The influence of exercise intensity on vascular health outcomes in 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, and the underlying atherosclerotic process has its origin in youth. Physical activity lowers future CVD risk, however few adolescents achieve the recommended minimum amount of daily activity and interventions fail to meaningfully increase activity levels in this group. It is therefore essential to identify how small volumes of exercise can be optimised for the primary prevention of CVD. The purpose of this thesis is to identify the influence of exercise intensity on vascular health outcomes in adolescents, and to assess the efficacy of 2 weeks of low volume, high-intensity interval training on CVD risk factors in this population.

Chapter 4 demonstrates that a single bout of high-intensity interval exercise (HIIE) performed one hour before a high fat meal elicits comparable reductions in postprandial lipaemia as a work-matched bout of moderate-intensity exercise (MIE) in girls. However, neither exercise attenuated postprandial lipaemia in the boys. Additionally, HIIE elicited a superior increase in postprandial fat oxidation and decrease in blood pressure, and this was sex independent. These findings are furthered in Chapter 5, which identified that accumulating HIIE, but not MIE, favourably modulates glycaemic control, postprandial blood pressure and fat oxidation in adolescents irrespective of sex.

A high fat meal was included in Chapter 6 in order to impair vascular function via oxidative stress. Postprandial vascular function was preserved following MIE, but improved after HIIE, and these changes were not related to changes in postprandial lipaemia or total antioxidant status. Chapter 7 addressed the time course of the changes in vascular function post exercise, and identified that HIIE promotes superior changes in vascular function than MIE.

Finally, Chapter 8 identified that 2 weeks of high-intensity interval training improved novel (endothelial function and heart rate variability), but not traditional CVD factors in adolescent boys and girls. However, most of these favourable changes were lost 3 days after training cessation.

Thus, this thesis demonstrates that vascular health outcomes are positively associated with exercise intensity. Given that HIIE was perceived to be more enjoyable than MIE in Chapters 4, 6 and 7, performing HIIE appears to be an effectual and feasible alternative to MIE for the primary prevention of CVD.

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It's a good trick that, I don't know anyone else who can do it.

Publications and conference presentations

The research presented within this thesis has been peer reviewed through the following publications and communications.

Publications

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Contents

List of figures

Figure permissions

List of tables

List of equations

Glossary of terms

3-OHB	3-hydroxybutyrate
95% CI	95% confidence interval for the true difference
AUC	Area under the curve
BMI	Body mass index
CON	Control trial (rest)
CV	Coefficient of variation
CVD	Cardiovascular disease
ECG	Electrocardiogram
EE	Energy expenditure
eNOS	Endothelial nitric oxide synthase
ES	Effect size
FA	Fatty acids
FMD	Flow mediated dilation
HDL	High-density lipoprotein
HIIE	High-intensity interval exercise
HIIT	High-intensity interval training
HRV	Heart rate variability
IAUC	Incremental area under the curve versus time
LDL	Low-density lipoprotein
L-NMMA	N^G-monomethyl-L-arginine
MIE	Moderate-intensity exercise
NO	Nitric oxide
PACES	Physical activity enjoyment scale
PRH	Peak reactive hyperaemia
RER	Respiratory exchange ratio
RMR	Resting metabolic rate
RMSSD	Root mean square of the squared differences between adjacent normal R-R intervals
RPE	Rating of perceived exertion
RPM	Revolutions per minute
SBP	Systolic blood pressure
SR_{AUC}	Area under the curve for shear versus time
TAG	Triacylglycerol
TAS	Total antioxidant status
TC	Total cholesterol
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}E$	Minute ventilation
VLDL	Very-low-density lipoprotein
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2\ max}$	Maximal oxygen uptake
$\dot{V}O_{2\ peak}$	Peak oxygen uptake

[square brackets] denote a concentration

$\dot{V}O_{2\ peak}$ is defined as the highest $\dot{V}O_2$ achieved during an incremental test to exhaustion

Mum,
this would
be my gift to
you if it weren’t
already your
gift to
me

Chapter 1

Introduction

1.1 Cardiovascular disease

"Mankind's greatest epidemic: coronary heart disease has reached enormous proportions striking more and more at younger subjects. It will result in coming years in the greatest epidemic mankind has faced unless we are able to reverse the trend by concentrated research into its cause and prevention."

World Health Organization 1973

When this warning from the World Health Organization was first published nearly half a century ago (WHO, 1973), cardiovascular diseases (CVD), including coronary heart disease, cerebrovascular disease and peripheral vascular disease, were responsible for more than half of all deaths (Cooper et al., 2000). Despite significant declines in CVD mortality, recent data published by the World Health Organization highlights that CVD remains the leading cause of mortality worldwide in both men and women, and is responsible for one third (17.8 million) of all deaths (WHO, 2011). This figure is predicted to rise to 30 million by 2030 due to the increasing incidence of CVD in low and middle income countries (Mathers and Loncar, 2006). In addition to these sobering mortality data, CVD also causes extensive disability. The total number of disability-adjusted life years lost globally is expected to rise from 134 million in 1990 to 204 million in 2020 (Neal, 2002). This projected increase is attributable not only to an anticipated increase in global CVD, but also due to an increase occurrence of these events from an earlier age. Thus, CVD has undoubtedly become "mankind's greatest epidemic" (WHO, 1973).

Atherosclerosis is the progressive disease which precedes overt CVD, and is characterised by endothelial dysfunction and the infiltration of lipids, cholesterol and cellular debris into the vascular wall, forming a fatty streak which may develop into a fibrous plaque (Figure 1.1). In the presence of continued lipid deposition and the proliferation of smooth muscle and connective tissue, fibrous plaques may enlarge and calcify before rupturing, thereby exposing the blood to thrombolytic, lipid-rich material promoting thrombotic occlusion and, in turn, a cardiovascular event.

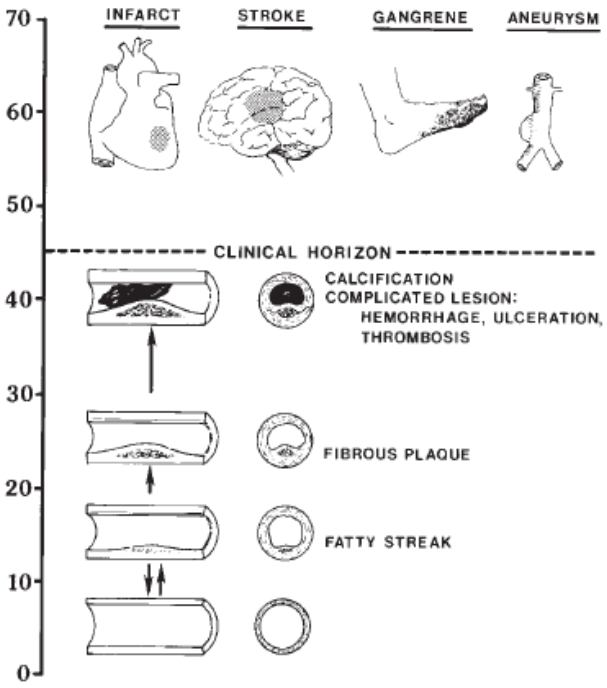

Figure 1.1 The progression of atherosclerosis. Reproduced from McGill *et al*. (2000a) with permission.

1.2 Atherosclerosis has paediatric origins

Although the clinical significance of atherosclerosis may not be apparent until the fourth or fifth decade of life, considerable evidence indicates that the atherosclerotic process has its origins in youth (Figure 1.1). While the existence of fatty streaks in children and adolescents was first demonstrated over a century ago (Klotz and Manning, 1911), the importance of the early origins of atherosclerosis did not become apparent until an autopsy study was published in 1953 on young soldiers killed in the Korean war (Enos et al., 1953). This landmark investigation reported that over 70% of the soldiers presented with some evidence of coronary atherosclerosis, despite a mean age of 22 years. At the same time, data from New Orleans demonstrated that fatty streaks were present in the aortas and coronary arteries of individuals in the first and second decades of life (Holman et al., 1958, Strong and McGill, 1962) and subsequent work has identified that 65% of 12-14 year olds have atherosclerotic lesions, with another 8% demonstrating advanced lesions (Stary, 1989).

In 1968, it was demonstrated across a range of ethnic and racial groups that children and adolescents had a greater frequency of atherosclerotic streaks in populations characterized by higher rates of adult coronary heart disease (Tejada et al., 1968), suggesting a link between the presence of fatty streaks in childhood and clinically significant fibrous plaques in adulthood. Direct evidence supporting this progression of fatty streaks to fibrous plaques and larger atheromas was provided in 1976 (Katz et al., 1976), prompting investigators to examine the incidence of established risk factors for CVD amongst children and adolescents in Louisiana (Berenson et al., 1978) and Iowa (Lauer et al., 1975) in the United States, and across Finland (Akerblom et al., 1985). These studies identified that elevated cholesterol levels in childhood were associated with a two-fold higher rate of coronary mortality in adulthood (Schrott et al., 1979), and that established risk factors for adult CVD, such as serum lipoproteins and blood pressure, tracked from youth into later life (Clarke et al., 1978, Webber et al., 1983). These findings resulted in the establishment of the Pathological Determinants of Atherosclerosis in Youth research group in 1985, which assessed atherosclerotic progression in the coronary arteries and aortas during autopsy in 2,876 teenagers and young adults between 1987 and 1994. This research group identified that hypertension, smoking, obesity, glucose and non high-density lipoprotein (HDL) levels in 15 to 34 year olds were positively associated with atherosclerotic lesions, whilst HDL concentration was negatively associated (McGill et al., 1997, McGill et al., 1998, McGill et al., 1995).

As the participants in these studies reached adulthood, more data and newer non-invasive techniques became available. The Muscatine study adopted ultrasound techniques and reported that carotid intima-media thickness, considered a clinically important marker of atherosclerotic progression (Touboul et al., 2004) and an independent predictor of future CVD in adolescents (Raitakari et al., 2003), in participants 33-42 years old was associated with childhood and adolescent (8-18 years old) cholesterol and body mass index (BMI) (Davis et al., 2001). Additionally, post-mortem analysis of participants in the Bogalusa cohort who had died of accidents, suicides or homicides further strengthened the association between CVD risk factors measured during youth (5-18 year olds) and atherosclerotic lesions (Berenson et al., 1992). More

recent findings suggest that non HDL levels, blood pressure, BMI and smoking status measured between 12-18 years of age are associated with adult carotid intima-media thickness even when adjusted for adult risk factor status (Raitakari et al., 2003), indicating that structural changes to the carotid arterial wall in adulthood may be attributable to the presence of CVD risk factors during the teenage years.

Given the above, the paediatric origins of atherosclerosis are well established, and whilst it has been demonstrated that some individual CVD risk factors in childhood and adolescence may track poorly into later life (Porkka et al., 1991, Mahoney et al., 1991, Twisk et al., 1997), evidence indicates that risk factors tend to cluster in adolescents (Andersen et al., 2003), and the tracking of clustered risk factors is stronger (Andersen et al., 2004). Indeed, findings from the Danish Youth and Sport cohort suggest that adolescents with clustered risk factors are six times more likely to present with clustered risk in adulthood (Andersen et al., 2004). Thus, there is a strong rationale for the identification of interventions which can modulate CVD risk factors in childhood and adolescence in order to facilitate the primary prevention of atherosclerosis across the lifespan.

1.3 Postprandial lipaemia and cardiovascular disease risk

Historically, the link between CVD and plasma triacylglycerol concentrations ([TAG]) has been concerned with fasting measures. However, whilst the two are positively associated with each other (Austin et al., 1998), this analysis has come under criticism as most of the day is spent in the postprandial state, and fasting [TAG] provide a poor reflection of postprandial TAG metabolism (Patsch et al., 1992, Miesenbock, 1992). Additionally, the relationship between CVD and fasted plasma [TAG] is often weakened by the inclusion of HDL into the multivariate analysis (Austin, 1991, Sarwar et al., 2007, Criqui et al., 1993), as [HDL] is determined by the metabolism of TAG-rich lipoproteins (Patsch et al., 1992, Patsch et al., 1984). Furthermore, postprandial TAG metabolism is mechanistically linked with the atherosclerotic process (in addition to reducing HDL concentrations) as postprandial hyperlipidaemia promotes transient endothelial dysfunction and oxidative stress (Bae et al., 2001). This is integral to

the atherogenic process (Bonetti et al., 2003, Juonala et al., 2004, Steinberg, 2009), and TAG-rich lipoprotein remnant particles can penetrate the endothelium and promote foam cell formation (Proctor and Mamo, 1998). Finally, a delayed clearance of plasma TAG following a meal with an appreciable fat content may reflect insulin resistance (Chen et al., 1993a, Ginsberg and Huang, 2000, DeFronzo and Ferrannini, 1991), which is also associated with CVD risk (Isomaa et al., 2001, Ford, 2005). As a result, identifying plasma [TAG] after a high fat meal has been argued to provide superior information regarding metabolic health than fasting TAG and a more appropriate assessment of CVD risk (Freiberg et al., 2008, Morrison et al., 2009).

It has been demonstrated that postprandial [TAG] is greater in men and women with coronary artery disease compared to healthy controls, even after correction for fasting [TAG] (Patsch et al., 1992, Meyer et al., 1996). Postprandial lipaemia has also repeatedly been shown to be positively associated with carotid intima-media thickness (Ryu et al., 1992, Karpe et al., 1998, Boquist et al., 1999). However, these studies do not establish the direction of causality between postprandial lipaemia and existing CVD, nor do they address the risk of developing CVD with repeated exposure to elevated postprandial [TAG]. Interestingly, data are available indicating that postprandial lipaemia is elevated in the apparently healthy sons of men with severe coronary artery disease compared to the healthy sons of a control group (Uiterwaal et al., 1994). This suggests a link between chronic exposure to elevated non-fasting [TAG] and future CVD risk, although such conclusions are clearly only speculative. Importantly, however, both groups of sons presented with comparable fasting [TAG], thereby highlighting the potential prognostic value of non-fasting TAG assessment.

Despite a plethora of studies indicating an association between non-fasting TAG and markers of CVD, the lack of large epidemiological studies and prospective evidence meant that this association remained controversial. It is only in recent years that postprandial lipaemia has been accepted as a risk factor for CVD in both men and women, and one which may be independent of other risk factors and more powerful than fasting [TAG] (Bansal et al., 2007, Freiberg et al., 2008, Morrison et al., 2009, Nordestgaard et al., 2007). Indeed,

after an 11-year follow up, data from the Women's Health Study demonstrated that non-fasting [TAG] was a better predictor of adverse cardiovascular events than fasting [TAG], and was strongly associated with cardiovascular events even when adjusted for smoking, blood pressure, hormone therapy, HDL, diabetes mellitus, BMI and C-reactive protein (Bansal et al., 2007). Non-fasting [TAG] was also identified as a significant predictor of future CVD events in the Copenhagen City Heart Study for both men and women (Nordestgaard et al., 2007, Freiberg et al., 2008), which corroborate with the data from earlier prospective studies in adults (Talmud et al., 2002, Walldius et al., 2001, Stampfer et al., 1996).

The establishment of non-fasting [TAG] as a more powerful determinant of CVD risk than fasting [TAG] was mirrored by an increase in research identifying the efficacy of lifestyle interventions to attenuate postprandial lipaemia in adults. However, although it was understood that fasted [TAG] during adolescence was related to future atherosclerosis (Davis et al., 2001, Raitakari et al., 2003), it was not until 2009 that longitudinal data were published identifying that non-fasting [TAG] determined during early adolescence was significantly associated with CVD events in the fourth and fifth decades of life (Morrison et al., 2009). This paper strengthened the rationale for adopting non-fasting plasma [TAG] as key marker for future CVD risk, and a growing body of research is now concerned with how postprandial [TAG] can be lowered following lifestyle interventions (i.e. physical activity promotion) in young people for the primary prevention of CVD.

1.4 Endothelial dysfunction and cardiovascular disease risk

An impairment in endothelial function is considered to be the earliest detectable manifestation of the atherosclerotic process (Ross, 1999), preceding structural adaptations to the vessel wall (Zeiher et al., 1991, Aggoun et al., 2008). In a landmark paper, Celermajer *et al.* (1992) demonstrated, using flow mediated dilation (FMD), that an impairment in endothelial function is present in asymptomatic children and adolescents with CVD risk factors. Subsequent studies have since established that an impairment in endothelial function is present in children with hypercholesterolaemia (Sorensen et al., 1994), type 1

diabetes mellitus (Jarvisalo et al., 2004), and who are overweight (Woo et al., 2004b) and obese (Aggoun et al., 2008, Tounian et al., 2001). This is particularly concerning as evidence from the Cardiovascular Risk in Young Finns Study indicates that endothelial dysfunction is a pre-requisite for the initiation of the formation of atherosclerotic lesions (Juonala et al., 2004). Specifically, this study demonstrated that the number of CVD risk factors in young adults with endothelial dysfunction is positively associated with an increased carotid intima-media thickness, however this association is abolished in the absence of endothelial dysfunction. Additionally, evidence indicates that changes in endothelial function do not correlate with the carotid intima-media thickness in children (Hopkins et al., 2013), which further suggests that impairments in vessel function precede structural remodelling. Therefore, endothelial function in youth is a pertinent health outcome for the primary prevention of CVD, and interventions which improve endothelial function, or reverse endothelial dysfunction are warranted from an early age.

