Kenney and Seals, 1993).

On the contrary to Chapter 8, in Chapter 10 BRS gain was decreased post-exercise without a concomitant fall in BP, suggesting a different mechanism orchestrating the drop in the gain of the BRS curve. A possible explanation is that to a same operation point (i.e. similar BP following exercise compared to baseline), BRS gain was lowered due to decreased central autonomic processing. Future studies using the neck suction technique would allow identification of the BRS gain over the whole baroreflex curve (Raven et al., 2006), and provide insight into the mechanisms by which exercise changes BRS acutely in youth.

Another novelty of the present work was to divide BRS into a vascular and autonomic determinant and investigate the acute effect of moderate and high-intensity exercise. This approach was limited to studies involving adults before the completion of this thesis, with a reported decrease in BRS gain up to 30 min following the exercise bout driven by a lowered vascular and autonomic determinant (Willie et al., 2011). The

present thesis observed a drop in BRS which was mainly driven by a decreased autonomic component. This may reflect discrepancies in the vascular response between young and old, with the former presenting an increase vascular compliance response to exercise (Chapter 8) and the latter a decrease in vascular compliance (Willie et al., 2011, Studinger et al., 2003). A higher elastic component of the arterial wall in youth, as evidenced by an elevated incremental elastic module (Lenard et al., 2004), is a candidate mechanism to explain the differences. Future studies are needed to investigate the effects of different vessel elasticity, caused by maturation or disease, on the BRS responses to exercise.

11.1.5 Chronic exercise

As suggested in section 11.1.2 HIIE training is an interesting strategy to deliver VPA and studies show promising results of this type of intervention to improve autonomic and vascular function in youth (Costigan et al., 2015, Logan et al., 2014, Bond et al., 2015a, Buchheit et al., 2008). With this rationale, Chapter 10 aimed to investigate the autonomic and vascular adaptations of BRS in response to four weeks of HIIE training and two weeks of detraining. Although in Chapters 5 VPA levels were positively associated with resting HRV, Chapter 10 did not show increases in this outcome after four weeks of HIIE training. The differences between the cross-sectional designs of Chapters 4 and 5, and the randomised controlled trial of Chapter 10 may explain these discrepant findings. Specifically, a higher amount of MPA and VPA can be associated with a given HRV, however, training may not cause increases in HRV in a healthy sample of adolescents who present an elevated HRV at baseline. This was observed in Chapter 10 and may reflect the participants' high baseline PA levels, influencing the ability of exercise to improve HRV. As such, improvements in HRV in youth may be dependent on the baseline values as previously shown by Nagai et al. (2004) who

divided participants into groups of low and high HRV (Ln of HF = 5.5 and 6.6 ms^2, respectively). Following 12 months of training, only the participants in the low-HRV group significantly increased HF. In Chapter 10, HF at pre HIIE intervention was 8.6 ms^2 which was similar to Chapter 5 (Ln of HF = 8.0 ms^2) where positive associations between HRV, MPA and VPA were observed. These values were also higher than the ones reported by Nagai et al. (2004). A possible explanation is the saturation phenomenon observed in participants with elevated parasympathetic modulation, which impedes further improvements in HRV (Kiviniemi et al., 2004). Future randomised trials are needed including a longer training intervention and lower HRV levels before the exercise intervention.

The work within this thesis also showed a lack of improvement in BRS gain following the exercise intervention. Unfortunately, further mechanistic explanation cannot be provided, but is likely that a possible increase in aortic BRS was superior to improvements of the carotid BRS, as described in trained compared to untrained adults (Smith et al., 2000). Future investigations are encouraged to elucidate different mechanisms of aortic and carotid BRS in youth which can be assessed by measuring aortic distensibility using ultrasound images of the aorta (Klassen et al., 2016). Another possible explanation, is a change in the amplitude of the total BRS response curve, without changing the operating point (Smith et al., 2000). However, our present BRS data are limited to the assessment of the BRS operating point, and future studies are encouraged to investigate possible alteration on the BRS curve caused by exercise in healthy adolescents using the neck suction method.

It can be also speculated that adaptations of BRS to exercise training are dependent on increases in the vascular component of BRS with consequent improvements in the

autonomic determinant. For example, following 12 weeks of aerobic training, increases in vascular compliance are necessary to lead to a better autonomic control of BP in adults aged 56 years old (Monahan et al., 2001a). If a ceiling effect for the vascular component exist in healthy youth, as recently evidenced in adults (Montero et al., 2017), the ability of exercise to increase overall resting BRS may be limited in healthy youth. Less clear is the autonomic determinant, as the central mechanisms controlling autonomic function in humans are difficult to assess. It is possible that central nervous system adaptations were not present, or saturation of vagal modulation also exist in healthy adolescents (Kiviniemi et al., 2006, Kiviniemi et al., 2004). To avoid a possible saturation effect, future studies are encouraged to investigate the autonomic and vascular determinants in children at a higher CVD risk, such as obese, diabetic or hypertensive youth, or youth with low levels of PA and CRF. Finally, this thesis was limited to the investigation of only one model of exercise and alternative models of interventions such as football, basketball, rugby, amongst others team sports are also encouraged due to the high-intensity characteristics of these exercises (Krustrup et al., 2014).