With regards to the scope of this thesis, endothelial function is also an attractive outcome as postprandial lipaemia transiently impairs endothelial function in adolescents (Sedgwick et al., 2013, Sedgwick et al., 2014, Sedgwick et al., 2012), and data from adult studies attribute this unfavourable response to an increase in oxidative stress (Bae et al., 2001, Anderson et al., 2001). Thus, the aforementioned link between postprandial lipaemia and future CVD risk may, in part, be related to repetitive periods of postprandial endothelial dysfunction occurring at the same time as elevations plasma [TAG] and oxidative stress (Wallace et al., 2010).

Given the above, there is a strong rationale for studies to either promote or prevent an impairment in endothelial function in paediatric groups for the promotion of vascular health across the lifespan. Data in adults identify that a preserved endothelial function attenuates the risk of cardiovascular events in individuals with a high plaque burden (Chan et al., 2003), and that pharmacological interventions which improve endothelial function reduce CVD risk (Modena et al., 2002). An increasing body of evidence demonstrates improvements in endothelial function are achievable following exercise training in both healthy young adults (Clarkson et al., 1999) and in clinical populations (Hambrecht et al., 2003, Hambrecht et al., 1998, Higashi et al., 1999). It is also

understood that physical activity reduces CVD risk independently to traditional risk factor modification (Berlin and Colditz, 1990, Hu et al., 2004, Paffenbarger et al., 1986), and that improvements in endothelial function may occur in the absence of any changes in traditional CVD risk factors (Green et al., 2003). Taken together, an impairment in endothelial function may be considered to be a novel risk factor for CVD (Green et al., 2011, Green et al., 2008).

1.5 Physical activity and cardiovascular disease risk

Despite the bleak prediction that CVD will account for 30 million deaths per annum by 2030 (Mathers and Loncar, 2006), the World Health Organization estimates that over three-quarters of all CVD mortality can be prevented through lifestyle interventions (Perk et al., 2012). Furthermore, epidemiological evidence indicates that regular exercise can lower total cardiac risk by 30-50% alone (Blair and Morris, 2009, Thompson et al., 2003, Warburton et al., 2006), whilst an increase in physical activity of 1000 kcal (4.2 MJ) per week confers a 20% survival benefit (Myers et al., 2004).

In accordance with the inverse association with exercise and CVD risk in adults, cross-sectional data from the European Youth Heart Study indicates that physical activity reduces the clustering of CVD risk factors in youth (Andersen et al., 2006). More recent longitudinal data demonstrates that time spent performing moderate to vigorous intensity physical activity in childhood and adolescence is inversely associated with CVD risk, regardless of sedentary time (Ekelund et al., 2012). Habitual physical activity has also been demonstrated to improve endothelial function in childhood (Abbott et al., 2002), whilst evidence in adolescents indicate that postprandial lipaemia can be attenuated (Tolfrey et al., 2014b), and the concomitant fall in postprandial endothelial function reduced (Sedgwick et al., 2012) or prevented (Sedgwick et al., 2013, Sedgwick et al., 2014), following a single bout of exercise. However, the most recent accelerometer data collected by the Department of Health indicate that only 7% of boys and < 1% of girls aged 11-15 years old in the UK achieve the recommended (Department of Health, 2011) minimum of 60 minutes of moderate to vigorous intensity physical activity per day (Department of Health, 2008). Indeed, the Avon Longitudinal Study of Parents and Children reported

that only 5.1% and 0.4% of the 5,965 11 year old boys and girls included in the study achieved this amount of physical activity, with the average time spent performing moderate to vigorous-intensity activities just 25 and 16 minutes, respectively (Riddoch et al., 2007). This is particularly sobering given that physical activity is thought to peak around this age, and then decline during adolescence (Kimm et al., 2005, Trost et al., 2002). Indeed, a systematic review of 26 cross-sectional studies (> 43,000 adolescent boys and girls) reported that physical activity declines by ~ 65% during the adolescent years (Dumith et al., 2011). Furthermore, 60 minutes is the recommended *minimum* amount of physical activity. Andersen *et al*. demonstrated that the odds ratio for clustered CVD risk was 3.29 (95% CI 1.96 to 5.52) times greater for adolescents performing 56 minutes compared to 131 minutes of daily moderate to vigorous-intensity exercise, and proposed that 90 minutes of daily exercise should be adopted as the recommended minimum (Andersen et al., 2006).

The consistent finding that very few adolescents achieve the recommended minimum amount of daily physical activity has encouraged interventions to promote physical activity. However, a recent meta-analysis highlighted that physical activity interventions may only have a small effect on increasing overall activity levels in children (Metcalf et al., 2012). Indeed, this meta-analysis reported that the mean increase in time spent performing moderate to vigorous activities across 30 studies (6,153 children) was 4 minutes per day. Therefore it is important to identify whether smaller volumes of physical activity can be optimised for the health of children and adolescents.

A growing body of literature indicates that time spent performing vigorous-intensity activities may promote cardiometabolic health and endothelial function in paediatric groups (Carson et al., 2014, Hay et al., 2012, Ortega et al., 2007, Ruiz et al., 2006, Hopkins et al., 2009). Cross-sectional data collected from 9-17 year olds in Canada identified that accumulating more than 7 minutes of vigorous-intensity activity per day was associated with significantly lower waist circumference, BMI z score, systolic blood pressure and increased aerobic fitness (Hay et al., 2012). The same research group have recently published longitudinal data demonstrating that performing just 4 minutes of vigorous-intensity activity per day significantly improved these cardiometabolic outcomes in 9-15 year olds (Carson et al., 2014).

These findings are striking not only due to the small amount of vigorous-intensity activity required, but also because time spent performing light-intensity physical activity may have a detrimental impact on adiposity. Indeed, in the aforementioned study, almost half of the participants classified as overweight or obese were in the top quartile for light-intensity activity (Carson et al., 2014). Data from the European Youth Heart Study similarly report that time spent performing vigorous-, but not moderate-, intensity activity is associated with lower adiposity in children (Ruiz et al., 2006) and adolescents (Ortega et al., 2007), whilst Hopkins *et al*. demonstrated that only vigorous-intensity activity is favourably associated with endothelial function in children (Hopkins et al., 2009). Therefore, the intensity of exercise appears to be an important consideration regarding health promotion in paediatric groups.

Accordingly, a growing body of evidence supports the adoption of low-volume, high-intensity interval exercise training (HIIT) programmes for the promotion of health in adolescents and children (Logan et al., 2014). Gutin *et al*. (2002) demonstrated that HIIT promoted greater improvements in physical fitness in obese adolescents compared to a moderate-intensity exercise training programme which was broadly matched for energy expenditure. Additionally, 12 weeks of HIIT (3 - 6 repeat 1 minute sprints) improved aerobic fitness, BMI and insulin sensitivity to a similar extent as traditional endurance training (30 - 60 minutes running at 80% peak heart rate) in obese 8 – 12 year olds, despite a ~ 70% lower training volume (Corte de Araujo et al., 2012). Racil *et al*. (2013) also recently reported that 12 weeks of HIIT improved fasted cholesterol profile, plasma [TAG], adiponectin concentrations (an adipocyte-derived peptide inversely associated with insulin resistance (Diez and Iglesias, 2003)), BMI and insulin resistance to a greater extent than moderate intensity exercise training in obese 14-16 year old girls. Eight weeks of HIIT has also been demonstrated to normalise endothelial dysfunction and improve central adiposity in obese 13-15 year olds (Watts et al., 2004a).

The benefits of HIIT, however, do not appear to be reserved to overweight and obese adolescents. Experimental data in normal weight adolescents indicate that 7 weeks of HIIT can promote either comparable or superior changes in physical fitness and systolic blood pressure than a traditional moderate-intensity training programme of the same length, despite an ~ 85% lower training volume

(Buchan et al., 2011). Other studies, which did not include a moderate-intensity exercise training group for comparison, also report encouraging improvements in aerobic fitness (Barker et al., 2014, Baquet et al., 2001) and systolic blood pressure (Buchan et al., 2012) in adolescents following HIIT. Importantly, the adherence rates to high-intensity exercise regimes appear to be high in adolescents and children (Buchan et al., 2011, Ratel et al., 2004), and the inclusion of repeated sprints to continuous exercise has been shown to increase enjoyment in both normal weight and overweight boys, without concomitant increases in perceived exertion (Crisp et al., 2012). Therefore, brief, high-intensity interval exercise (HIIE) might provide an attractive, time-efficient alternative to traditional moderate-intensity exercise in paediatric groups, a concept which is also supported by adult data (Gibala, 2007, Bartlett et al., 2011).

It is thought that the acute response to a single exercise bout underlies the benefit of exercise training (Dawson et al., 2013, Freese et al., 2015). It is therefore consistent that data are available which indicate that a single bout of HIIE can attenuate postprandial lipaemia (Thackray et al., 2013) and preserve postprandial endothelial function (Sedgwick et al., 2014) in adolescent boys. However, this area of research is in its infancy, and no study has compared the efficacy of a single bout of HIIE or moderate-intensity exercise (MIE) on these outcomes in adolescent boys or girls.

1.6 Thesis rationale

In light of the cited literature, there is a clear rationale for identifying exercise interventions which can favourably modulate postprandial lipaemia and endothelial function in adolescents. Given that few adolescents achieve the recommended (Department of Health, 2011) minimum amount of physical activity, and rarely sustain exercise for longer than 10 minutes (Riddoch et al., 2007), it is pertinent to assess how smaller volumes of exercise can be optimised for the primary prevention of atherosclerosis in youth. Therefore, this thesis investigates the influence of exercise intensity on vascular health outcomes in adolescents.

Chapter 2

Literature Review

2.1 Introduction

This section provides an overview of TAG digestion and lipoprotein metabolism in order to provide a mechanistic insight into the relationship between postprandial lipaemia and the atherosclerotic process. This is followed by a brief discussion of the methodological concerns regarding postprandial investigations and a review of the key adult studies concerned with how exercise may modulate the lipaemic response to a high fat meal. Finally, this section will provide a thorough examination of the evidence concerning the effect of acute exercise on postprandial lipaemia in young people.

2.1.1 Digestion and absorption of triacylglycerol

Triacylglycerols account for 90-95% of all dietary fats, with the remainder comprised of phospholipids, sterols (e.g. cholesterol) and fat-soluble vitamins (Patsch, 1987). The digestion of TAG starts in the mouth via the mechanical process of chewing and the secretion of lingual lipase (Hamosh and Scow, 1973). However, the influence of this enzyme in the oral cavity is considered to be negligible as the activity of lingual lipase is optimised by acidic environments (Hamosh and Burns, 1977). In the stomach, the acidic (pH ~4) gastric lipase (and the swallowed lingual lipase) start to hydrolyse the TAG into free fatty acids (FA) and glycerol molecules. This process is aided by gastric churning, which mechanically disrupts the ingested lipid into an emulsion of smaller hydrophobic droplets in water.

Cholecystokinin and gastric inhibitory polypeptide are secreted once the FA leave the stomach and enter the duodenum. Cholecystokinin promotes the flow of bile by simultaneously stimulating the sphincter of Oddi to relax and contracting the gall bladder (Byrnes et al., 1981) and increases the secretion of pancreatic lipase which hydrolyses the remaining TAG (Patsch, 1987). The liberated medium- and short-chain FA are able to passively diffuse across the intestinal wall and into the blood, where they are predominantly bound to albumin and passed to the liver via the hepatic portal vein (Patsch, 1987). However, long-chain FA, the most abundant FA in the Western diet (Patsch, 1987), are preferentially bound by FA-binding protein in the cytosol (Ockner and

Manning, 1974) and then transferred to the smooth endoplasmic reticulum to be re-esterified back into TAG in a reaction catalysed by acetyl CoA synthetase (Iqbal and Hussain, 2009). This reaction uses either absorbed glycerol as a substrate during the postprandial period, or glycerol-3-phosphate derived from intestinal glucose metabolism when fasted (Patsch, 1987).

Triacylglycerols are insoluble in water, and would coalesce in the predominantly aqueous blood if secreted by the small intestine in its current state. Consequently, the enterocyte must package the TAG into soluble lipoproteins for distribution around the body. Accordingly, the enterocyte also synthesises apoproteins in the rough endoplasmic reticulum, and re-esterifies cholesterol via cholesterol acyl transferase (Norum et al., 1979). These apoproteins are transferred to the smooth endoplasmic reticulum and associate with the newly synthesized TAG and cholesterol esters, forming a nascent chylomicron with a phospholipid surface (Hussain et al., 1996). The apoproteins are then glycosylated by the Golgi apparatus (Mahley et al., 1971), before the chylomicron is transported to the basolateral membrane of the enterocyte and secreted into the lymphatic capillaries which eventually drains into the blood stream via the left subclavian vein.

2.1.2 Digestion and synthesis of cholesterol

In the absence of a cell wall, animal cells incorporate cholesterol into the cell membrane in order to promote structural integrity (Ohvo-Rekila et al., 2002). Consequently, dietary cholesterol is predominantly sourced from animal products. Exogenous cholesterol is mostly esterified, and is subsequently hydrolysed by pancreatic cholesterol esterase in the intestinal lumen to form cholesterol and FA (Patsch, 1987). These products are then re-esterified and packaged into lipoproteins as described in Section 2.1.1. Additionally, some of the cholesterol present in the small intestine has been recycled in the formation of bile acids (Grundy and Metzger, 1972), and this biliary cholesterol pool either suffers the same fate as the exogenous cholesterol or is excreted (Grundy, 1983). Finally, cholesterol can also be derived by *de novo* synthesis in the enterocytes (and hepatocytes) (Patsch, 1987), and this manufacturing of cholesterol is inversely proportional to dietary availability (Grundy, 1983).

2.1.3 Lipoprotein metabolism

Lipoproteins vary in size, TAG and cholesterol ester content, apoprotein expression, site of origin and role. However they are unified by their most basic structural necessity; a hydrophobic waxy core and a hydrophilic outer membrane comprised of phospholipids, unesterified cholesterol and apoproteins (Mills et al., 1984). Lipoproteins are typically classed into four groups according to their Svedberg flotation rate; chylomicrons, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and HDL. An overview of the properties of these lipoprotein classes is provided in Table 2.1, and lipoprotein metabolism is summarised in Figure 2.1. In addition to these four groups, chylomicron remnants and intermediate-density lipoproteins are frequently cited in the literature. These are formed by the degradation or incomplete hydrolysis of chylomicron and VLDL respectively, and as such are denser than their parent lipoproteins. Intermediate-density lipoprotein particles will be referred to as VLDL remnants throughout this thesis.

2.1.4 Chylomicrons

Chylomicrons are the largest and least dense of the lipoproteins. Their primary purpose is to transport re-esterified dietary TAG away from the small intestine into the lymphatic system before draining into the bloodstream. Nascent chylomicrons are synthesized with apoproteins (apo) A-1 and B-48, before acquiring apo C-I, C-II, C-III and E from VLDL and LDL, and transferring some apo A-I to HDL in the circulation (Mills et al., 1984). Of these apoproteins, apo B-48 is unique to chylomicrons and their remnants and can therefore be used to identify the number of intestinally-derived lipoproteins following a meal (Havel, 1994).

Table 2.1 The four main lipoprotein classes. Adapted from Mills *et al*. (1984).

	Chylomicron	VLDL	LDL	HDL
Sveberg flotation rate (Sf)	>400	20-400	0-12	-
Density ($g \cdot mL^{-1}$)	<0.95	0.95-1.01	1.02-1.06	1.06-1.21
Diameter (nm)	>70	30-80	18-22	5-12
Apoproteins	A-I, B-48, C-I, C-II, C-III, E	B-100, C, E	B-100	A-I, A-II, C, E
Origin	Small intestine	Liver	Peripheral capillaries	Small intestine and liver
Primary function	Transport of exogenous TAG	Transport of endogenous TAG	Transport of cholesterol to periphery	Reverse cholesterol transport
TAG (% of mass)	83	50	10	8
Cholesterol (% of mass)	8	22	48	20
Protein (% of mass)	2	7	20	50

VLDL, very-low-density lipoproteins; LDL, low-density lipoproteins; HDL, high-density lipoproteins; TAG, triacyglycerol.

Chylomicrons scavenge apo C-II from HDL in the blood (Havel, 1994) which is a cofactor for lipoprotein lipase (LaRosa et al., 1970), the enzyme which resides on the capillary endothelium and catalyses the hydrolysis of TAG. Consequently, individuals with an inherited deficiency in apo C-II are characterised by hypertriglyceridaemia and other associated co-morbidities (Cox et al., 1978). The hydrolysis of TAG by lipoprotein lipase facilitates the rapid diffusion of FA across the endothelium for storage or oxidation. Any FA released into the blood during this interaction are bound to albumin and

transported to the liver for re-esterification (and subsequently repackaged into VLDL) or oxidation in the liver and extra-hepatic tissues (Bergman et al., 1971). After most (~ 90%) of the TAG is hydrolysed by lipoprotein lipase (Havel, 1994), surface phospholipids and apoproteins C-II and A are scavenged by HDL (Mjos et al., 1975, Lewis and Rader, 2005), forming a chylomicron remnant. This remnant is further modified in the blood by LDL and HDL which exchange TAG for cholesterol ester via cholesteryl ester transfer protein (Barter et al., 2003).

The loss of apo C-II from the chylomicron remnant to HDL prevents further TAG hydrolysis by lipoprotein lipase (Lewis and Rader, 2005), however some of the remaining TAG can be hydrolysed by hepatic lipase expressed on the cell surface of the liver (Shafi et al., 1994). It is thought that the hydrolysis of TAG by lipoprotein lipase and hepatic lipase may facilitate the binding of apo E on the surface of the chylomicron remnant to the LDL receptors (Brasaemle et al., 1993) and LDL receptor related proteins (Beisiegel et al., 1991) expressed by the liver. Evidence also suggests that the periphery may remove the largest chylomicron remnants from the blood, as they may be too large to penetrate the fenestrated hepatic sinusoidal endothelium and reach the hepatic LDL and LDL receptor related proteins (Karpe et al., 1997, Fraser et al., 1995).

Chylomicrons also transport esterified cholesterol from the small intestines. Due to the hydrolysis of TAG and the transfer of cholesterol esters from LDL and HDL (Barter et al., 2003), chylomicron remnants have a greater concentration of cholesterol esters than their precursor lipoprotein particles. The cholesterol ester in the remnant chylomicron is eventually hydrolysed by hepatic lysosomes, and the resulting cholesterol is either re-esterified and stored, packaged into VLDL, or excreted in the bile as free cholesterol or after conversion to bile acids (Havel and Hamilton, 1988).

2.1.5 Very-low-density lipoproteins

Very-low-density lipoproteins are predominantly formed in the liver, although a minor amount (~ 10%) may originate from the small intestine in the postabsorptive state (Risser et al., 1978). The primary function of VLDL is to transport endogenous TAG to the periphery. Each VLDL expresses apo B-100,

a longer apo B- isoform than apo B-100 on the surface of chylomicrons, but similarly scavenge apoproteins C-II and E from HDL in the blood (Hamilton et al., 1991, Lewis and Rader, 2005).

The TAG content of VLDL is determined not only by the re-packaging of the remaining TAG present in chylomicron remnants and VLDL remnants taken up by the liver (Yamada et al., 1988), but also by hepatic *de novo* synthesis of TAG (Hellerstein et al., 1991), particularly during prolonged hyperglycaemia (Aarsland et al., 1996). Furthermore, VLDL synthesis is also determined by hepatic uptake and re-esterification of FA liberated during lipolysis by hormone sensitive lipase in adipose tissue and the "spill over" of FA following interactions between lipoprotein lipase and TAG-rich lipoproteins (e.g. chylomicrons and VLDL) (Frayn et al., 1994, Miles et al., 2004). The rate of VLDL secretion by the liver is determined not only by these factors, but also by the balance between hepatic FA oxidation and re-esterification, and the demand for TAG at the periphery (i.e. when fasted).

VLDL are catabolised in much the same way as chylomicrons, with apo C-II facilitating the hydrolysis of VLDL TAG by lipoprotein lipase (LaRosa et al., 1970), and cholesterol ester transfer protein catalysing the exchange of TAG and cholesterol ester between VLDL and HDL respectively (Barter et al., 2003). However, the lipolysis of VLDL is much slower than chylomicrons, with a half-life of 2-4 hours in the blood (Durstine and Haskell, 1994) compared to <10 minutes (Cohen, 1989, Cohen et al., 1989), which likely reflects the preferential hydrolysis of TAG packaged in chylomicrons by lipoprotein lipase (Havel, 1994, Schneeman et al., 1993). Accordingly, the resulting VLDL remnant particle contains a greater amount of cholesterol ester due to increased exposure time to cholesterol ester transfer protein in the circulation (Havel, 1994).

The presence of apoproteins B-100 and E enable the largest VLDL remnants to be taken up by the liver via the LDL receptor (Yamada et al., 1988). About half of all VLDL remnants are able to complete receptor-mediated endocytosis at the liver (Mahley and Ji, 1999), whilst the remaining VLDL remnants are small enough to penetrate the fenestrated hepatic sinusoidal endothelium and be further hydrolysed by hepatic lipase expressed on the liver cell surface (Zambon et al., 2003, Packard et al., 1984). The subsequent loss of the remaining TAG

promotes the formation of an LDL particle, predominantly characterised by an enriched cholesterol ester core and apo B-100 (Sigurdsson et al., 1975, Havel, 1984).

2.1.6 Low-density lipoproteins

The primary function of LDL particles is the distribution of cholesterol to places of need. Due to the prior removal of apo E, LDL are only slowly taken up by the liver and extra-hepatic tissues via interaction with apo B-100 and the LDL receptor (Havel, 1994). Furthermore, LDL receptor-related proteins are not thought to remove LDL from the circulation (Kowal et al., 1989). Thus, the cholesterol-dense LDL particle has a half-life of 2-3 days (Hussain et al., 1996), and the number of LDL particles in the blood greatly exceeds that of its parent lipoprotein as a consequence (Havel, 1994).

In addition to LDL receptor-mediated uptake, ~ 10% of LDL can be removed from the bloodstream by a receptor-independent process (Osono et al., 1995), whilst LDL which is modified in the circulation may undergo phagocytosis via scavenger receptors expressed on the surface of macrophages (Kelley, 1991). This pathway is widely acknowledged to be atherogenic (Ross, 1993, Bobryshev, 2006, Steinberg, 2009), as macrophages are not able to down regulate their scavenger receptor expression despite excessive increases in intracellular cholesterol. This lack of negative feedback promotes the formation of foam cells (Bobryshev, 2006), which are able to penetrate the endothelium and proliferate in the blood vessel wall (Yla-Herttuala et al., 1989). The accumulation of foam cells is also aided by the long half-life of LDL (Hussain et al., 1996), and the increased exposure to an environment which encourages oxidative modification of the LDL particle (Steinbrecher et al., 1984). Indeed, the apparent resistance to atherosclerosis in individuals with CVD risk factors but genetically predisposed to low levels of LDL highlights the importance of LDL in the progression of atherosclerosis (Cohen et al., 2006).

2.1.7 High-density lipoproteins

The HDL fraction in human plasma is heterogeneous, and can more accurately be classed into a series of minor HDL species (Francone and Fielding, 1990, Barter, 2002). However, the primary function of each of these lipoproteins is to transport excess cholesterol from the periphery back to the liver for catabolism ("reverse cholesterol transport") (Fielding and Fielding, 1995). As such, these minor species will often be grouped and referred to as HDL throughout this thesis.

Phospholipid-rich and cholesterol-poor nascent HDL particles with apo A are secreted by the liver and intestine (Lewis and Rader, 2005). This is followed by the acquisition of unesterified cholesterol and phospholipids from extra hepatic cellular efflux (Fielding and Fielding, 1995) and from the degradation of circulating chylomicrons and VLDL particles (Barter et al., 2003). This cholesterol is esterified by lecithin-cholesterol acyltransferase (Jonas, 2000), which is activated by apo A (Grundy, 1983) and essential for normal HDL maturation (Lewis and Rader, 2005). Thus, the fate of HDL is inexorably linked with TAG-rich chylomicron and VLDL metabolism. Indeed, genetic lecithin-cholesterol acyltransferase deficiency syndromes are characterised by a marked reduction in circulating HDL (Kuivenhoven et al., 1997).

Mature HDL particles are involved in the aforementioned exchange of surface apoproteins with chylomicrons and VLDL (Lewis and Rader, 2005), forming HDL_2. This interaction between HDL and TAG-rich lipoproteins is essential for TAG hydrolysis and subsequent removal of chylomicrons and VLDL from the circulation. Consequently, high HDL_2 concentrations are inversely associated with CVD risk (Patsch et al., 1983).

High-density lipoproteins also exchange cholesterol ester for TAG between lipid-rich lipoproteins in the presence of cholesterol ester transfer protein (Barter et al., 2003). However, the magnitude of cholesterol ester lost by HDL is greater than the amount of TAG gained (Rye et al., 1995), thus there is a net reduction in the HDL lipid core. The resulting TAG-rich, cholesterol-depleted HDL particle is now preferentially hydrolysed by hepatic lipase bound to the liver sinusoidal capillaries (Barter, 2002), releasing apo A and denser HDL_3 particles (Clay et al., 1992). These HDL remnant particles are taken up by the liver and

catabolised (Lewis and Rader, 2005), thereby delivering cholesterol to the hepatocytes for re-esterification and storage, re-packaging into VLDL or the formation of bile acids (Grundy, 1983). Therefore, prolonged elevations in circulating TAG-rich lipoproteins (i.e. postprandial lipaemia) lowers total HDL concentrations and unfavourably increases the ratio of HDL_3 to HDL_2 fractions (Patsch, 1987).

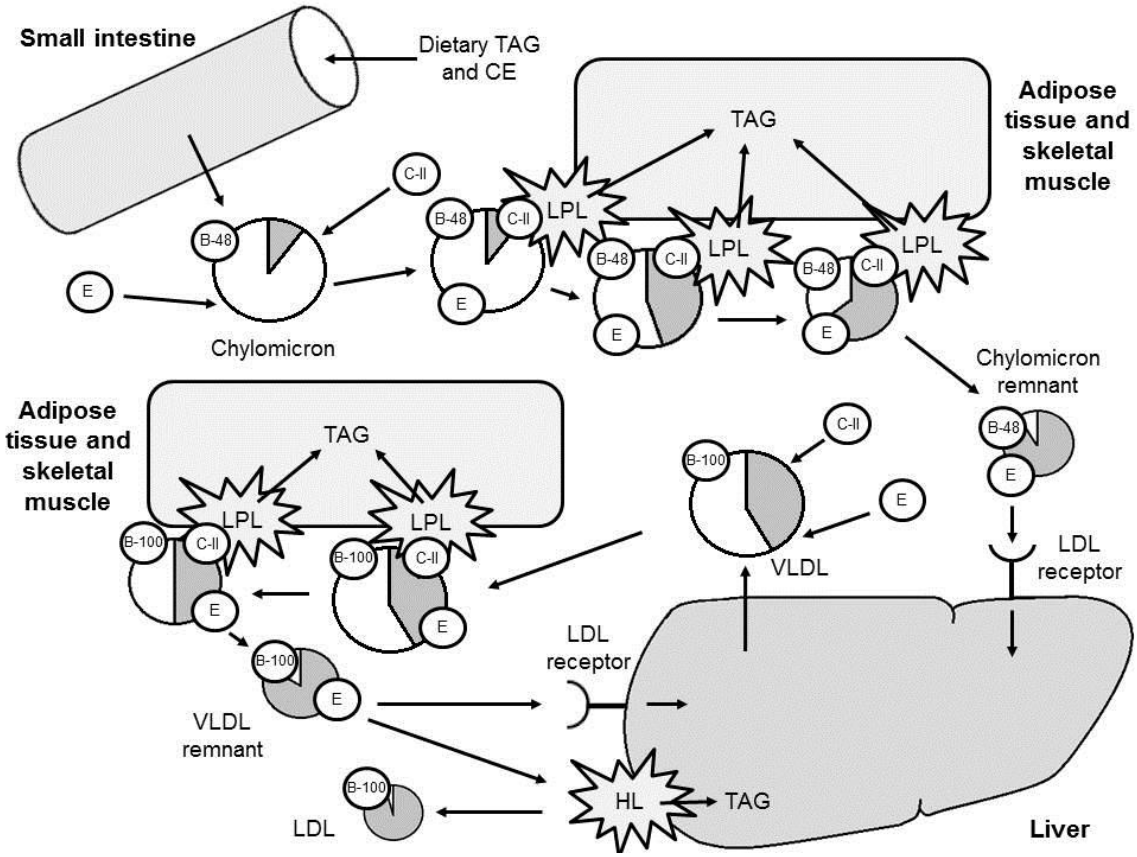

Figure 2.1 Lipoprotein metabolism. This schematic provides a simplified overview of triacylglycerol (TAG) rich lipoprotein metabolism. For clarity, high-density lipoproteins have not been included, but note that they are instrumental in the scavenging of apoproteins E and C-II. The shaded portions of each lipoprotein describe the relative ratio of cholesterol ester (CE) to TAG content. LPL, lipoprotein lipase; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HL, hepatic lipase. Adapted from Jackson et al., (2012).

2.1.8 Postprandial lipaemia and promotion of an atherogenic phenotype

Circulating VLDL are the only TAG-rich lipoproteins present during the postabsorptive state. However, following a meal containing fat, TAG is packaged into chylomicrons and secreted into the bloodstream, and this

lipoprotein competes with VLDL for clearance via the lipoprotein lipase pathway (Brunzell et al., 1973).

There is currently some debate regarding which lipoprotein fraction is associated with postprandial lipaemia, likely due to difficulties in determining chylomicron particle size (Nakajima et al., 2011). In the early 1990s it was demonstrated that chylomicrons account for only 20% of the postprandial increase in circulating lipoproteins (Schneeman et al., 1993), but 80% of the increase in total plasma TAG concentration (Cohn et al., 1993). Therefore, only a relatively small number of TAG-rich chylomicrons were thought to be responsible for the increase in postprandial lipaemia. However, the recent development of new immunoseparation techniques indicate that the major remnant lipoproteins associated with postprandial lipaemia are VLDL particles, although these make a smaller contribution to the total TAG concentration (Nakajima et al., 2011). Regardless, the aforementioned lipid exchange between TAG-rich lipoproteins via cholesterol ester transferase protein is enhanced during exaggerated lipaemia (Groener et al., 1984), and this increases the cholesterol content of remnant chylomicrons and the abundance of VLDL remnants. It is thought that these particles become more atherogenic as a result of a greater cholesterol core (Zilversmit, 1995). Indeed, it has been demonstrated that chylomicron remnants can penetrate the arterial wall and remain trapped within the sub-endothelial space (Mamo et al., 1998), whilst VLDL remnants have been identified within fibrous plaques (Pal et al., 2003, Rapp et al., 1994). Thus, it is possible that a causal relationship may exist between repeated periods of elevated TAG, fatty streak incidence and the development of larger atherosclerotic plaques (the “Zilversmit hypothesis” (Zilversmit, 1979)).

It is now apparent that the Zilversmit hypothesis does not fully explain the link between repeated postprandial lipaemia and atherosclerosis, as the evidence supporting the ability of chylomicron remnants to directly contribute to fatty streak development is not universal (Karpe and Hamsten, 1995, Nordestgaard and Nielsen, 1994). The more recent “triglyceride intolerance hypothesis” (Patsch et al., 1992, Miesenbock, 1992) suggests that the mechanistic link between postprandial lipaemia and atherosclerosis extends to the whole

constellation of lipoproteins and their metabolic cascade, rather than just the carriers of TAG. A summary of these processes is provided in Figure 2.2.

It is understood that the preferential hydrolysis of TAG-rich chylomicrons by lipoprotein lipase (Potts et al., 1991) results in a slower clearance of VLDL. This reduction in postprandial VLDL catabolism (rather than an increase in hepatic VLDL secretion (Bjorkegren et al., 1996)) extends VLDL residence time and facilitate further exchanges of TAG and cholesterol ester between VLDL and cholesterol ester transferase (Barter et al., 2003, Havel, 1994). Accordingly, the main postprandial increase in cholesterol ester concentrations in TAG-rich lipoproteins occur in VLDL, not chylomicrons (Schneeman et al., 1993). The increased activity of cholesterol ester transferase during hypertriglyceridaemia (Groener et al., 1984), also promotes the formation of the denser HDL_3 particle (Clay et al., 1992), and eventual catabolism of HDL (Lewis and Rader, 2005).

A reduction in circulating HDL fractions is problematic as this compromises the hydrolysis of future TAG-rich chylomicrons and VLDL via the attenuated transfer of apo C-II (Havel, 1994, Lewis and Rader, 2005). Furthermore, fewer HDL are available for the exchange of TAG and cholesterol via cholesterol ester transferase (Barter et al., 2003), thereby interfering with the lypotic cascade of chylomicrons and VLDL and increasing the transit time of these TAG-rich lipoproteins. As a consequence, elevated postprandial lipaemia is thought to be the “driving force” in the reduction of HDL (Patsch et al., 1984, Patsch et al., 1992), and that the inverse associated between HDL and CVD (Tall, 1990, Cooney et al., 2009, Gordon et al., 1989, Gordon et al., 1977) is related, in part, to the positive association between postprandial lipaemia and CVD (Patsch et al., 1992).

In concert with unfavourable changes in circulating HDL fractions, elevated lipaemia promotes the formation of smaller and more dense LDL particles (McNamara et al., 1992) due to the slower catabolism of VLDL (Bjorkegren et al., 1996), and the increased interaction (Schneeman et al., 1993) and activity (Groener et al., 1984) of cholesterol ester transferase. The density of LDL is known to be positively associated with a greater oxidative susceptibility (Chait et al., 1993, Tribble et al., 1992), thereby encouraging macrophage endocytosis of the now modified, dense LDL particle (Kelley, 1991) and ultimately foam cell

formation (Bobryshev, 2006) and proliferation in the blood vessel wall (Yla-Herttuala et al., 1989). As such, the smaller, denser LDL particles associated with exaggerated lipaemia (McNamara et al., 1992) are strongly and positively related to CVD risk (Austin et al., 1988, Lamarche et al., 1997).