11.1.6 Postprandial state

Given that increases in [TAG] and [GLU] during the postprandial state in youth are associated with mortality in adulthood (Morrison et al., 2009, Franks et al., 2010), the work within thesis also addressed the postprandial state. Chapter 6 aimed to investigate the associations between PA intensities, CRF and postprandial TAG, HRV, and arterial stiffness. There were no significant associations between postprandial lipaemia and MPA, VPA and CRF. It is worth highlighting that participants were asked to refrain from any strenuous exercise 48 h before completing the HFM protocol, and the findings replicate adult cross-sectional investigations were lack of exercise ~ 60 h

before the ingestion of the HFM was the main determinant of postprandial lipaemia compared to training status (Tsetsonis et al., 1997, Peddie et al., 2012, Herd et al., 2000). The present work in healthy adolescents further contributes to this topic and indicates that the effects of exercise on postprandial lipaemia is due to the last bout effect (Maraki and Sidossis, 2013). Future work is encouraged to investigate whether the lack of association between MPA, VPA and postprandial [TAG] in the present cross-sectional study is indeed due to the fact participants refrained from exercise in the 48 h before data collection took place.

Although extensions of these new and original findings are warranted, the lack of significant associations between PA levels and postprandial lipaemia might indicate that PA does not improve mechanisms by which exercise decrease postprandial responses. Candidate mechanisms are increases in lipoprotein lipase activity (Seip and Semenkovich, 1998), when exercise is performed around 16 h before the ingestion of the HFM (Tolfrey et al., 2014), or a greater hepatic fatty acid oxidation evidenced by a positive association (r = 0.61) between 3-hydroxybutyrate and TAG responses when exercise is performed around one hour before the HFM (Bond et al., 2015b). Future investigations isolating the mechanisms by which PA and CRF may alter postprandial lipaemia will further contribute to the identification of exercise protocols or duration of weekly PA needed to modulate postprandial lipaemia through PA.

In addition to the HFM in Chapter 6, Chapter 9 aimed to investigate whether the autonomic and vascular determinants of BRS are altered following an oral glucose challenge. This chapter also investigates whether exercise performed before the glucose challenge modifies the postprandial outcomes and is dependent on exercise

intensity. It was noted that in Chapter 6 HRV decreased following the ingestion of a HFM, whereas in Chapter 9 the ingestion of a glucose load led to an increase in HRV. The mechanisms by which a fat or glucose load may differentially alter the autonomic indices in healthy adolescents in unknown, however previous investigations have shown an increase in vascular function following the ingestion of GLU in children (Dengel et al., 2007). This is also somewhat dissonant of the observed drop in the BRS autonomic determinant following the ingestion of a glucose load in Chapter 9. It is likely that the rises in [GLU] led to increases in the sympathetic control of the vasomotor activity, reflected in an increase in the low-frequency component of BP variability (Floras, 2013). On the contrary, no concomitant effect of [GLU] was observed on the low-frequency of HRV (Chapter 9). As a consequence, the different effects of GLU on BP variability and HRV may explain the decrease of the overall BRS (Chapter 9), without modification of HRV.

In addition to the associations between habitual PA and postprandial state, the acute effects of exercise on postprandial responses were addressed in this thesis. It was hypothesised that an increase in oxidative stress caused either by the glucose or lipid load would decrease arterial compliance, via reduced NO bioavailability (Lacroix et al., 2012). However, in the present thesis vascular compliance and stiffness did not change in the postprandial state. Several aspects may underpin these findings. First, a decrease in NO bioavailability may not be associated with arterial stiffening (Horvath et al., 2012); second, glucose ingestion has been demonstrated to increase arterial function in healthy children (Dengel et al., 2007); and third, the local and regional arterial stiffness assessments in the present thesis may not suffer from postprandial effects, as different responses were reported in adults in the femoral but not peripheral arteries (Baynard et al., 2009). The lack of effects of the meals arterial function in the

present thesis therefore impede the hypothesis of the protective effects of exercise to be tested and future investigations are needed to investigate a possible dissociation between vascular function and stiffness following the ingestion of glucose or lipids and the role of exercise, PA and CRF.

11.2 Practical applications

11.2.1 Physical activity

Lack of PA is considered one of the main health problems in the world (WHO, 2014), with over 80% of adolescents worldwide failing to achieve a minimum of 60 min·day^{-1} of MVPA (Hallal et al., 2012). As such, it is important to highlight the amount of VPA performed by the adolescents in Chapter 5 in which positive associations were observed with rest and recovery autonomic function. Girls and boys who took part in Chapter 5 performed a total of ~ 6 and ~ 15 min·day^{-1} of VPA. This thesis also provided novel mechanisms by which a small dose of PA can alter overall health via its associations with cardiac autonomic function, as opposed to traditional CVD risk factors. Importantly, the identification of novel CVD risk factors is supported by the American Heart Association (Balagopal et al., 2011).Based on these findings, adolescents are encouraged to perform around 15 min·day^{-1} of VPA aiming to have high resting and recovery autonomic functions. It is also likely that girls benefit from a lower dose given that in Chapter 5 girls who performed 2 min·day^{-1} of VPA presented faster parasympathetic reactivation following exercise.