2.1.9 Postprandial lipaemia and oxidative stress

Oxidative stress is an umbrella term used to describe a number of conditions which alter the pro/anti-oxidant balance toward oxidative damage (Powers et al., 2011). Whilst the continuous interactions between free radicals are legion and difficult to fully elucidate, it is thought that the enhanced influx of FA into the muscle, adipose and hepatic tissues during periods of hypertriglyceridemia (Goldberg et al., 2009) augments FA metabolism via β–oxidation and the tricarboxylic acid cycle. This causes an overproduction of electron donors which in turn may overload the electron transport chain and cause an accumulation of electrons (Wallace et al., 2010). This surplus of electrons may be donated to molecular oxygen by coenzyme Q, thereby increasing mitochondrial production of superoxide radicals (O_2^-) and augmenting oxidative stress (Brownlee, 2005, Wallace et al., 2010). Denser LDL particles are prominent targets for oxidative modification (Chait et al., 1993, Steinbrecher et al., 1984), and this process is considered to play an integral role in the development of CVD (Pentikainen et al., 2000, Steinberg, 2009).

Data are also available linking postprandial oxidative stress with transient periods of endothelial dysfunction (Anderson et al., 2001, Bae et al., 2001). This process is thought to be a key event in the progression of atherosclerosis (Ross, 1993) and is reviewed in Section 2.9. Indeed, endothelial dysfunction appears to be a requirement for the initiation and subsequent development of atherosclerosis (Juonala et al., 2004), as discussed in Chapter 1 and described in Figure 2.2. Taken together, the association between postprandial lipaemia and CVD (Bansal et al., 2007, Iso et al., 2001, Nordestgaard et al., 2007, Sarwar et al., 2007) may be explained not only by the development of an atherogenic lipoprotein phenotype (Austin et al., 1990), but also by the unfavourable modification of these denser LDL particles via oxidative stress

(Sies et al., 2005), during periods of impaired endothelial function (Wallace et al., 2010).

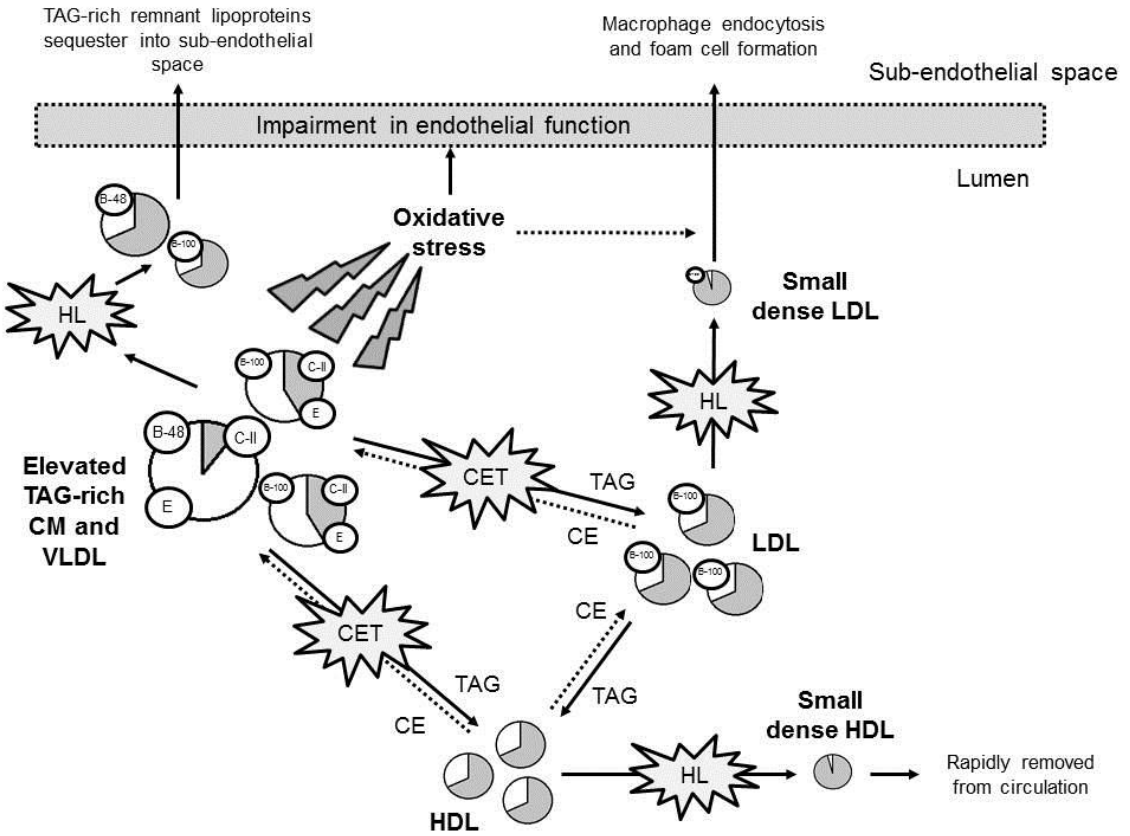

Figure 2.2 Postprandial lipaemia and the development of an atherogenic phenotype. This schematic provides an overview of the consequences of delayed clearance/increased residence time of triacylglycerol (TAG) rich chylomicrons (CM) and very-low-density lipoproteins (VLDL), both of which have may penetrate the endothelium and contribute to fatty streak development. Cholesterol ester (CE) is exchanged for TAG between the increased CM and VLDL pool and low-density lipoproteins (LDL) and high-density lipoproteins (HDL) via cholesterol ester transferase (CET). The hydrolysis of this TAG via hepatic lipase (HL) encourages the formation of smaller, denser HDL which are rapidly removed from the circulation. In turn, this limits the scavenging of apoproteins E and C-II from HDL by CM and VLDL, thus further delaying clearance of TAG-rich lipoproteins. Smaller, denser LDL are more susceptible to oxidative modification and encourage foam cell formation via receptor-mediated macrophage endocytosis. These processes occur in concert with an impairment in endothelial function via oxidative stress, which is considered to be integral to the atherosclerotic cascade.

2.2 Factors affecting postprandial lipaemia

In comparison to the number of studies with adults, limited data are available identifying the factors which influence postprandial lipaemia in children and adolescents. Thus, much of our understanding is derived from literature in adults and will therefore be discussed in the following sections.

2.2.1 Assessment and interpretation

Whilst intravenous fat tolerance tests have been adopted for research purposes (Cohen, 1989, Hallberg, 1965), postprandial lipaemia is commonly assessed via the consumption of a high fat meal, and as such is conceptually similar to the oral glucose tolerance test. However, unlike the latter, there are no official guidelines for an oral fat tolerance test, which has resulted in the use of a wide range of study designs (Kolovou et al., 2011).

Following a high fat meal, postprandial investigations typically report that plasma [TAG] slowly increases until a zenith is reached 3 to 4 hours later, and return to baseline after ~ 8 hours provided that no other food is consumed (Patsch, 1987, Peddie et al., 2012). However, it is likely that this well documented plasma TAG curve is a function of infrequent blood sampling, as the postprandial response may be more accurately defined as bi- (Mattes, 2002, Fielding et al., 1996) or even tri-phasic (Cohn et al., 1988) in nature, with the first peak occurring ~ 1 hour after ingestion and predominantly representing the TAG consumed in the previous meal (Fielding et al., 1996). Thus, standardisation (or replication) of diet before postprandial assessment is encouraged, and a preceding ~ 12 hour fast is considered to be mandatory (Kolovou et al., 2011). Consequently, studies in this field typically take place in the morning, following an overnight fast. This has the added benefit of broadly standardising the time of the meal ingestion between studies, as it is known that postprandial lipaemia is exaggerated following the consumption of a high fat meal in the morning compared to an identical meal in the afternoon (Burdge et al., 2003).

Conventionally, the postprandial observational time invariably lasts 6-8 hours and includes regular (often hourly) blood samples (Peddie et al., 2012). This assessment of an oral fat tolerance test has been shown to provide a high level of reproducibility in men (a within-subject coefficient of variation (CV) of 10.1%) (Gill et al., 2005). In order to minimise the burden of testing for participants, some studies have investigated the validity of a shorter, 4 hour postprandial period (Maraki et al., 2011, Weiss et al., 2008) and protocols which require three (Guerci et al., 2001) or just one (Maraki et al., 2011, Rector et al., 2009) blood sample(s). A truncated 4 hour observation period has been demonstrated

to provide a valid surrogate of a 6 (Maraki et al., 2011) or 8 hour (Weiss et al., 2008) postprandial observational period in lean and obese normolipidaemic men ($R^2 > 0.70$, $P < 0.01$ for all analyses). In contrast, limited success has been reported using a three (Carstensen et al., 2003, Guerci et al., 2001) or single (Maraki et al., 2011, Rector et al., 2009) sample protocol, probably due to the large inter-individual variation in the kinetics of TAG handling even in normolipidaemic individuals (Patsch et al., 1983).

Postprandial lipaemia is most commonly quantified as an area under the plasma TAG curve (AUC) versus time using the trapezoid rule, which has the statistical benefit of collating the entire TAG response into a single value (Matthews et al., 1990). The AUC analysis is typically expressed either as a total (TAUC) or incremental (IAUC) AUC, with the former often identified as the primary outcome measure in postprandial studies (Gill et al., 1998, Koutsari and Hardman, 2001, Malkova et al., 2000). Whilst the TAUC analysis expresses the gross lipaemic response following a meal, and is therefore the most reflective measure of the total physiological challenge, its use in studies where baseline TAG may be manipulated (i.e. by prior exercise) has come under criticism (Carstensen et al., 2003, Kolifa et al., 2004) as the TAUC analysis strongly correlates with fasting plasma [TAG] (Lewis et al., 1991, Potts et al., 1994, Gill et al., 2007). Indeed, a change in baseline TAG the day after exercise has been shown to account for 79% of the difference in TAUC for TAG between exercise and non-exercise conditions (Kolifa et al., 2004). In contrast, the IAUC analysis reflects the net lipaemic response to a high fat meal as the AUC is adjusted for baseline plasma [TAG]. Therefore, the IAUC analysis may be considered to better describe any changes in TAG handling following an intervention by partitioning out the confounding influence of modified baseline [TAG] (Carstensen et al., 2003). Regardless of these differences, it is common practice to report both AUC outcomes.

2.2.2 Test meal composition

It has been demonstrated in adults that a meal containing ≤ 15 g of fat is not sufficient to increase postprandial plasma TAG (Dubois et al., 1998). In contrast, 30 – 50 g of fat increases the TAUC for plasma TAG in a dose-

dependent manner (Cohen et al., 1988, Dubois et al., 1998), and while the magnitude of the postprandial response is even greater following meals ≥ 80 g of fat, this is no longer dose-dependent (Cohen et al., 1988, Murphy et al., 1995).

The type of fat is also essential to the postprandial response, as short and medium chain FA enter the circulation via the portal route and therefore are not associated with enhanced chylomicron secretion (Patsch, 1987). Consequently, the use of dairy fat as the predominant source of fat in a test meal is thought to elicit a lower postprandial response than other types of fat (Lopez-Miranda et al., 2007, Peddie et al., 2012). Additionally, meals containing greater amounts of n-3 polyunsaturated FA may lower the lipaemic response compared to n-6 polyunsaturated FA, monounsaturated FA and saturated FA (Lopez-Miranda et al., 2007, Williams, 1998).

The inclusion of carbohydrate in a test meal is associated with profound effects on the subsequent postprandial lipaemic response. Postprandial lipaemia has been shown to be increased (Singleton et al., 1999), unchanged (Cohen and Schall, 1988), attenuated (Cohen and Berger, 1990) or abolished (Albrink et al., 1958) with the co-ingestion of 17.5 g, 50 g, 100 g and 100-250 g of glucose respectively. The mechanisms behind these relationships are not well defined and difficult to decipher, partly due to differences in the fat loads between studies. However, factors such as a delay in gastric emptying and subsequent slowing of chylomicron release (Westphal et al., 2002) and a reduction in hepatic VLDL secretion due to the suppression of FA released from adipose tissue during periods of elevated insulin (Lewis et al., 1995) are likely to be influential. Importantly, the manipulation of postprandial lipaemia following the co-ingestion of different carbohydrate sources does not appear to be uniform as the addition of fructose to a meal increases the subsequent lipaemic response (Grant et al., 1994), even if the meal contains only 5 g of fat (Jeppesen et al., 1995a).

Several sources of dietary fibre have been shown to lower postprandial lipaemia (Cara et al., 1992, Khossousi et al., 2008, Sandstrom et al., 1994), probably by reducing the intestinal transit time of the consumed fat (Lairon et al., 2007). Finally, the co-ingestion of protein, particularly whey protein (Mortensen et al.,

2009), has also been shown attenuate postprandial lipaemia (Mortensen et al., 2009). Consequently, disparity in test meals (i.e. milkshake (Sedgwick et al., 2012) vs. pizza (Tyldum et al., 2009)) is likely to cloud direct comparisons between studies.

2.2.3 Age and sex

It is understood that postprandial lipaemia is exaggerated with increasing age (Cohn et al., 1988). Indeed, chylomicron residence time has been demonstrated to be nearly twice as long in men and women over 60 years of age compared to those aged 18-30 years (Krasinski et al., 1990). This reduced chylomicron clearance in the elderly is consistent with data indicating an inverse relationship between age and lipoprotein lipase activity (Huttunen et al., 1976). It is also know that children are characterised by a slower gastric emptying time than adults when fed full cream milk (Maes et al., 1995), which may be of importance considering that the majority of postprandial investigations in paediatric populations incorporate dairy products (e.g. double cream, ice cream, whipping cream) as the primary source of fat in the test meal (Barrett et al., 2007, MacEneaney et al., 2009, Thackray et al., 2013, Tolfrey et al., 2012, Tolfrey et al., 2008, Tolfrey et al., 2014a).

It is widely reported that women experience an attenuated lipaemic response following a high fat meal compared to men, regardless of age (Cohn et al., 1988, Couillard et al., 1999, Horton et al., 2002, Koutsari et al., 2004). Whilst the precise mechanisms and their relative contributions behind this disparity are yet to be fully elucidated, evidence suggests that these differences extend beyond the sex hormones (Wang et al., 2011). Indeed, it is thought that the dyslipidaemia associated with the menopause (Derby et al., 2009) can be predominantly attributed to the aforementioned effect of aging (Cohn et al., 1988), rather than loss of ovarian function *per se* (Wang et al., 2011, Casiglia et al., 2008). However, it is likely that sex hormones are partly responsible for the differences in lipaemic response, as it has been demonstrated that the TAG excursion following a test meal can be modified by menstrual phase (Gill et al., 2005).

Data are available which identify that this sexual dimorphism is unrelated to fasting [TAG] (Demacker et al., 1982), insulin sensitivity (Jensen, 1995), total body fat (Koutsari et al., 2000), body mass (Baggio et al., 1980), intestinal TAG absorption (Horton et al., 2002), or postprandial substrate oxidation (Jensen et al., 1998), but may be related to differences in visceral body fat (Couillard et al., 1999). It has also been demonstrated that women are characterised by a greater uptake of TAG by the skeletal muscle following a meal (Horton et al., 2002), and superior total body (Despres et al., 1999) and adipose tissue (St-Amand et al., 1995) lipoprotein lipase activity, although these findings are not universal (Perreault et al., 2004). Clearance of TAG may further be enhanced in women by differences in VLDL metabolism. Specifically, women produce fewer VLDL particles, which are larger and richer in TAG than men (Magkos et al., 2007). This is thought to be important as it has been demonstrated *in vitro* that the lipolysis of larger, TAG-richer lipoproteins by lipoprotein lipase is more efficient than their smaller, TAG-poorer counterparts (Fisher et al., 1995).

2.2.4 Postprandial lipaemia in children and adolescents

Despite the consistent evidence supporting an independent effect of sex on the postprandial response to a high fat meal in adults (Couillard et al., 1999, Koutsari et al., 2004), no study has directly compared the lipaemic response to a test meal between boys and girls. In truth, limited data are available identifying the factors which influence postprandial lipaemia in young people, which is surprising considering the effect of age on postprandial lipaemia (Cohn et al., 1988), the link between non-fasting TAG in childhood and future CVD (Morrison et al., 2009), and the understanding that atherosclerosis starts in youth (Berenson et al., 1992, Stary, 1989).

In 1994, Uiterwaal *et al*. (1994) demonstrated that healthy young men (mean age 25 ± 5 years) with parental history of CVD were characterised by higher postprandial TAG concentrations than a control group. Whilst this cohort does not qualify as a paediatric population, this investigation suggested that unfavourable changes in postprandial lipoprotein metabolism could be identified many years before overt CVD. Similar findings have been reported in the normolipidaemic offspring (mean age 22 ± 5 years) of parents with combined

familial hyperlipidaemia (Reiber et al., 2003). However, the increased lipaemic response in “at risk” offspring (mean age 23 ± <1 years) is not a universal finding (Tiret et al., 2000), although these authors did note that exaggerated lipaemia was related to elevated fasting [TAG], which is in agreement with adult data (O'Meara et al., 1992).

The Columbia University Biomarkers Study was the first to investigate the correlates of postprandial lipaemia in children and adolescents (Couch et al., 2000). This study assessed the lipaemic response to a high fat meal (52.5 g) in adolescents with or without a family history of premature coronary heart disease and identified that low [HDL] and high fasting [TAG] were associated with elevated lipaemia. In contrast to Uiterwaal *et al.* (1994), family history had no influence on the postprandial response. Low [HDL] (Patsch et al., 1983) and elevated fasting [TAG] (O'Meara et al., 1992) are known to be associated with an exaggerated lipaemic response in adults. Thus, the authors concluded that the predictors of postprandial lipaemia might be similar between children and adults.

Support for this hypothesis was provided a year later when data were published identifying that postprandial lipaemia is exaggerated in adolescents with central obesity (Moreno et al., 2001), a finding which is also apparent in adults (Ryu et al., 1994, Wideman et al., 1996). Additionally, elevated postprandial lipaemia is apparent in adolescents with type 2 diabetes mellitus and elevated fasted [TAG] (Umpaichitra et al., 2004), both of which corroborate with the extant literature in adults (O'Meara et al., 1992, Chen et al., 1993a). Provisional evidence is also available which indicates that the lower lipaemic excursion following a high fat meal in African American adults compared to Caucasian adults (Friday et al., 1999) may be present in adolescents (Lee et al., 2013).

In summary, there is a growing body of evidence indicating that factors which are known to exaggerate postprandial lipaemia in adulthood (i.e. obesity, hypertriglyceridaemia and hyperinsulinaemia) may have similar effects during childhood and adolescence. However, this field of research remains in its infancy and more studies are needed to partition out the relationship between CVD risk factors and the lipaemic response to a fat challenge in youth. Furthermore, no paediatric data are available concerning other known

influences on postprandial lipaemia, such as preceding diet (Blades and Garg, 1995), smoking (Sharrett et al., 2001), alcohol use (Fielding et al., 2000), sex (Cohn et al., 1988, Couillard et al., 1999, Koutsari et al., 2004), changes in sex hormones at the onset of puberty (Wang et al., 2011), and apolipoprotein phenotype (Lopez-Miranda et al., 2007).

2.3 Physical activity and postprandial lipaemia

The relationship between physical activity and postprandial lipaemia in youth remains to be elucidated. Cross-sectional studies have frequently reported that adults who regularly engage in physical activity are characterised by a lower lipaemic response following a test meal (Cohen et al., 1989, Cohen et al., 1991, Merrill et al., 1989, Ziogas et al., 1997), and this has been attributed to an observed increase in lipoprotein lipase activity in endurance-trained individuals (Podl et al., 1994). However, none of these studies required the participants to refrain from exercise in the days before the postprandial assessment. This is a substantial methodological flaw, as a single bout of exercise performed the day before a fat tolerance test profoundly affects the postprandial response (see section 2.4). Consequently, it is not possible from these studies to identify whether the benefit of regular physical activity on postprandial lipaemia is related to the last bout of exercise or habitual activity *per se*.

Two studies have since provided clarification on this issue by requiring participants to refrain from exercise for at least 48 hours prior to the consumption of the high fat test meal (Herd et al., 2000, Tsetsonis et al., 1997). Both of these studies failed to report any differences in postprandial lipaemia between trained and untrained adult groups. A similar finding has been reported Aldred *et al*. (1995) who also included a 48 hour abstention from exercise period between the completion of a 12 week exercise training intervention and the high fat test meal in adult women. This lack of improvement in postprandial lipaemia occurred in the presence of favourable changes in physical fitness and insulin sensitivity. Finally, Freese *et al*. (2015) recently demonstrated that the magnitude of the reduction following a single bout of sprint interval cycling is comparable to that observed the day after 6 weeks of sprint interval training (13.1% vs 9.7% respectively) in women at risk of the metabolic syndrome.

Whilst these authors did not determine whether the sprint interval cycling protocol improved physical fitness, this study provides evidence that the favourable lipaemic response to a test meal observed after an acute bout of exercise cannot be "trained". Therefore, it appears habitual physical activity and exercise training does not directly influence postprandial lipaemia in adults beyond a "last bout effect". Furthermore, no data are available indicating that the magnitude of the lipaemic response to a single bout of exercise is influenced by training status.

2.4 Acute exercise and postprandial lipaemia

A single bout of exercise has repeatedly been demonstrated to lower the lipaemic response to a high fat meal in adults. It is outside the scope of this thesis to include an exhaustive critique of this evidence base, and the reader is referred to a number of excellent review articles for more detail (Freese et al., 2014, Maraki and Sidossis, 2013, Peddie et al., 2012). However, this section will briefly draw upon the adult literature in order to discuss how characteristics of the exercise bout (i.e. energy expenditure, intensity, mode and timing) have been shown to influence the subsequent postprandial lipaemic response.

2.4.1 Exercise-induced energy expenditure

In a seminal study, Tsetsonis and Hardman (1996b) reported comparable reductions in postprandial lipaemia following 3 hours of walking at 32% of the maximal oxygen uptake ($\dot{V}O_{2\ max}$) and 1.5 hours of walking at 63% $\dot{V}O_{2\ max}$, thereby demonstrating that the energy expended during an exercise bout was the most important determinant of the subsequent reduction in postprandial lipaemia. Two years later, Gill *et al*. (1998) highlighted the importance of energy expenditure by identifying that the reduction in postprandial lipaemia was the same (~ 18%) when 90 minutes of walking at 60% $\dot{V}O_{2\ max}$ was completed in one bout or three 30 minute sessions.

Several investigations identified a dose-response between the exercise-induced energy expenditure and magnitude of the reduction in postprandial lipaemia.

Doubling energy expenditure by performing 90 minutes of exercise at 30% and 60% $\dot{V}O_{2\ max}$ has been shown to attenuate postprandial lipaemia by 16% and 26% respectively (Tsetsonis and Hardman, 1996a). A similar effect occurs when energy expenditure is manipulated by increasing exercise time but keeping exercise intensity constant (Gill et al., 2002a). In 2003, a meta-analysis identified a significant inverse relationship between the magnitude of the reduction in postprandial lipaemia as an effect size (*ES*) and energy expenditure (r=-0.62, P=0.02), and highlighted that only energy expenditure, but not exercise intensity, duration or timing, influenced the postprandial response (Petitt and Cureton, 2003). However, at the time data from only 13 studies were available for this analysis, and the authors reported a weak relationship between the magnitude of the reduction in postprandial lipaemia and the exercise-induced energy expenditure (r=-0.35, P value not provided) once two outlying studies were removed.

A more recent meta-analysis of 53 studies by Freese *et al.* (2014) reported a similar relationship between the change in postprandial lipaemia and the energy expended during aerobic exercise (r=-0.31, P<0.01). Interestingly, these authors reported that no relationship was present between the attenuation in postprandial lipaemia and the energy expended during resistance (r=-0.28, P=0.37) or high-intensity interval exercise (r=-0.27, P=0.60). It is plausible that this dissociation is partly due to the difficulty in accurately determining energy expenditure via indirect calorimetry during these activities, however given that these two reviews indicate that the energy expended during moderate-intensity exercise only accounts for approximately 10 – 12% of the variance in postprandial lipaemia, it is likely that other characteristics of the exercise bout mediate the postprandial response. Indeed, Peddie *et al.* (2012) demonstrated in their review that any relationship between exercise-induced energy expenditure and postprandial lipaemia is lost when the IAUC for plasma [TAG] is adopted instead of the TAUC analysis. Thus, energy expended during prior exercise may lower baseline plasma [TAG] the following day, but not the net lipaemic response following consumption of the test meal.

Data are available indicating that energy expended during exercise promotes a greater reduction in postprandial lipaemia than dietary restriction (Bellou et al., 2013, Gill and Hardman, 2000, Maraki and Sidossis, 2010). Gill and Hardman

(2000) demonstrated that 90 minutes of walking at ~ 63% $\dot{V}O_{2\ max}$ significantly attenuated postprandial lipaemia by 20%, whilst an isocaloric energy deficit (via dietary restriction) only elicited a 7% reduction. Additionally, replacing the energy expended during exercise does not completely diminish the reduction in postprandial lipaemia (Burton et al., 2008, Freese et al., 2011, Harrison et al., 2009, Maraki and Sidossis, 2010). Thus, the energy deficit induced by exercise may not fully explain the subsequent postprandial response.

2.4.2 Exercise intensity and modality

Whilst the energy expended during low- to moderate-intensity exercise appears to be a determinant of the subsequent postprandial response (Freese et al., 2014, Petitt and Cureton, 2003), data are available indicating that this relationship is lost when high-intensity exercise (Gabriel et al., 2012, Trombold et al., 2013) or resistance exercise (Magkos et al., 2008, Petitt et al., 2003) precedes the test meal.

Trombold *et al*. (2013) identified that high-intensity interval cycling elicited significantly greater reductions in postprandial lipaemia than an isoenergetic moderate-intensity bout (45% and 25% respectively). One of the strengths of this study is that the authors quantified exercise-induced changes in excess post oxygen consumption and demonstrated that the total energy expenditure was comparable between trials. A similar finding was reported by Gabriel *et al*. (2012) who demonstrated that high-intensity interval cycling elicited comparable reductions in lipaemia as 30 minutes of walking despite a ~ 60% lower energy expenditure. Indeed, a recent review highlighted that high-intensity exercise may be the most effectual at lowering the IAUC-TAG outcome (Freese et al., 2014).

Resistance exercise also appears to provide a potent stimulus for favourable reductions in postprandial lipaemia. Petitt *et al*. (2003) demonstrated that 1.7 MJ (~ 400 kcal) of resistance exercise significantly attenuated postprandial lipaemia by 18% compared to a walk matched for duration and energy expenditure. Similarly, Magkos *et al*. (2008) identified that ~ 400 kcal of resistance exercise significantly increased the rate of VLDL clearance

compared to an isoenergetic walk (24%) and a non-exercise control trial (30%). Interestingly, the intensity of resistance exercise does not appear to modulate the postprandial response provided that the two exercise trials are matched for energy expenditure (Singhal et al., 2009). Further studies identifying the influence of exercise intensity are needed, but the aforementioned investigations provide evidence that the characteristics of the exercise bout may affect the subsequent lipaemic response.

2.4.3 Accumulated exercise

Public health guidelines include accumulating exercise over the course of the day as a feasible alternative to a single bout of exercise (Haskell et al., 2007, Pate et al., 1995), and data are available indicating that this pattern of exercise can increase adherence to physical activity interventions in adults (Jakicic et al., 1995). Consequently, it is pertinent to address whether accumulating exercise can attenuate postprandial lipaemia, and whether this pattern of exercise is more effectual than a single exercise bout.

In the first study to address this question, Gill *et al*. (1998) demonstrated that three 30 minute bouts of running at 60% $\dot{V}O_{2\ max}$ the day before a test meal elicited comparable reductions (~ 18%) in postprandial lipaemia as one intensity-matched 90 minute bout in men. Two years later, Murphy *et al*. (2000) demonstrated that three 10 minute bouts of walking at ~ 60% $\dot{V}O_{2\ max}$ lowered the average plasma [TAG] response to multiple meals to a similar extent as one 30 minute walk (~ 12%) in sedentary men and women. This finding is encouraging as small volumes of walking exercise should be feasible for most.

In the years that followed, several studies confirmed that accumulating exercise is as effectual as a comparable exercise stimulus performed in a single bout for the attenuation of postprandial lipaemia in healthy (Miyashita et al., 2006, Miyashita et al., 2008), and obese (Miyashita, 2008) men. One notable exception is the study performed by Altena *et al*. (2004), who reported that three 10 minute bouts of running at 60% $\dot{V}O_{2\ max}$ reduced postprandial lipaemia by 27% in women, whilst an equivalent 30 minute bout elicited only a 16% reduction. These authors speculated that this difference might be related to a

greater total excess post-exercise oxygen consumption when exercise is accumulated, however this was not measured in this study. Additionally, these lipaemic responses were not significantly different between exercise trials (*P* value not provided). Regrettably, the authors also do not provide the means and standard deviations for plasma [TAG] (TAUC or IAUC) in their manuscript, so it is not possible to determine the magnitude of this difference as an effect size. Thus, the weight of evidence indicates that accumulating exercise elicits comparable reductions in lipaemia provided that the total exercise stimulus is the same.

2.4.4 Exercise timing

Data are available indicating that the timing of the exercise bout(s) in relation to the high fat meal is a key determinant of the postprandial response. In a landmark study, Zhang *et al*. (1998) reported a significant 51% and 38% decrease in postprandial lipaemia when 1 hour of exercise at 60% $\dot{V}O_{2\ max}$ was performed either 12 hours or 1 hour before a high fat meal compared to a resting control trial ($P>0.05$ for the difference between trials). However, postprandial lipaemia was only attenuated by 5% when this exercise bout was performed 1 hour after the meal, which was not significantly different compared to the control trial. These results were attributed to the delayed peak in lipoprotein lipase activity (Seip et al., 1997, Seip and Semenkovich, 1998, Zhang et al., 2002) which is discussed later in Section 2.5.1. Other studies (Petridou et al., 2004, Pfeiffer et al., 2005), but not all (Katsanos et al., 2004, Hardman and Aldred, 1995), have since reported that exercise (which would reasonably be expected to lower postprandial lipaemia) performed immediately before or during the postprandial period failed to attenuate the lipaemic response to a test meal. Further research is needed to identify the mechanistic basis of this disparity. However, there is preliminary evidence to suggest that sex might modulate the reduction in postprandial lipaemia when the time between the exercise bout and the test meal is short (Henderson et al., 2010). Specifically, these authors demonstrated postprandial lipaemia is lowered in the immediate hours after exercise in women but not men, possibly due to sex differences in VLDL metabolism (see Section 2.5.6).

2.5 Mechanisms underlying the reduction in postprandial lipaemia after exercise

A lower postprandial TAG excursion following exercise is logically related to either a reduced appearance or increased clearance of TAG from the blood. Almost all of our understanding regarding the mechanisms responsible for this reduction in postprandial lipaemia, and how this interaction can be modulated by manipulating the exercise bout, is derived from research with adult groups. This is predominantly a result of ethical considerations and the obvious need to restrict laboratory techniques to those that are suitable for the study population. Accordingly, evidence for the proposed mechanism(s) responsible for an exercise-induced modulation in postprandial [TAG] is drawn from the extant adult literature.

2.5.1 Lipoprotein lipase

Typically, postprandial investigations adopt a 2-day protocol, whereby the exercise bout is completed in the afternoon or evening and the participants consume a standardised evening meal before being transported to the laboratory the following morning after an overnight fast. In addition to controlling for the confounding effects of previous meal composition (Chen et al., 1993b, Weintraub et al., 1988), baseline [TAG] (Lewis et al., 1991, Potts et al., 1994), prior exercise (Maraki and Sidossis, 2013, Peddie et al., 2012), and diurnal variation (Burdge et al., 2003), the benefit of this design is that it coincides with the delayed peak in lipoprotein lipase activity post exercise (Seip and Semenkovich, 1998, Zhang et al., 2002), which is responsible for the hydrolysis of TAG-rich lipoproteins and thus conceptually linked to attenuation of postprandial lipaemia.

Two early investigations in the 1980s suggested that an increase in lipoprotein lipase activity following endurance running was responsible for the subsequent attenuation in postprandial plasma [TAG] (Kantor et al., 1984, Sady et al., 1986). In the years that followed, it was demonstrated that skeletal muscle contractions promote a transient (Seip et al., 1997, Seip and Semenkovich, 1998) and tissue-specific (Seip et al., 1995) increase in lipoprotein lipase activity. The time course of the increase in lipoprotein lipase activity post

exercise is not well defined, but the activity of this enzyme has been demonstrated to increase 4 hours post exercise and peak between 8-24 hours after exercise (Seip et al., 1997, Seip and Semenkovich, 1998, Zhang et al., 2002). Furthermore, this increased activity of lipoprotein lipase has been shown to correlate strongly (r >0.77, P<0.05) with the reduction in postprandial lipaemia observed after exercise (Gill et al., 2003b, Herd et al., 2001). The mechanism(s) behind this upregulation is(are) not fully understood, however studies which manipulated exercise intensity (and thus fibre type recruitment) in rats indicate that lipoprotein lipase activity is increased only in the corresponding fast- or slow-twitch muscle fibres (Bey and Hamilton, 2003, Hamilton et al., 1998), thereby suggesting the importance of contractile activity.