Given that the postprandial state in youth is positively associated with CVD risk in adults (Morrison et al., 2009, Franks et al., 2010), strategies to modulate postprandial lipaemia and glycaemia may decrease CVD burden in this population. Chapter 6 failed to demonstrate a significant association between VPA and MPA with postprandial

[TAG]. This was attributed to the fact that participants avoided exercise for 48 h. Based on these findings, and other experimental studies showing a blunted postprandial [TAG] and [GLU] response when exercise is performed on the day before (Tolfrey et al., 2014, Cockcroft et al., 2017b) or on the same day (Bond et al., 2014, Bond et al., 2015b, Cockcroft et al., 2015) (see also Chapter 9 where a moderate decrease in GLU was noted for HIIE), adolescents are encouraged to perform MPA and VPA on a daily basis, with it likely that VPA may promote a superior effect(Bond et al., 2015b).

11.2.2 Exercise intervention

The work within Chapter 10 has practical applications in a broad context of conducting an exercise intervention in a school setting. This is especially important as adolescents spend most of the day at school, and a recent review of systematic reviews concluded that school-based interventions are promising to increase PA and CRF in healthy youth (Kriemler et al., 2011). Furthermore, the work in Chapter 10 becomes especially interesting given the findings of a recent narrative review investigating the effects of HIIE interventions in a school setting (Bond et al., 2017a), highlighting the growth in number and quality of investigations in the last decade involving HIIE. The results of the review suggest that this type of intervention is promising for improvements in traditional and potentially novel CVD risk factors in youth (Bond et al., 2017a).

As such, given the excellent compliance (100%) with the HIIE protocol adopted in a school setting in Chapter 10, together with the fact that adolescents have reported to prefer HIIE over MIIE, and also find HIIE more enjoyable (Malik et al., 2018, Malik et al., 2017), the current HIIE regimen may be used in schools as a training intervention aiming to target VPA (total duration of at least 23 $min \cdot day^{-1}$). This protocol is

particularly attractive as it has minimum cost and can be performed before school activities with minimal equipment (e.g., cones and whistles).

Additionally, adolescents in a school setting typically face stressful situations such as mental arithmetic, public speaking and reaction time tasks. An exaggerated BP response to these situations is associated with CVD development in youth via changes in autonomic and vascular functions (Roemmich et al., 2014). Consequently, based on the findings from Chapter 8 in which a sustained decrease in BP lasting at least one hour was observed following 8 x 1 min of high-intensity running, HIIE at school is encouraged to this population, reinforcing another possible pathway that HIIE training at school can be used to target CVD risk reduction.

11.3 Strengths and limitations

One strength of this thesis is the variety of research designs, such as a systematic review, cross-sectional investigations, and experimental cross-over and randomised controlled trials, which were applied. Therefore, the presented work provides a significant and original contribution to the literature on changes in the autonomic and vascular systems through PA, CRF and exercise in adolescents. Additionally, a multitude of methodological approaches were used to investigate autonomic function, vascular stiffness and compliance, and the possible interplay between these systems at rest, in the postprandial state, and following acute and chronic exercise.

However, as in all research investigations, the findings should be interpreted considering several limitations. This is especially accentuated when working with paediatric groups which limits physiological measurements to non-invasive approaches. This is also extended to recruitment challenges at schools which inevitably leads to the inclusion of a biased sample of healthy and active volunteers.

For example, the levels of habitual PA measured in the participants in Chapters 5 and 6 are higher compared to the adolescent literature, as just one participant was not meeting the current PA guideline of at least 60 min·day^{-1} of MVPA. Similarly, only 13 of the 72 participants who took part in Chapters 5 – 9 presented CRF below the ideal cardiovascular health threshold suggested by Ruiz et al. (2014). In addition, only 21 (18%) of the 95 participants in Chapters 5 – 10 were classified as overweight/obese based on the BF%. As a consequence, data in the present thesis is mainly limited to active and fit participants with a healthy body weight status. This limitation is an important caveat, as CVD risk is increased in physically inactive, unfit, and overweight/obese adolescents.

This recruitment bias might reflect the obtained BRS and HRV data. For example, participants presented resting BRS of 24.4 ± 5.6 ms·mmHg^{-1} (Chapters 7 – 9) and 22.5 ± 8.3 ms·mmHg^{-1}(Chapter 10) which are higher than the percentile 95th obtained from a sample of 229 adolescents boys and girls (Zavodna et al., 2006). Similarly, participants in Chapter 5 were above the 75th percentile and participants in Chapter 10 were above 90th percentile for HRV indices taken from normative values published in a large (n = 1,152) sample of healthy adolescents (Farah et al., 2014).

Additionally, it is possible that the cut-off points used in the present thesis to quantify PA intensities may have miss-classified MVPA in Chapters 5 and 6. The validation was performed in laboratory conditions using a different sample of adolescents (Phillips et al., 2013), which limits the between-individual application of cut-off points. Cut-off points, however, are the best approach to divide PA into intensities considering feasibility of daily PA assessment (Loprinzi and Cardinal, 2011). Likewise, accelerometer data were treated using 60 second epochs which might have

underestimated the PA levels, especially VPA (Sanders et al., 2014). However, this is unclear as the literature has not yet addressed the effect of epoch lengths obtained from GENEActiv accelerometers. Similarly, although the ramp test applied to measure CRF in Chapters 5 and 6 has been validated previously (Bongers et al., 2013), CRF in Chapters 5 and 6 was obtained using an indirect assessment, which may have led to different estimations of CRF. The choice of an indirect CRF approach was based on the feasibility of performing the study in a school-based laboratory. Indirect assessment of CRF is also common practice and most of the literature shows associations between indirectly obtained CRF and CVD risk in youth (including Chapter 5) across a range of approaches such as peak power, shuttle run, and submaximal protocols (Ekelund et al., 2007).

Another limitation is that Chapters 7 – 10 included only adolescent boys, and it is not clear whether the findings extend to girls. Future studies involving girls are needed as when age matched to boys, girls are typically less active and less fit (i.e. findings in Chapter 4 and 5). Likewise, maturation has been shown to differently decrease arterial stiffness of girls, whereas in boys stiffening of the arteries are observed (Ahimastos et al., 2003). These observations suggest that responses to exercise training and PA may vary between sexes.

Although the present thesis provides initial ideas on the effects of PA intensities, CRF and exercise training on autonomic function in adolescents, the clinical significance of autonomic function in this population is not clear. Longitudinal studies are still warranted to identify the optimal autonomic function associated with a lowered CVD risk. However, as evidenced in the adult literature, which demonstrates the clinical significance of cardiac autonomic measures and its association with overt CVD

(Thayer and Lane, 2007), the present findings suggest protective effects of PA intensities in youth.

The postprandial lipaemia approach in Chapter 6 was completed using a two-hour protocol, with a single assessment of lipid concentration following the ingestion of the HFM. This inevitably led to an underrepresentation of the lipaemic response, which has been shown to be elevated up to four hours following the ingestion of a HFM in adolescents (Bond et al., 2014). This approach was used due to feasibility of performing the study in a school setting and presents ecological validity compared to laboratory approaches.

In Chapters 7 – 10 CCA compliance was determined using pulse pressure derived from the Finometer. The validity of determining CCA with pressure measures obtained distant from the carotid has been questioned (Steinback et al., 2005). This is likely to have influenced the measurements obtained. Likewise, measurements of endothelial/arterial function were not performed limiting the current approach to compliance assessments. Additionally, the autonomic determinant of BRS obtained in the present thesis, although used previously in adolescents (Lenard et al., 2004), has not been validated. Furthermore, the spontaneous BRS assessment is limited to the investigation of the operating point of the BRS curve (Schwartz et al., 2013), and the factors associated with increases and decreases in the gain may differ between chapters.

Finally, the HIIE intervention in the present thesis lasted only for four weeks and it is likely that a longer period of HIIE training would cause adaptations in the autonomic and vascular systems. However, to date a time-course of adaptation of the autonomic

and vascular system in youth has not been demonstrated and future studies are needed in this area.

11.4 Conclusions

The present thesis is the first to comprehensively investigate the role of PA intensities, CRF and exercise on cardiac autonomic and vascular stiffness in healthy adolescents. The work within this thesis shows that previous literature was unclear about the possible cross-sectional associations between PA intensities, CRF and resting cardiac autonomic function. The experimental chapters extend current knowledge showing a positive association for VPA and MPA on resting cardiovagal function, and a stronger influence of VPA on recovery of vagal activity following exercise. It was also demonstrated that the control of BP by the baroreceptors can be determined reliably in healthy adolescents. Furthermore, the intensity of the exercise stimuli is responsible for the acute observed differences in the interplay between the vascular and autonomic systems, assessed as BRS control of BP. Postprandial hyperglycaemia and hyperlipaemia had little influence on autonomic function, vascular stiffness, and the interplay between these systems via the BRS. Finally, HIIE training did not change BRS, arterial compliance and cardiac autonomic function at rest or at acute recovery following exercise.

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Appendix

Appendix 1 – Ethical approval

College of Life and Environmental Sciences
SPORT AND HEALTH SCIENCES

St. Luke's Campus
University of Exeter
Heavitree Road Exeter

EX1 2LU
United Kingdom

Certificate of Ethical Approval

Proposal Ref No: 160217/B/04

Title: The effect and dose-response-relationship of an acute bout of high-intensity interval running on cardiovascular disease risk factors in adolescents

Applicants: Sascha Kranen, Ricardo Santos Oliveira (PhD), Adam Abdul Malik (PHD), Emma Cockcroft (PhD), Owen Tomlinson (PhD), Dimitris Vlachopoulos (PhD), Kelly Wilkinson (MSc)

The proposal was reviewed by the Sport and Health Sciences Ethics Committee.

Decision: This proposal has been approved until November 2016

Signature: Melvyn Hillsdon Date: 15/3/2016

Name/Title of Ethics Committee Reviewer: Dr Melvyn Hillsdon

Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.