Data are available indicating that exercise intensity and the exercise-induced energy expenditure may both modulate the upregulation in lipoprotein lipase activity. Gordon *et al*. (1996) demonstrated that lipoprotein lipase activity increased 24 hours after treadmill running at 75% $\dot{V}O_{2\ max}$, but not after an isoenergetic bout (800 kcal) at 60% $\dot{V}O_{2\ max}$. Elsewhere, an expenditure of 800 kcal at 70% $\dot{V}O_{2\ max}$ failed to increase lipoprotein lipase activity 24 hours later, although a 33% increase was observed when 1100 kcal were expended at this intensity (Ferguson et al., 1998). Interestingly, these authors reported a 26% reduction in plasma TAG concentrations despite no change in lipoprotein lipase activity following the 800 kcal exercise bout, indicating that attenuations in postprandial lipaemia may be possible without a concomitant upregulation of lipoprotein lipase activity. Indeed, of the available studies which observed favourable changes in postprandial lipaemia after exercise and measured lipoprotein lipase activity (Herd et al., 2001, Thomas et al., 2001, Zhang et al., 2002, Gill et al., 2003b, Miyashita and Tokuyama, 2008, Katsanos et al., 2004, Ferguson et al., 1998), only one identified an increase in lipoprotein lipase activity after exercise compared to a resting control trial (Zhang et al., 2002). However, it is pertinent that one of these studies did observe a strong, inverse relationship between lipoprotein lipase activity and the difference in lipaemic response to the test meal between trials (r = -0.79; P<0.05) despite no apparent changes in lipoprotein lipase activity at a group level (Herd et al., 2001). Thus, it seems likely that the increase in lipoprotein lipase activity has an additive, but not exclusive, role in modulating the lipaemic response to a high fat meal.

2.5.2 Very-low-density lipoprotein metabolism

The lower fasted plasma [TAG] observed the day after acute exercise predominantly reflect a reduction in the [TAG] of VLDL particles (Magkos, 2009). Additionally, recent evidence indicate that the major remnant lipoproteins associated with postprandial lipaemia might be VLDL (Nakajima et al., 2011), rather than chylomicrons as previously thought (Cohn et al., 1993). Thus, it has been proposed that changes in VLDL metabolism may play an important role in reducing postprandial lipaemia after exercise (Katsanos, 2006, Gill et al., 2001, Malkova et al., 2000).

Malkova *et al*. (2000) demonstrated that 70% of the reduction in postprandial lipaemia (33%) achieved the morning after prolonged exercise (i.e. during the period of likely increase lipoprotein lipase activity (Seip et al., 1997, Seip and Semenkovich, 1998)) was attributable to a reduction VLDL-TAG. These findings were corroborated a year later by Gill *et al.* (2001) who reported that 79% of the reduction in plasma TAG concentration (25%) the day after 90 min of brisk walking was due to a reduction in VLDL-TAG.

A reduction in both VLDL concentration (38%) and VLDL-TAG content (40%) has also been observed 4.5 hours after prolonged moderate-intensity exercise (Borsheim et al., 1999). Considering that VLDL and chylomicrons are both hydrolysed by lipoprotein lipase (Brunzell et al., 1973), which has a greater affinity for the latter (Potts et al., 1991), it is likely that this reduction in lipaemia could reflect an attenuated hepatic VLDL-TAG output. Indeed, Gill *et al*. (2001) reported that serum [3-hydroxybutyrate] (3-OHB) increased by over 50% following exercise, which is indicative of a greater rate of hepatic fatty acid oxidation (Gill et al., 2001, Malkova et al., 2000). In turn, this could plausibly limit the availability of fatty acids to be re-esterified and packaged into VLDL (Gorski et al., 1990). Indeed, in a separate study, Gill *et al*. (2007) identified a strong and inverse correlation between the change in [3-OHB] due to exercise and the exercise-induced fall in $VLDL_1$ concentration (r=-0.72, P=0.02).

Magkos *et al*. (2006) observed a reduction in number of VLDL-apo B particles secreted by the liver, but not the total VLDL-TAG excursion, and an increase in VLDL-TAG clearance rate the day after 2 hours of cycling at 60% $\dot{V}O_{2\ peak}$. Recent progress in this field of research was achieved by Al-Shayji *et al*. (2012),

who isolated $VLDL_1$, the major determinant of postprandial lipaemia (Tan et al., 1995), rather than documenting changes in the total VLDL fraction. These authors identified that $VLDL_1$ clearance rate, but not secretion, was responsible for the reduction in postprandial lipaemia the day after 2 hours of walking at ~ 50% $\dot{V}O_{2\ max}$. Larger VLDL particles are more likely to be hydrolysed by lipoprotein lipase (Fisher et al., 1995, Karpe et al., 2007), which would explain why an increased VLDL-TAG clearance rate has been observed in the absence of an increase in lipoprotein lipase activity (Malkova et al., 2000). Indeed, Al-Shayji *et al*. (2012) demonstrated that $VLDL_1$ particles were 26% bigger and ~ 25% TAG-richer the day after exercise, and that the fractional catabolic rate of VLDL was strongly, and positively, correlated with this increase in size and TAG-enrichment. Finally, it has recently been demonstrated *in vitro* that a prior bout of moderate-intensity exercise increases the affinity of $VLDL_1$ for hydrolysis by lipoprotein lipase (Ghafouri et al., 2015). Consequently, the attenuated lipaemic response observed following exercise is likely related to an increase in the efficiency of VLDL-TAG clearance due to the production of fewer, but larger and TAG-richer, VLDL particles (Magkos, 2009, Magkos et al., 2007, Magkos et al., 2006).

2.5.3 Insulin

Insulin controls VLDL secretion from the liver (Lewis and Steiner, 1996), stimulates lipoprotein lipase activity in the adipose tissue in the postprandial period (Picard et al., 1999), but diminishes this activity in skeletal muscle (Kiens et al., 1989). Furthermore, insulin resistance is associated with exaggerated lipaemia (Jeppesen et al., 1995b, Chen et al., 1993a), possibly because insulin inhibits muscle lipoprotein lipase activity in the postprandial state (Kiens et al., 1989). Despite these mechanistic links, Gill *et al.* (2002b) demonstrated that whilst the postprandial lipaemic response correlated with postprandial insulin in a resting control trial (r=0.48, P<0.05), no relationship existed between the change in postprandial TAG and postprandial insulin (r=0.04, P=0.70) the day after 90 minutes of brisk walking at 60% $\dot{V}O_{2\ max}$. A lack of a significant relationship between TAUC-TAG and TAUC-insulin has also been reported following resistance training (Petitt et al., 2003). Thus the beneficial effect of

prior exercise on postprandial lipaemia appears to be independent to changes in [insulin].

2.5.4 Substrate oxidation

In an elegant design, Malkova *et al.* (1999) used acipimox (an inhibitor of lipolysis (Fuccella et al., 1980)) to demonstrate that the reduction in postprandial lipaemia (~ 20%) the day after 90 minutes of jogging at 60% $\dot{V}O_{2\,max}$ was unrelated to the metabolism of fat during the exercise bout. Indeed, several studies have demonstrated that the lipaemic response to a test meal is lower following high-intensity interval exercise (Ferreira et al., 2011, Freese et al., 2011, Gabriel et al., 2012, Trombold et al., 2013), where the reliance of fat oxidation is appreciably lower (van Loon et al., 2001). However, a single exercise bout promotes fat oxidation for ~ 24 hours (Hansen et al., 2005), and this phenomenon may (Phelain et al., 1997), or may not (Kuo et al., 2005) be positively associated with exercise intensity. Several authors have suggested that this shift in substrate metabolism during the postprandial period may account for some of the reduction in postprandial lipaemia (Ferreira et al., 2011, Pfeiffer et al., 2006, Tsetsonis and Hardman, 1996b, Petitt et al., 2003, Trombold et al., 2013). Gill *et al*. (2007) demonstrated that a post-exercise increase in hepatic fat oxidation, as demonstrated via [3-OHB], during the postprandial period accounted for nearly half of the variance in the lipaemic response in men with type 2 diabetes. Additionally, significant inverse relationships have been observed between postprandial fat oxidation and lipaemia following moderate-intensity (r=-0.58) and high-intensity (r=-0.67) exercise (Burton et al., 2008, Trombold et al., 2013). Therefore, more studies which assess the relationship between the post exercise change in substrate oxidation during the postprandial period and the difference in lipaemia are warranted.

2.5.5 Blood flow

In addition to an upregulation in lipoprotein lipase activity, an increase in plasma TAG delivery, either to skeletal muscle, the liver or adipose tissue, may

augment TAG clearance. It has been demonstrated that 2 hours of running at ~ 65% $\dot{V}O_{2\ max}$ increased calf blood flow and TAG clearance rate the following day (Malkova et al., 2000). More recently, Hurren *et al*. (2011) demonstrated in overweight men that a 22% reduction in postprandial lipaemia was associated with a 19% and 16% increase in blood flow through the femoral artery and hepatic portal vein the day after performing 90 minutes of brisk walking at 60% $\dot{V}O_{2\ max}$. A redistribution of blood flow may facilitate an increase in substrate delivery to lipoprotein lipase, and thus have an additive effect to the increased VLDL clearance rate observed after exercise (Al-Shayji et al., 2012) with or without a concomitant upregulation in lipoprotein lipase activity (Seip and Semenkovich, 1998).

2.5.6 Sexual dimorphism in the postprandial response following exercise

A recent meta-analysis identified a greater effect of prior exercise on postprandial lipaemia in women (*ES*=0.96) compared to men (*ES*=0.57) (Freese et al., 2014). Interestingly, this disparity was lost when comparing the IAUC-TAG responses to exercise between sexes, suggesting that the lower lipaemic response in women may relate to differences in baseline (i.e. fasted) [TAG]. However, Bellou *et al*. (2013) demonstrated that the reduction in lipaemia with prior exercise in women was due to an increased clearance and decreased secretion of VLDL-TAG, whilst the same research group identified that men experience an increased clearance in VLDL-TAG only (Magkos et al., 2006). Furthermore, evidence indicates that postprandial lipaemia may be lowered in the immediate hours after exercise in women but not men (Henderson et al., 2010). These authors demonstrated that postprandial lipaemia was attenuated in females, but not males, in the hours following 90 minutes and 60 minutes of cycling at 45% and 65% of $\dot{V}O_{2\ peak}$. A possible explanation for this finding is that women have been shown to experience a smaller increase in plasma free [FA] post exercise compared to men (Henderson et al., 2007), and a blunted delivery of FA to the liver during exercise recovery could plausibly lower hepatic VLDL-TAG output post exercise.

Therefore, it appears that women are not only characterised by a lower lipaemic response to a meal at rest (Cohn et al., 1988, Couillard et al., 1999, Horton et

al., 2002, Koutsari et al., 2004), but may also experience a greater reduction in postprandial lipaemia following exercise than men (Freese et al., 2014). Consequently, it is likely inappropriate to assume that the observed benefits of an exercise bout on postprandial lipaemia can be translated between the sexes.

2.6 Acute exercise and postprandial lipaemia in paediatric populations

This section examines the available evidence concerning the interactions between exercise and postprandial lipaemia in paediatric populations. A summary of the existing data is provided in Table 2.2.

In comparison to the adult literature, there is a paucity of studies which have addressed the influence of exercise on postprandial lipaemia in children and adolescents. Excluding the experimental chapters of this thesis, only twelve studies have investigated the effect of acute exercise on postprandial lipaemia in young people (Barrett et al., 2007, Lee et al., 2013, MacEneaney et al., 2009, Sedgwick et al., 2013, Sedgwick et al., 2014, Sedgwick et al., 2012, Sisson et al., 2013, Thackray et al., 2013, Tolfrey et al., 2012, Tolfrey et al., 2008, Tolfrey et al., 2014a, Thackray et al., 2014), and these have recently been reviewed (Tolfrey et al., 2014b). Thus, this area of research is in its infancy and more work needs to be conducted to identify whether the factors which have been shown to modulate the postprandial response following exercise in adults are present in youth. However, these adolescent studies do demonstrate that a single session of moderate to vigorous intensity exercise with an energy expenditure ≥ 1 MJ (240 kcal) performed the day before a high fat meal can meaningfully attenuate postprandial lipaemia in this group. Furthermore, the magnitude of this effect (*ES* typically range from “small” to “moderate” (Cohen, 1988)) is in line with the adult literature (Freese et al., 2014, Maraki and Sidossis, 2013, Peddie et al., 2012).

Table 2.2 Existing exercise and postprandial lipaemia studies in paediatric populations

Study	Sex	*n*	Age (years)	Intervention	EE (MJ)	Hours between exercise and HFM	Amount of fat (g kg^{-1} BM)	TAUC-TAG *ES*	IAUC-TAG *ES*
Barrett *et al*. (2007)	M	10	15.3	4 x 15 min TMW at 59% $\dot{V}O_{2\ peak}$	2.0	16.0	1.3	0.46*	0.07
	M	9	15.4	4 x 18 min LIST at 69% $\dot{V}O_{2\ peak}$	-	16.0	1.3	0.78*	1.17*
Tolfrey *et al*. (2008)	M	8	12.9	6 x 10 min TMR at 53% $\dot{V}O_{2\ peak}$ 6 x 10 min TMR at 75% $\dot{V}O_{2\ peak}$	1.5 2.2	14.7	1.5	0.86* 0.67	0.37* 0.18
Mac Eneaney *et al*. (2009)	M	10 NW	15.6	59 min TM at 65% $\dot{V}O_{2\ peak}$	2.5	12.0 to 14.0	97 (g/2 m^2 BSA)	0.59* (pooled)	0.49* (pooled)
	M	8 OW	15.9	52 min TM at 65% $\dot{V}O_{2\ peak}$	2.5				
Tolfrey *et al*. (2012)	M	11	13.3	3 x 10 min TMR at 55% $\dot{V}O_{2\ peak}$	1.0	14.5	1.5	0.26	-
				6 x 10 min TMR at 55% $\dot{V}O_{2\ peak}$	2.0			0.32*	-
Sedgwick *et al*. (2012)	M	13	13.6	60 min TMR at 72% $\dot{V}O_{2\ peak}$	1.9	18.0	B 1.5 L 1.1	0.71*	0.49
Lee *et al*. (2013)	M + F	21 AA	15.4	60 min cycling at 50% $\dot{V}O_{2\ peak}$	1.9	14.0	64	0.18	-
	M + F	17 C	14.5		2.1		66 (g/2 m^2 BSA)	0.46*	-

Sedgwick *et al.* (2013)	M	14	12.9	6 x 10 min TMR at 72% $\dot{V}O_{2\ peak}$ (each separated by 50 min)	1.9	18.0	B 1.5 L 1.1	0.38	0.50
Sisson *et al*. (2013)	M + F	18	14.8	3 x 45 min TMW at 1.5-3 METS (each separated by 15 min)	-	Exercise immediately after meal	~ 0.3	0.17	-
Thackray *et al*. (2013)	M	15	11.8	10 x 1 min TMR at 100% $\dot{V}O_{2\ peak}$	-	15.5	B 1.5 L 1.1	0.50*	0.39
Sedgwick *et al.* (2014)	M	9	13.1	4 blocks of 10 x 6 s cycle sprints (each sprint separated by 90 s with 3 min between blocks)	99 kJ (mechan-ical work)	15.0	B 1.5 L 1.1	0.40*	0.39*
Thackray *et al*. (2014)	F	11	12.1	TMW at 60% $\dot{V}O_{2\ peak}$ Equivalent dietary restriction	1.55 1.51	14.5	B 1.5 L 1.1	0.71*† 0.39	NS
Tolfrey *et al*. (2014b)	F	18	10-14	3 x 10 min TMR at 55% $\dot{V}O_{2\ peak}$ 6 x 10 min TMR at 56% $\dot{V}O_{2\ peak}$	777 kJ 1536 kJ	15.5	1.5	0.10 0.40*†	0.30 0.15

* A statistically significant difference exists between the exercise and resting control trial; † A statistically significant difference exists between intervention trials. No statistical comparison between exercise groups was made by Barrett *et al*. (Barrett et al., 2007) due to a between-measures design. NS = not statistically significant difference exists between intervention and control trial (the author provides medians and the interquartile range so an *ES* cannot be calculated). M = male; F = female; LIST, Loughborough Intermittent Shuttle Test; TMR, treadmill running; TMW, treadmill walking; AA, African American; C, Caucasian; B, breakfast; L, lunch; BSA, body surface area.

2.6.1 Participant characteristics

Currently, no data are available comparing the lipaemic response post exercise between boys and girls despite the aforementioned effect of sex in adults (Freese et al., 2014, Henderson et al., 2010). Although girls have been included with boys in one study (Lee et al., 2013), the authors did not identify whether sex had an interaction effect on the lipaemic response post exercise. Considering that non-fasting [TAG] has been shown to be associated with CVD in women independently of other risk factors (Bansal et al., 2007), and that girls are less likely to meet current physical activity guidelines than boys (Riddoch et al., 2007), it is pertinent to identify effective exercise interventions which attenuate postprandial lipaemia in this group. Indeed, two of the latest publications in this field exclusively recruited girls (Thackray et al., 2014, Tolfrey et al., 2014a). Whilst any comparisons between studies are made with caution, it is interesting to note that in two separate studies by the same research group (Tolfrey et al., 2012, Tolfrey et al., 2014a), the mean TAUC-TAG in the control trials following identical high fat meals (1.5 g of fat kg^{-1} body mass) were comparable between boys and girls (7.06 and 7.09 $mmol \cdot L^{-1}$ 6 h, respectively) who were of a similar age and body mass. Thus, the sexual dimorphism in the lipaemic response observed in adults (Cohn et al., 1988, Couillard et al., 1999, Horton et al., 2002, Koutsari et al., 2004) might not be present in youth. However, following identical exercise bouts (six, 10 minute bouts at ~ 55% $\dot{V}O_{2\ peak}$) girls experienced a similar reduction in postprandial lipaemia (~ 19%, $P<0.05$ (based on 95% CI, P values not provided), $ES=0.40$) compared to boys (~ 16%, $P=0.02$, $ES=0.32$) despite an exercise-induced energy expenditure of 1.5 MJ (Tolfrey et al., 2014a) compared to 2 MJ (Tolfrey et al., 2012). Limited conclusions can only be tentatively drawn from this comparison, especially given that the relationship between the attenuation in postprandial lipaemia and the energy-expended during the exercise bout is not well established (see Sections 2.4.1 and 2.6.3). Thus, further work is required to elucidate the influence of sex on the lipaemic response to a test meal following exercise in pre-pubertal and pubertal boys and girls.