Appendix 2 – Recruitment documents

Children's Health and Exercise Research Centre
Sport and Health Sciences
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Participant Information Sheet

Study: The health benefits of 4 weeks of running training in adolescents
Researcher: Ricardo Santos Oliveira
Organisation: The University of Exeter
Version: V1. 12.01.2017

Thank you for your interest. Please read this information carefully and discuss

1. What is the purpose of the study?

This study will investigate how running training at a high intensity affects your blood vessels (in the arm and neck), blood pressure, heartbeats, and the amount of fat and sugar in your blood after training for four weeks.

2. What does this study involve?

This study will be completed in the laboratories of the Children's Health and Exercise Research Centre (University of Exeter), and in your school. You will be asked to complete four visits of tests at the university. Each day will last between 2 and 6 hours, and you will be picked up either from home or school in the morning and returned to home or school in the afternoon. In addition, you will be randomly drawn to take part in either a training group involving high-intensity exercise or a non-training (control) group. Details of each visit and the exercise training are outlined below:

Visit 1 (University of Exeter)

You will be required to not eat anything after 8 pm in the night before the visit and not eat breakfast on the morning of the visit. We will take a small amount of blood from your fingertip. Then, your height and weight will be measured, followed by the measurement of your body fatness. For the body fatness assessment, you will be asked to wear light clothes and enter in a capsule machine that looks like a spaceship. You will then be asked to lie down

for 10 minutes when your blood pressure will be monitored using a cuff around your finger, and after your neck and arm blood vessels will be scanned using ultrasound. A cuff will be inflated around your arm for 5 min during one of the scans. Afterwards, you will be asked to complete a shuttle run test. The test will start at a low speed, and then the speed will increase slowly until you reach exhaustion. During this test, you will wear a strap around your chest to measure your heart rate.

Visit 2 (University of Exeter)
You will be required to not eat anything after 8 pm as in the visit 1. On arrival at the laboratories, you will be asked to asked to lie down for 10 minutes when your blood pressure will be monitored using a cuff around your finger. Afterwards, your neck and arm blood vessels will be scanned using ultrasound. A cuff will be inflated around your arm for 5 min during one of the scans, as in visit 1. Then you will perform 8 high intensity runs of 1 minute with 75 s between the runs. After this, we will repeat the measurements of blood pressure, and the scans of the arteries in your neck and arm for two more times.

Visit 3 (University of Exeter)
This will be identical as visit 2, and will happen after four weeks of training.

Visit 4 (University of Exeter)
This will take place two weeks after the last training session and will be identical to visit 1, apart from you will not be asked to complete the running test to exhaustion.

After completing visit 1, you will be randomly drawn to join either a training or non-training (control) group.

If you are in the training group, you will complete running training for four weeks divided into 3 training sessions per week. The training will be performed and supervised at the school and will last 30 – 40 min:

- Weeks 1 and 2: One set of eight runs of 1 minute, with 75 seconds of rest between the runs
- Week 3: One set of ten runs of 1 minute, with 75 seconds of rest between the runs
- Week 4: One set of twelve runs of 1 minute, with 75 seconds of rest between the runs

If you are in the control group, you will be asked to complete the visits at the university. You will be completing your normal daily routines, but will be asked not to engage in exercise training programs for the duration of the study.

3. What else do I have to do?

You will need to bring suitable kit for exercise during the university visits (shorts, t-shirt and trainers). You will also be asked to wear a watch for several days during the study. You will also be asked to fill a food diary detailing the amount and type of food you eat for two days. Both food diary and watch devices will be delivered to you at your school and/or send by post to your home address. Participants in the training group will be asked to bring exercise kit for all training sessions which will take place at school. Finally, we will show you five pictures of

physical maturation and you will be asked to choose the one that best represents your current physical development. You will self-assess your physical development in privacy. It is a routine measure in studies involving children and adolescents. We would also encourage you to ask as many questions as you please. We hope that your participation in the project inspires you to think about your health and the potential of higher education.

4. What are the possible risks for me if I decide to take part?

All the procedures used in the study are regularly used in research with adolescents. The minimal risks include tiredness after the shuttle run test and you may feel light muscle pain in the two days following. You may experience a feeling of 'pins and needles' in your left hand during the cuff inflation which will disappear after cuff deflation. The blood sampling may cause a feeling of a slight pin prick. The completion of a health assessment form by you, together with your parents, prior to the study, will ensure your safety to participate.

5. What will be my gains from taking part in this study?

This study will look at how four weeks of high-intensity running training affects the health of your heart and blood vessels. Whilst this may not immediately benefit you, we hope that you will enjoy your participation in the project and the chance to be part of a scientific study. At the Children's Health and Exercise Research Centre, we pride ourselves on ensuring that each volunteer has an enjoyable and informative experience throughout every research project. We hope that we can inspire you to take an interest in your health and in the science of exercise, and that this project will be both interesting and fun. You will also receive certificates for participation in a scientific project that you can use in your CV, as well as feedback with your health status.

6. What will happen to the results of the study?

Your data will be stored in coded form to protect anonymity and will be completely confidential. This research will form part of a PhD thesis, and this study will also be submitted to relevant scientific journals for publication. Your information and data will not be identifiable in either of these instances. You will be sent a summary of the research findings once all data have been collected and analysed, as well as your individual data with a full explanation of what it represents should you so wish.

7. What should I do if I would like to take part?

Please note that your participation must be decided together with your parents. If you would like to take part in the study you and your parents must give your permission by completing the following forms which are included in this information pack.

- The contact information form
- The parental consent form
- The participant assent form
- The health screen questionnaire

You should then return these forms to the school reception. We will then make contact with the teachers and the school to arrange the schedule for tests.

Taking part is entirely voluntary and it is up to you to decide whether or not you will be involved. If you want to take part you are still free to withdraw at any time, without giving a reason. If you have any questions regarding the nature or purpose of this study, please feel free to contact Ricardo Oliveira (primary investigator).

Primary investigators:
Ricardo Santos Oliveira
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Parent/Guardian Consent Form

Study: The health benefits of 4 weeks of running training in adolescents
Researcher: Ricardo Santos Oliveira
Organisation: The University of Exeter
Version: 1. 12.01.2017

I have read the information sheet, version V1. 12.01.2017, regarding this project and understand the rationale for the study and what my child will be asked to do. I have had the chance to ask questions about the study, and I have received satisfactory answers to any questions I have asked.

Please put your initials in the small boxes to indicate you understand that:

- My child will complete four days of tests at the University of Exeter and he can withdraw whenever he wants. ☐
- My child will have his height, weight, and body fat measured. ☐
- My child will assess their own pubertal status using scientific drawings of secondary sexual characteristics. The purpose of this has been made clear to me. ☐
- My child will be asked to complete a shuttle run test until exhaustion. ☐
- My child will perform a bout of high-intensity running on two occasions. ☐
- My child will be randomly assigned to a training group or a control group. ☐
- If my child is allocated to the training group, he will complete 3 running training sessions at a high-intensity per week (~ 30 – 40 min each) for 4 weeks. ☐
- My child's arteries in the left upper arm and the neck will be scanned via ultrasound. ☐
- A cuff will be placed around my child's arm and will be inflated for five minutes. He ☐

might feel a sensation of 'pins and needles' in his/her hand.

- A small amount of blood will be taken from my child's fingertip on the testing days. He might feel a slight pinprick in his/her finger during this procedure. ☐
- My child will be asked to wear an accelerometer (a watch-like device to measure his physical activity levels) for several days during the study period. ☐
- My child will be asked to not eat after 8 pm the night before and to not have breakfast on the testing day to that I will be informed. 48 hours before the test day my child will be ask to complete a food diary. ☐
- I am free to request further information at any stage. ☐

I know that:

- My child's participation in the project is entirely voluntary and he is free to withdraw from the project at any time without giving reason. ☐
- The results will be stored confidentially on a computer for sole use by the Children's Health and Exercise Research Centre, University of Exeter. ☐
- The results of the project may be published but my child's anonymity will be preserved. ☐

Name……

Signed ……. (Parent/Guardian)

Date…….

On behalf of my child ……

Name……

Signed ……. (Researcher)

Date…….

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Participant Assent Form (to be completed by the child)

Study: The health benefits of 4 weeks of running training in adolescents
Researcher: Ricardo Santos Oliveira
Organisation: The University of Exeter
Version: 1. 12.01.2017

I agree to take part in the study as described in the information sheet version V1. 12.01.2017. The study has been clearly explained to me and I have had the opportunity to ask any questions I may have about my involvement in the study.

Please write your initials in the small boxes to indicate you understand that:

- I will complete four days of tests at the University of Exeter and I can withdraw from them whenever I want to. ☐
- I will have my height, weight, and body fat measured. ☐
- I will assess my pubertal status using scientific drawings of physical development. The purpose of this has been made clear to me. ☐
- I will be asked to complete a shuttle run test until exhaustion. ☐
- I will perform a high-intensity running session on two visits at the University. ☐
- I will be randomly drawn to a training group or a control group. ☐
- In case I will take part in the training group, I will complete 3 running training sessions at a high-intensity per week (~ 30 – 40 min each) for 4 weeks. ☐
- My arteries in the left upper arm and the neck will be scanned using an ultrasound. ☐
- A cuff will be placed around my arm that will be inflated for five minutes. I might feel a sensation of 'pins and needles' in my hand. ☐

- A small amount of blood will be taken from my fingertip on the testing days. I might feel a slight pinprick in my finger during this procedure. ☐

- I will be asked not to eat after 8 pm the night before and not to have breakfast on the testing day to that I will be informed. I will also be asked to complete a food diary 2 days before the visits to the university ☐

- I will be asked to wear an accelerometer, a watch-like device that will measure my physical activity levels, for several days during the study period. ☐

- I am free to ask any questions at any time. ☐

I know that:

- I can withdraw from the study at any point with no questions asked. ☐

Name ……

Signed …… (Participant)

Date ……

Name ……

Signed …… (Researcher)

Date ……

HEALTH SCREEN FOR CHILD VOLUNTEERS (PARENTAL FORM)

Name of child: ……..
Height: …… (please provide in cm or feet)
Weight: …….. (please provide in kg or stone)

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is:

i) To ensure their own continuing well-being
ii) To avoid the possibility of individual health issues confounding study outcomes

Your answers to the questions in this questionnaire, on behalf of your child, are strictly **confidential**.