One study with adolescents has identified that ethnicity may influence the postprandial response following exercise (Lee et al., 2013). In this study, Lee *et al*. (2013) identified that 60 min of cycling at 50% $\dot{V}O_{2\ peak}$ lowered postprandial

lipaemia by a greater extent in overweight Caucasian (19%) than overweight African American (8%) adolescents. This finding is at odds with existing data in adults (Shannon et al., 2008), which demonstrate that African Americans may experience a greater reduction in postprandial lipaemia following exercise than Caucasians, possibly due to a greater activity of lipoprotein lipase (Despres et al., 2000). However, the TAUC-TAG was significantly lower both in the control (27%) and exercise trial (19%) in African American adolescents compared to Caucasians. Therefore, the authors hypothesised that the reduced attenuation in lipaemia observed in the overweight African American group could be attributable to their already lower lipaemic response and fasted plasma [TAG] the day after exercise, although no IAUC-TAG analysis was performed which would have accounted for this disparity in baseline TAG between groups.

Interestingly, Lee *et al*. (2013) identified a significant interaction effect of visceral fat on the postprandial response in Caucasian (but not African American) adolescents, with visceral fat explaining 56% and 25% of the variance in TAUC-TAG in the control and exercise trials respectively. Only one other study has recruited overweight adolescents (MacEneaney et al., 2009), and these authors also observed a significant association between body fat (as determined by sum of skinfolds measured at 7 anatomical sites) and TAUC-TAG in a resting control trial (r=0.49, P<0.05) and the day after expending 600 kcal at 65% $\dot{V}O_{2\ peak}$ (r=0.47, P<0.05) in boys. Furthermore, plasma [TAG] remained significantly elevated above baseline values 6 hours after the high fat meal in the overweight group in both the control and exercise trial, but had returned to baseline in both trials in the normal weight cohort. Thus, it appears that body composition influences the postprandial response to a high fat meal in adolescence. However, in contrast to Lee *et al*. (2013), these authors observed similar reductions in postprandial lipaemia in the exercise trial between overweight and normal weight boys (~ 20%). This disparity may be due to the quantification of total body fat using skinfolds compared to the determination of visceral fat by dual-energy X-ray absorptiometry, as body fat distribution is a known predictor of the postprandial response in adults (Couillard et al., 1999, Mekki et al., 1999). More data are therefore needed to identify the interaction effect of prior exercise and body fat distribution, and whether this is affected by sex.

Finally, data in adults demonstrate that postprandial lipaemia increases with age (Cohn et al., 1988). However, no study has identified whether age or maturity modulates the postprandial lipaemic response to a test meal either at rest or following exercise in paediatric populations.

2.6.2 Potential mechanisms

All studies with adolescents bar one (Sisson et al., 2013) have used a 2-day protocol whereby the test meal is consumed the day after the prescribed exercise bout. Currently no study has provided any insight regarding the mechanisms underlying the favourable postprandial response post exercise by quantifying changes in lipoprotein subfractions, lipoprotein lipase activity, VLDL-TAG metabolism, resting fat oxidation and energy expenditure, or skeletal blood flow during the postprandial period. Seven investigations have, however, measured postprandial [insulin], although this outcome was not lowered following the exercise intervention in any of these studies (MacEneaney et al., 2009, Sedgwick et al., 2013, Sedgwick et al., 2014, Sedgwick et al., 2012, Sisson et al., 2013, Thackray et al., 2013, Thackray et al., 2014). Consequently, none of these studies demonstrated a meaningful relationship between changes in [insulin] and the attenuation of postprandial lipaemia following exercise. These findings corroborate with data in adults (Gill et al., 2002b), and together indicate that changes in postprandial [insulin] following exercise do not explain the lipaemic response.

2.6.3 Exercise-induced energy expenditure

In the first study to be published in this field with adolescent boys, Barrett *et al*. (2007) identified that postprandial lipaemia was attenuated following a single session of continuous treadmill walking (14%) and intermittent games activity (26%). The authors cautiously attributed the larger fall in postprandial lipaemia following intermittent games activity to a greater exercise-induced energy expenditure. However, energy expenditure was not determined, and a comparison between exercise trials is limited by the between-measures design.

In order to determine the influence of energy expenditure on the subsequent lipaemic response, Tolfrey *et al.* (2008) compared the efficacy of 60 minutes of treadmill running either at 53% or 75% $\dot{V}O_{2\ peak}$ in adolescent boys. These authors reported that postprandial lipaemia was equally reduced compared to a non-exercise control trial by 24% and 21% despite exercise-induced energy expenditures of 1.5 and 2.2 MJ respectively. In a further study, the same research group manipulated energy expenditure by keeping exercise intensity constant (~55% $\dot{V}O_{2\ peak}$) and doubling the duration of exercise (Tolfrey et al., 2012). In agreement with their earlier finding, these authors failed to observe a dose-response relationship in boys as the difference in lipaemia between expending 777 and 1536 kJ was trivial (4%, *P*=0.58). More recently, these authors conducted the same study design (Tolfrey et al., 2012) with adolescent girls and demonstrated that 60, but not 30, minutes of treadmill exercise reduced plasma [TAG], and this was attributed to considerable heterogeneity in the individual postprandial responses (Tolfrey et al., 2014a). This study indicates that an energy expenditure of >1500 kJ may be necessary to attenuate postprandial lipaemia in adolescent girls, however again failed to identify a dose-response relationship between exercise-induced energy expenditure and postprandial lipaemia. Finally, MacEneaney *et al*. (2009) also failed to observe a significant relationship between the magnitude of the reduction in postprandial lipaemia and energy expenditure or substrate utilisation during exercise. No other study paediatric study has identified whether substrate oxidation during exercise is related to the subsequent reduction in postprandial lipaemia.

The available paediatric data concerning the relationship between the exercise-induced energy expenditure and the *ES* of the change in TAUC-TAG (14 exercise interventions from 9 studies) and IAUC-TAG (8 exercise interventions from 5 studies) is presented in Figure 2.3. Bivariate analysis demonstrates non-significant relationships between the exercise-induced energy expenditure and the *ES* for the change in TAUC-TAG (*r*=0.39, *P*=0.16) and IAUC-TAG (*r*=0.16, *P*=0.71). While caution is warranted when interpreting the significance of this analysis due to the low sample size and different experimental designs, it is pertinent to note that the correlation coefficient for the change in the TAUC-TAG outcome is similar to that reported in adults (Freese et al., 2014, Petitt and

Cureton, 2003) (Section 2.4.1). Consequently, it appears that the energy expended during the exercise bout is only responsible for part of the subsequent reduction in postprandial lipaemia in adolescents.

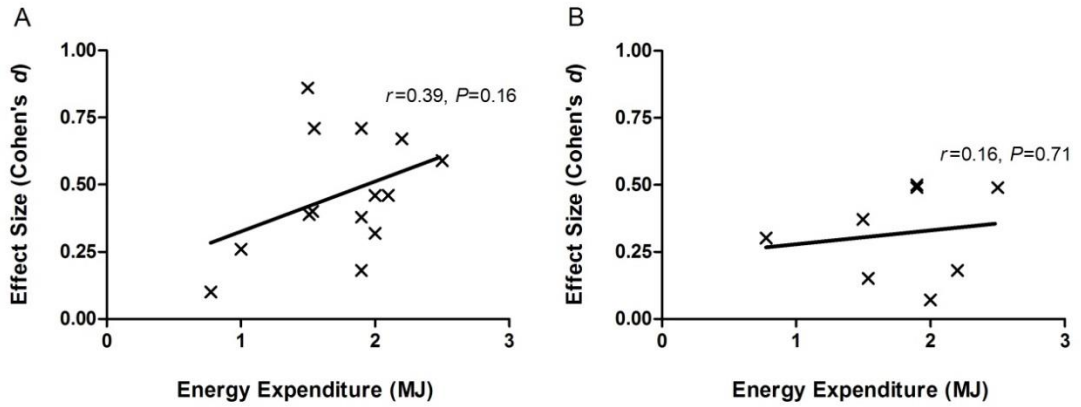

Figure 2.3 Exercise-induced energy expenditure and postprandial lipaemia in paediatric populations. The relationship between energy expenditure and the lipaemic response is calculated using the magnitude of the change in the total (A) and incremental (B) area under the plasma triacylglycerol curve versus time. Data in panel A are sourced from 9 studies (95 boys and 46 girls). Data in panel B are sourced from 6 studies (63 boys and 18 girls).

2.6.4 Energy deficit

Whilst the weight of evidence indicates that exercise-induced energy expenditure does not appear to be the primary determinant of the postprandial response, adult studies have shown that the physiological origin of an energy deficit may play an important role (Maraki et al., 2010). Thackray *et al.* (2014) demonstrated in adolescent girls that expending 1.55 MJ at 60% $\dot{V}O_{2\ peak}$ reduced postprandial lipaemia compared to a non-exercise control condition by 22%, whilst an isoenergetic diet-induced energy deficit significantly attenuated lipaemic response by 9%. The difference between trials was significant (14%, $P<0.001$), which is conceptually important as it further highlights that the benefit of prior exercise on subsequent lipaemia may extend beyond the exercise-induced energy expenditure.

2.6.5 High-intensity interval exercise

In line with the evidence indicating that the exercise-induced energy expenditure is not the sole determinant of the lipaemic response, two paediatric studies have identified that reductions in postprandial lipaemia are achievable even if the total duration of exercise is low (≤ 10 min), provided that the exercise intensity is high (Sedgwick et al., 2014, Thackray et al., 2013). These findings corroborate with a recent review of the available data from adult studies which identifies that low-volume, high-intensity interval exercise provides an effectual stimulus for the attenuation of postprandial lipaemia (Freese et al., 2014).

In the first of these investigations, Thackray *et al*. (2013) demonstrated that 10 repetitions of treadmill running at maximal aerobic speed for 1-minute significantly attenuated lipaemia by ~ 11% in adolescent boys. The following year, Sedgwick *et al*. (2014) reported that 40 x 6-second "all-out" cycle sprints lowered the total lipaemic response to a high fat breakfast and lunch by 13% (*P*=0.02, *ES*=0.40) in adolescent boys. This was achieved despite an average total mechanical work of just 99 kJ.

Importantly, Thackray *et al*. (2013) reported that the treadmill running exercise intervention was well tolerated by their participants, and concluded that this exercise design may provide an attractive and time-efficient alternative to traditional continuous, moderate-intensity exercise. However, such a comparative exercise trial was not included in the study design. In contrast, Sedgwick *et al*. (2014) conceded that 5 of the 15 participants were unable to complete the sprint cycling protocol, and 2 of these vomited. Therefore, it is likely that low-volume, high-intensity (but not sprint) interval exercise may have some utility in lowering postprandial lipaemia in adolescents, however further work is needed to establish how this type of exercise can be optimised whilst remaining feasible and attractive to a range of paediatric groups.

2.6.6 Accumulated exercise

Considering that adolescents rarely perform exercise for longer than 10 minutes at a time (Riddoch et al., 2007), it is important to identify whether accumulating small bouts of exercise can lower the lipaemic response to a test meal. Whilst

many paediatric studies have incorporated an intermittent exercise protocol in order to improve exercise tolerance (Barrett et al., 2007, Sedgwick et al., 2014, Thackray et al., 2013, Tolfrey et al., 2012, Tolfrey et al., 2008, Tolfrey et al., 2014a), only one study has identified the effect of accumulating exercise throughout the day on postprandial lipaemia (Sedgwick et al., 2013). These authors reported that 6 bouts of 10 minutes of treadmill running at 72% $\dot{V}O_{2\ peak}$ (each separated by 50 minutes) reduced the total lipaemic response to a high fat breakfast and lunch by 11%, although this was not significantly different from a resting control trial (*P*=0.18, *ES*=0.38). Unfortunately, no comparable continuous exercise bout was included in this study. However, the same research group demonstrated in a separate investigation that this exercise stimulus completed in a single bout attenuated postprandial lipaemia following an identical breakfast and lunch by 22% (*P*=0.18, *ES*=0.38) compared to a resting control trial. Whilst comparing between groups is not ideal, these data suggest that performing exercise in a single bout may be more effectual than accumulating the same total exercise stimulus throughout the day. However, not study has adopted a within-measures design to address the efficacy of accumulated exercise in paediatric groups. Furthermore, no data are available identifying whether the intensity of accumulated exercise is important for postprandial health in this population.

2.6.7 Exercise timing

To date, only one study with adolescents has prescribed exercise on the same day as the high fat test meal (Sisson et al., 2013). Considering that the activity of lipoprotein lipase has a delayed peak after exercise (Seip and Semenkovich, 1998, Zhang et al., 2002), and this is purported by some to play a role in the clearance of plasma TAG and subsequent fall in lipaemia in adults (Gill et al., 2003b, Herd et al., 2001, Kantor et al., 1984, Sady et al., 1986, Zhang et al., 2002), such a design could conceivably isolate a different mechanistic pathway underlying the postprandial response. These authors reported that a total of 135 minutes of light treadmill walking (1.5-3 metabolic equivalents) 1 hour after a meal failed to reduce the lipaemic response (*P* value not provided, *ES*=0.17) in adolescent boys and girls. However, postprandial [TAG] was not the primary

outcome of this paper, and as such there are a number of methodological differences between this study and the extant postprandial literature that must be considered.

Firstly, the test meal prescribed in this study contained only 20.5 g of fat, which is far lower than other paediatric investigations (see Table 2.2). This is important considering that the amount of fat consumed is a key determinant of the lipaemic response (Cohen et al., 1988). Indeed, the ingestion of ≤ 15 g of fat fails to increase plasma [TAG] in adults (Dubois et al., 1998). Additionally, the authors only adopted a three hour postprandial observation period, which arguably skews their analysis more towards the rate of TAG appearance rather than clearance. This is even more problematic considering that the initial rise in plasma [TAG] might reflect the TAG content of the previous evening meal (Fielding et al., 1996), which was not standardised or monitored in this study. A further criticism is that the authors did not analyse whether sex modulated the postprandial response, which would have been a judicious choice considering that sex differences exist in adults when the time between the exercise bout and the test meal is short (Henderson et al., 2010). Finally, data are available in adults which indicate that exercise during the postprandial period is not as effectual as an identical exercise bout performed 1 hour before the test meal (Zhang et al., 1998). Thus, the efficacy of exercise performed the same day but before the consumption of a high fat meal remains to be fully elucidated.

2.7 Endothelial function

Acting as the interface between vessel and blood, the vascular endothelium is an active endocrine, paracrine and autocrine organ, and considered to be essential in regulating a variety of homeostatic functions (Bonetti et al., 2003, Petty and Pearson, 1989). Under normal conditions, the endothelium has anti-thrombotic, fibrinolytic and anti-coagulant properties (Rubanyi, 1993), inhibits cell growth and proliferation (Garg and Hassid, 1989), and reduces leukocyte and platelet adhesion to the vessel wall (Kubes et al., 1991). The endothelium is integral in modulating vascular tone in accordance with metabolic demand by secreting the vasodilators bradykinin (Cherry et al., 1982), endothelial-derived hyperpolarising factors (Cohen and Vanhoutte, 1995), nitric oxide (NO) (Ignarro,

1989) and prostacyclin (Moncada and Vane, 1978), and vasoconstrictors endothelin (Yanagisawa et al., 1988) and angiotensin II (Dzau, 1988).

Of these vasoactive substances, NO has been the most comprehensively studied. Initially discovered by Furchgott and Zawadzki (1980), who demonstrated that denuded blood vessels failed to dilate following the administration of acetylcholine, NO is a labile, lipid-soluble gas, continually synthesised (Vallance et al., 1989) by endothelial NO synthase (eNOS) from the amino acid L-arginine (Palmer et al., 1988), and also produced via the nitrite-nitrate-NO pathway (Lundberg et al., 2008). NO is a potent inhibitor of platelet aggregation and leukocyte adhesion to the endothelium (Mellion et al., 1981). Additionally, NO rapidly diffuses into the smooth muscle of the tunica media and binds with guanylate cyclase (Ignarro et al., 1986), forming cyclic guanosine monophosphate which causes the smooth muscle to relax (Furchgott and Jothianandan, 1991) and induce vasodilation. Therefore, NO plays an essential role in vessel homeostasis.