Please complete this brief questionnaire to confirm your child's fitness to participate:

1. **At present**, does your child have any health problem for which they are:
   (a) On medication, prescribed or otherwise ……. YES ☐ NO ☐
   (b) Attending a general practitioner ……. YES ☐ NO ☐
   (c) On a hospital waiting list …… YES ☐ NO ☐

2. **In the past two years**, has your child had any illness that required them to:
   (a) Consult your family GP…….. YES ☐ NO ☐
   (b) Attend a hospital outpatient department …… YES ☐ NO ☐
   (c) Be admitted to hospital……... YES ☐ NO ☐

3. **Has your child ever** had any of the following:
   (a) Convulsions/epilepsy ……. YES ☐ NO ☐
   (b) Asthma ……... YES ☐ NO ☐
   (c) Eczema ……... YES ☐ NO ☐
   (d) Diabetes …….. YES ☐ NO ☐
   (e) A blood disorder ……. YES ☐ NO ☐
   (f) Head injury ……. YES ☐ NO ☐

(g) Digestive problems ……. YES ☐ NO ☐

(h) Heart problems …… YES ☐ NO ☐

(i) Lung problems …… YES ☐ NO ☐

(j) Problems with bones or joints ……. YES ☐ NO ☐

(k) Disturbance of balance/coordination …….. YES ☐ NO ☐

(l) Numbness in hands or feet ……. YES ☐ NO ☐

(m) Disturbance of vision …….. YES ☐ NO ☐

(n) Ear/hearing problems …….. YES ☐ NO ☐

(o) Thyroid problems …… YES ☐ NO ☐

(p) Kidney or liver problems …… YES ☐ NO ☐

(q) Allergy to nuts …… YES ☐ NO ☐

(r) Eating disorder …… YES ☐ NO ☐

4. Do you know of any other reason why your child should not engage in physical activity?

YES ☐ NO ☐

If **YES** to any question, please describe briefly (for example, to confirm the problem was/is short-lived, insignificant or well controlled).

A member of our research team may contact you if we have any further questions.

Primary investigators:
Ricardo Santos Oliveira
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Sascha Kranen
shk205@exeter.ac.uk
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Thank you for your cooperation

Project coordinators:
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Study: The health benefits of 4 weeks of running training in adolescents
Researcher: Ricardo Santos Oliveira
Organisation: The University of Exeter
Version: 1. 12.01.2017

Contact details

Child's name: _____

Parent's/guardian's name: _____

Address: _____

_____

Postcode: _____

Home telephone: _____

Mobile telephone: _____

Email address: _____

Best time to contact you: _____

Appendix 3 – Search strategy

1 heart rate.ti,ab.
2 (resting or variability or recovery).ti,ab.
3 1 adj2 2
4 spectral analysis.ti,ab.
5 (high or low) adj frequency.ti,ab.
6 time-domain.ti,ab.
7 frequency-domain.ti,ab.
8 autonomic modulation.ti,ab.
9 autonomic nervous system.ti,ab.
10 cardiac autonomic function.ti,ab.
11 cardiovagal modulation.ti,ab.
12 vagal adj (activity or tonus or modulation).ti,ab.
13 baroreflex.ti,ab.
14 (sympathetic or parasympathetic).ti,ab.
15 *autonomic nervous system/ or *parasympathetic nervous system/ or *vagus nerve/ or *sympathetic nervous system/ or exp vasomotor system/
16 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15
17 arterial adj (stiffness or compliance).ti,ab.
18 pulse adj2 velocity.ti,ab.
19 flow adj2 dilation.ti,ab.
20 endothelial adj (function or health).ti,ab.
21 endothelium.ti,ab.
22 vascular adj (function or health or stiffness).ti,ab.
23 vasodilation.ti,ab.
24 intima media thickness.ti,ab.
25 atherosclerosis.ti,ab.
26 carotid.ti,ab.
27 risk factor*.ti,ab.
28 (cluster or cardiovascular or metabolic).ti,ab.
29 27 adj2 28
30 pressure adj (blood or arterial).ti,ab.
31 hypercholesterolemia.ti,ab.
32 cholesterol.ti,ab.
33 (HDL or LDL).ti,ab.
34 (high or low) adj2 lipoprotein.ti,ab.
35lipidemia.ti,ab.
36 blood lipids.ti,ab.
37 body composition.ti,ab.
38 (body adj2 index).ti,ab.
39 overweight.ti,ab.
40 percent* body fat.ti,ab.
41 fitness.ti,ab.
42 acceleromet*.ti,ab.
43 peak oxygen consumption.ti,ab.
44 maximal oxygen uptake.ti,ab.

45 aerob* adj (performance or capacity or power).ti,ab.
46 physical* adj (activ* or inactiv*).ti,ab.
47 sedentar*.ti,ab.
48 sedentary adj (time or behavio?r).ti,ab.
49 (unfit or low fitness).ti,ab.
50 (metabolic adj (syndrome or health)).ti,ab.

51 exp physical endurance/ or *physical fitness/
52 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51
53 infan*.ti,ab.
54 child*.ti,ab.
55 schoolchild*.ti,ab.
56 pupil*.ti,ab.
57 adolescen*.ti,ab.
58 (prepubescent or post-pubescent or pubescent).ti,ab.
59 puberty.ti,ab.
60 (pubertal or post-pubertal or pre-pubertal).ti,ab.
61 teen*.ti,ab.
62 kid*1.ti,ab.
63 (girl*1 or boy*1).ti,ab.
64 exp child/
65 exp adolescent/
66 (young or youth).ti,ab.
67 p?ediatrics.ti,ab.
68 53 or 54 or 55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67
69 16 and 52 and 68

Appendix 4 – Modified Newcastle-Ottawa Scale

Adapted version of a modified Newcastle-Ottawa Scale for a single use in a specific context

Modified Newcastle-Ottawa Scale

Legend

0= Definitely No (high risk of bias)
1= Mostly No
2= Mostly Yes
3= Definitely Yes (low risk of bias)

Domain of evaluation: Methods for selecting study participants (i.e. Selection Bias)

Is the source population (cases, controls, cohorts) appropriate and representative of the population of interest?

0 (high risk of bias) 1 2 3 (low risk of bias)

Example of low risk of bias: A consecutive sample or random selection from a population that is representative of the condition under study.

Example of moderate risk of bias: A consecutive sample or random selection from a population that is not highly representative of the outcome of interest.

Example of high risk of bias: The source population cannot be defined or enumerated (i.e. volunteering or self-recruitment).

Domain of evaluation: Methods to control for confounding (i.e. Performance Bias)

Is the sample size sufficient and is there sufficient power to detect a meaningful difference in the outcome of interest?

0	1	2	3
(high risk of bias)			(low risk of bias)

Example of low risk of bias: Sample size was adequate and there was sufficient power to detect a difference in the outcome.

Example of high risk of bias: Sample size was small and there was not enough power to test the outcome of interest.

Did the study adjust for any variables or confounders that may influence the outcome?

0	1	2	3
(high risk of bias)			(low risk of bias)

Example of low risk of bias: The study identified and adjusted for all possible confounders that may influence the estimates of association between exposure and outcome.

Examples of moderate risk bias: The study identified and reported possible variables that may influence the outcome but did not statistically explore their influence.

Example of high risk of bias: The study either did not report any variables of influence or acknowledge any variables of influence when it was clear they were present.

Domain of evaluation: Statistical methods (i.e. Detection Bias)

Did the study use appropriate statistical analysis methods relative to the outcome of interest?

0	1	2	3
(high risk of bias)			(low risk of bias)

Example of low risk of bias: The study reported use of appropriate statistical analysis as required.

Examples of moderate risk bias: The study used either correct statistical methods but did not report them well, or used the incorrect methods but reported them in detail.

Example of high risk of bias: The study did not use appropriate statistical analysis as required.

Is there little missing data and did the study handle it accordingly?

0 1 2 3
(high risk of bias) (low risk of bias)

Example of low risk of bias: The study acknowledged missing data to be less than 10% and specified the method of handling it.

Examples of moderate risk bias: The study either had greater than 15% of missing data but they specified the method used to handle it.

Example of high risk of bias: The study had greater than 15% of missing data and did not handle it at all.

Domain of evaluation: Methods of measuring outcome variables (i.e. Information bias)

Is the methodology of the outcome measurement explicitly stated and is it appropriate?

0 1 2 3
(high risk of bias) (low risk of bias)

Example of low risk of bias: The study provides a detailed description of the outcome measure(s) which are appropriate for the outcome of interest.

Examples of moderate risk bias: The study provides a somewhat complete description of outcome measurements that are justified.

Example of high risk of bias: The study provides limited information on the methods of measuring the outcome and the measure is not appropriate considering the outcome.

Is there an objective assessment of the outcome of interest?

Example of low risk of bias: The study used objective methods to discern the outcome status of participants (*i.e. laboratory measurements, medical records*)

Examples of moderate risk bias: The study relied on subjective data as the primary method to discern the outcome status of participants (*i.e. self-report*)

Example of high risk of bias: The study had limited reporting about assessment of outcomes.

Domain of evaluation: Subject Follow-up

Was the follow-up sufficiently long enough for the outcome to occur?

0	1	2	3
(high risk of bias)			(low risk of bias)

Was there minimal loss to follow-up and are subjects lost to follow-up unlikely to introduce bias?

0	1	2	3
(high risk of bias)			(low risk of bias)

Example of low risk of bias: Follow-up was completed for all, or nearly all subjects, and reasons for losses to follow-up were well documented.

Example of moderate risk of bias: Losses to follow-up are not excessive, and reasons for losses to follow-up are well documented and mostly unrelated to the outcome.

Example of high risk of bias: Significant loss to follow-up, reasons for losses to followup not reported, suspect that reasons for dropouts are related to the outcome.