Endothelial dysfunction, caused by insult to the vascular wall, is considered to be a key early event in the atherosclerotic process (Ross, 1993), which precedes structural changes to the vessel wall (Hopkins et al., 2013, Juonala et al., 2004) and is linked to the clinical manifestations of CVD (Vita et al., 1990, Zeiher et al., 1991). Endothelial dysfunction facilitates the development of atherosclerotic plaques via increased adherence and permeability to monocytes and lipoproteins, which then accumulate in the vessel wall (Steinberg, 1987), increased platelet adhesion and smooth muscle cell migration and subsequent proliferation (Henderson, 1991). Endothelial dysfunction is also characterised by a decreased bioavailability of NO, which has been accepted as a sentinel atherogenic event (Cooke and Tsao, 1994, Ganz and Vita, 2003, Ross, 1999). Support for this theory is provided by studies concerned with L-arginine supplementation (a precursor for NO synthesis (Palmer et al., 1988)), which has been shown to reduce platelet aggregation (Adams et al., 1995) and monocyte adhesion to the endothelium (Adams et al., 1997). Consequently, the endothelium may adopt a phenotype which promotes inflammation, vasoconstriction, thrombosis, and atherosclerotic plaque formation and progression under the continued presence of CVD risk factors (Celermajer, 1997). This includes childhood overweight and obesity (Tounian et al., 2001,

Woo et al., 2004b), low levels of physical activity (Abbott et al., 2002, Pahkala et al., 2008), type 1 diabetes (Jarvisalo et al., 2004), and elevated cholesterol (Jarvisalo et al., 2002). Thus, the endothelium is much more than an inert barrier, and the bioavailability of NO can be used as a practical surrogate of determining endothelial function.

2.7.1 Measuring nitric oxide in vivo *via flow mediated dilation*

In a seminal paper, Celermajer *et al*. (1992) published details of a technique which purported to non-invasively assess endothelial function *in vivo* via high resolution ultrasound of the brachial artery in response to an increase in shear stress induced by cuff occlusion. Termed flow mediated dilation (FMD), this presented an attractive technique to determine endothelial function. However, whilst data were available from animal studies indicating that the vasodilator response was endothelium-dependent (Pohl et al., 1986, Smiesko et al., 1985), at the time it was only an assumption that this was mediated by NO.

In the years that followed, two research groups demonstrated that the post occlusion vasodilation was abolished following the infusion of the eNOS antagonist N^G-monomethyl-L-arginine (L-NMMA) (Joannides et al., 1995, Lieberman et al., 1996), indicating that FMD is NO-mediated. However, Doshi *et al*. (2001) reported that L-NMMA only partly prevented post-occlusive vasodilation when the cuff was positioned proximal (i.e. upstream) to the ultrasound probe, suggesting that other dilators are responsible for this response when the brachial artery is included in the ischaemic stimulus. Additionally, Mullen *et al*. (2001) demonstrated using L-NMMA infusion that NO-mediated the FMD response after 5, but not 15, minutes of cuff occlusion.

It is now generally accepted that FMD is NO-mediated provided that the cuff is positioned distal to the ultrasound probe and the occlusion stimulus is 5 minutes in length (Green, 2005), as advocated in several FMD guidelines (Corretti et al., 2002, Harris et al., 2010, Thijssen et al., 2011).

2.7.2 Methodological considerations regarding flow mediated dilation

In addition to cuff placement (Doshi et al., 2001) and occlusion time (Mullen et al., 2001), there are several other considerations regarding the assessment and interpretation of FMD. The first of these is the trade-off between accurately determining vessel diameter and blood velocity. The brachial artery runs parallel to the skin surface, and as such the ultrasound probe bisects the vessel at 90°. Whilst this may produce a high quality longitudinal image of the vessel, parallel blood flow to the probe prevents the Doppler assessment of blood velocity. A compromise is possible by the asynchronous firing of the phased array of the crystals which transmit and receive the Doppler signal; “steering” the beam to achieve a more appropriate angle of insonation. It is understood that the error associated with estimating blood velocity increases exponentially when the angle of insonation is > 60° (Logason et al., 2001, Rizzo et al., 1990). Consequently, an angle steer of ≤ 60° is recommended (Harris et al., 2010, Thijssen et al., 2011), and has been widely adopted in the paediatric literature (Hopkins et al., 2009, Hopkins et al., 2011, Hopkins et al., 2013).

The assessment of blood velocity is further complicated by the laminar flow of blood through a vessel. Blood velocity can be calculated using either the peak or mean velocity, and there is no common consensus regarding which outcome to adopt (Thijssen et al., 2011, Harris et al., 2010). Additionally, the slower moving blood nearest the vessel walls may not be taken into account even if the Doppler sample gate is widened to encompass the whole lumen (Thijssen et al., 2011). Therefore, current guidelines recommend consistency within a research laboratory until a standardised method becomes apparent.

Optimising the image of the vessel wall is technically demanding and requires a transducer with a minimum frequency of 7 MHz (Corretti et al., 2002), whilst a 10-14 MHz probe may be more appropriate for paediatric populations when the distance between the brachial artery and skin is smaller (Harris et al., 2010). Capturing an acceptable image is also complicated due to changes in artery diameter across the cardiac cycle. Consequently, an electrocardiogram (ECG) gating system is recommended (Harris et al., 2010), whereby arterial diameter is determined during ventricular systole (R wave). Current guidelines also highlight that edge-detection software more accurately determine arterial

diameter and FMD than manual image analysis (Thijssen et al., 2011), which has been shown to be highly operator dependent (Mancini et al., 2002, Woodman et al., 2001). This is now commonplace in the paediatric literature (Hopkins et al., 2009, Hopkins et al., 2011, Hopkins et al., 2013, Sedgwick et al., 2013, Sedgwick et al., 2014, Sedgwick et al., 2012).

Flow mediated dilation is calculated as the difference between peak and baseline brachial artery diameter, expressed as a percentage of baseline diameter (Equation 2.1). However, this interpretation of a vessel's ability to vasodilate has come under criticism due to the dependence of the FMD statistic on baseline diameter (Atkinson and Batterham, 2012, Atkinson and Batterham, 2013, Atkinson et al., 2009). Specifically, FMD is inversely correlated with baseline diameter (Thijssen et al., 2008, Celermajer et al., 1992), which means that individuals with a wider arterial diameter at baseline will be penalised (i.e. the FMD statistic will be lower) compared to those with a smaller calibre vessel. Consequently, the ratio-scaling of this outcome is inappropriate as the confounding influence of baseline diameter is not partitioned out (Packard and Boardman, 1999). Indeed, Celermajer *et al*. (1992) reported a correlation coefficient of r=0.8 between FMD (%) and baseline artery diameter, indicating that baseline diameter is accountable for 64% of the variability in FMD.

$$\text{FMD (\%)} = \frac{\text{peak diameter} - \text{baseline diameter}}{\text{baseline diameter}} \times 100$$

Equation 2.1 The ratio-scaled flow mediated dilation statistic.

The appropriateness of using the ratio-scaled FMD statistic can be examined using the slope of the relationship between the logarithmically transformed baseline and peak diameters. Unless the 95% confidence interval of this slope spans unity (i.e. 1.0), the ratio-scaling method should be rejected. Allometric scaling has been proposed for the unbiased assessment of FMD should the assumptions of ratio-scaling be violated (Atkinson and Batterham, 2013,

Atkinson et al., 2013), whereby the difference between these logged values is the outcome in an analysis of covariance, with the logged baseline diameter as the covariate. This process abolishes the relationship between FMD and baseline diameter, creating a variable which no longer bears a significant relationship with size (Nevill and Holder, 1995). The subsequent allometrically scaled FMD statistic is then interpreted in the usual manner. This approach is essential for the valid comparison of endothelial function where differences in baseline diameter are expected, such as following exercise, or when comparing between individuals or groups (i.e. paediatric populations and adults), however the prognostic utility of the allometrically-scaled FMD statistic remains to be addressed.

The magnitude of change in brachial artery diameter post-occlusion relies not only on the health of the endothelium, but also the imposed shear stimulus (Pyke et al., 2004). The post-occlusive shear is influenced by many factors and has been shown to have a high inter-individual variability (Joannides et al., 2002, Mitchell et al., 2004, Pyke et al., 2004). Consequently, it is recommended to normalise FMD for shear in order to determine whether a small FMD value is indicative of endothelial dysfunction or the result of a low shear stimulus (Harris et al., 2010, Pyke and Tschakovsky, 2007, Thijssen et al., 2011). However, the relationship between FMD and shear is not apparent in paediatric groups (Thijssen et al., 2009a), or following exercise in adults (Llewellyn et al., 2012). Indeed these authors reported that shear explained just 2% and 3% of the variance in FMD, respectively. Current guidelines recommend that the shear stimulus is quantified and presented, although normalising FMD for shear is not appropriate if the two are not related (Thijssen et al., 2011).

2.7.3 Prognostic relevance of flow mediated dilation

Andersen *et al*. (1995) provided the first data indicating that peripheral conduit artery function provided a surrogate of coronary artery function in 50 patients undergoing catheterisation for the evaluation of coronary artery disease. The authors reported a significant, but weak, relationship (r=0.36, R^2=13%, P=0.01) between serial inter-coronary infusions of acetylcholine and FMD. However, a criticism of this initial investigation is that the stimuli to compare the agreement

in peripheral and coronary endothelial function were different. Accordingly, Takase *et al*. (1998) induced endothelial-dependent vasodilation by increasing blood flow and shear stress in both vessels and reported that brachial artery function explained 62% of the variance in coronary artery function (r=0.79, P<0.001) in patients with coronary artery disease. Additionally, the same research group identified that the vasodilator response of the brachial and coronary arteries to a moderate dose of acetylcholine were closely related in individuals with (r=0.68, P<0.001) and without (r=0.72, P<0.001) coronary artery disease (Takase et al., 2005).

Taken together, these data indicate that the assessment of brachial artery vasodilation by FMD provides a valid surrogate of coronary arterial endothelial function. However, this claim has recently been criticised as these authors failed to account for the aforementioned confounding influence of baseline vessel diameter on the FMD statistic (Atkinson and Batterham, 2015). Accordingly, Atkinson and Batterham re-analysed the data provided by Andersen *et al.* (1995) using the now recommended (Atkinson et al., 2013, Thijssen et al., 2011) allometric scaling techniques, and revealed that brachial artery function explained only 7% and 29% of the variation in coronary artery vasodilation in participants with and without coronary artery disease, respectively. Additionally, these authors highlight that the power of FMD to predict coronary artery vasodilation is associated with wide confidence limits. For example, by re-analysing the data provided by Takase *et al.* (1998), Atkinson and Batterham (2015) demonstrate that an individual FMD of 5% predicts a coronary artery vasodilation of -10 to 27%, and suggest that the strength of the original correlation between brachial and coronary artery vasodilation in this seminal paper might be due to the substantial heterogeneity in disease status in the sample population.

Despite this recent criticism of the clinical relevance of FMD, a plethora of studies have reported that FMD independently predicts cardiovascular events in populations at risk of CVD (Brevetti et al., 2003, Chan et al., 2003, Gokce et al., 2002, Gokce et al., 2003, Neunteufl et al., 2000, Wang et al., 2009, Meyer et al., 2005). In contrast, the evidence regarding the predictive use of FMD in asymptomatic groups is less clear, with some studies reporting that FMD has independent prognostic value (Rossi et al., 2008, Shechter et al., 2009), and

others reporting that the predictive power of FMD is no greater than traditional CVD risk factor assessment (Shimbo et al., 2007, Yeboah et al., 2007). A criticism of the latter paper is that the participant age ranged from 72 to 98 years, and an increased arterial stiffness with advancing age (Lind et al., 1999) is known to interfere with valid measurements of FMD (Witte et al., 2005a). Accordingly, a more recent investigation by the same research group in a younger cohort (45-84 years) demonstrated that FMD is a predictor of cardiovascular events, and that this relationship remained significant even after the adjustment of multiple CVD risk factors and Framingham score (Yeboah et al., 2009). Furthermore, evidence in 45 to 66 year olds indicates that only FMD, but not CVD risk factors or Framingham score, predicted the progression of carotid intima-media thickness (Halcox et al., 2009). Given the progressive nature of CVD, the assessment of endothelial function via FMD may therefore provide a predictive measure of the evolution of atherosclerosis.

Two meta-analyses have demonstrated that FMD is significantly and inversely related to future CVD events (Inaba et al., 2010, Ras et al., 2013), and it has recently been estimated that every 1% increase in FMD reduces CVD risk by 13% (95% CI 9% to 17%) in adults (Green et al., 2011). However, these meta-analyses did not differentiate between studies which placed the cuff above or below the ultrasound probe, which is important as whilst both approaches determine endothelial function, the latter is more NO-dependent (section 2.8.1). In order to identify whether the prognostic relevance of FMD reflects NO-mediated endothelial function, Green *et al*. (2011) re-analysed the data included in this meta-analysis and demonstrated that a 1% increase in distal cuff placement (i.e. NO-mediated) FMD reduced CVD risk by 9% (95% CI 4% to 13%). In contrast, a 1% increase in FMD determined with the cuff placed above the ultrasound probe reduced CVD risk by 17% (95% CI 12% to 22%), and the differences in prognostic power between these two methodological approaches was significant (P=0.01). This is somewhat surprising, given that current FMD guidelines recommend distal cuff placement (Corretti et al., 2002, Harris et al., 2010, Thijssen et al., 2011), however both techniques are considered to be endothelium-dependent, and as such highlight the importance of this single cell lining as a “barometer” of CVD risk (Vita and Keaney, 2002).

Advocates of the FMD technique (Green et al., 2008) have highlighted that modification of traditional CVD risk factors with exercise fails to explain the subsequent magnitude of risk reduction (Berlin and Colditz, 1990, Blair et al., 1989, Hu et al., 2004, Paffenbarger et al., 1986). Indeed, in an 11 year longitudinal study of ~ 27,000 nurses, Mora *et al*. (2007) identified that favourable changes in traditional CVD risk factors with exercise only accounted for 59% of the reduction in CVD risk. Thus, ~ 40% of the benefit of exercise on CVD risk remains unaccounted for. It is thought that endothelial function may account for some of this "risk factor gap" (Green et al., 2003, Joyner and Green, 2009), possibly because traditional CVD risk factors often fail to predict plaque rupture (Naghavi et al., 2003), a key CVD event, whilst endothelial dysfunction might (Green et al., 2011), but also because improvements in endothelial function determined by FMD may occur in the absence of any changes in traditional CVD risk factors (Green et al., 2003) due to structural and functional changes of the vasculature observed following regular exercise (Green et al., 2008).

Currently, there are no established normative values for FMD in youth. According to one review, the FMD in apparently healthy, non-obese children is typically between 8-11% (Fernhall and Agiovlasitis, 2008), although the span of the 95% confidence interval is large; 4-18% (Tounian et al., 2001, Woo et al., 2004a). The work of one research group indicates that the mean FMD in 36 adolescent boys is between 7-9% (Sedgwick et al., 2013, Sedgwick et al., 2014, Sedgwick et al., 2012), although they do not provide any data in adolescent girls which is a limitation considering that FMD has been shown to be lower in 24-39 year old males than age-matched females, even after adjustment for baseline brachial artery diameter and CVD risk factors (Juonala et al., 2008). It is also problematic that empirical evidence regarding the clinical implications of low FMD in youth are lacking. However, a meta-analysis of 211 studies (~ 12,000 participants) documented that the prospective strength of FMD regarding vascular health is strongest in individuals considered to be low risk (Witte et al., 2005b). Given that FMD has already been shown to be impaired in children (Celermajer et al., 1992), FMD may be a powerful tool to assess vascular health and future CVD risk in youth.

In summary, FMD is considered to predict cardiovascular events, and is at least as predictive as traditional risk factors in adults (Green et al., 2011). Whilst no longitudinal data are available regarding FMD measured in early life and future cardiovascular risk, CVD is a progressive disease which has its origins in youth (McGill et al., 2000), and impairments in endothelial function are a requirement for the formation of atherosclerotic lesions (Juonala et al., 2004, Halcox et al., 2009). Furthermore, the landmark paper published by Celermajer *et al*. (1992) identified an impairment in endothelial function in children with CVD risk factors. Therefore, the ability to non-invasively determine endothelial function *in vivo* identifies the FMD technique as an attractive and pertinent outcome measure.

2.8 Exercise, shear and endothelial function

Vallance *et al.* (1989) demonstrated that NO is continuously secreted by the endothelium, however secretion rates can be augmented pharmacologically (i.e. with an infusion of acetylcholine (Furchgott and Zawadzki, 1980)), and in response to an increase in shear stress during exercise (Ranjan et al., 1995, Uematsu et al., 1995) as the endothelium acts as a mechanotransducer (Rubanyi et al., 1986). The mechanisms underlying how greater shear facilitates vasodilation are yet to be fully elucidated. However, data from *in vitro* studies indicate that shear is positively associated with endothelial potassium channel activation (Olesen et al., 1988), calcium influx into the endothelial cell (Dull and Davies, 1991) eNOS sensitivity to calcium (Dimmeler et al., 1999) and bradykinin release (Hecker et al., 1993). These processes ultimately stimulate eNOS activity and thus increase NO bioavailability (Figure 2.4). Regardless of the precise signalling pathway, shear stress is understood to be responsible for the maintenance of a favourable endothelial phenotype (Jenkins et al., 2012).

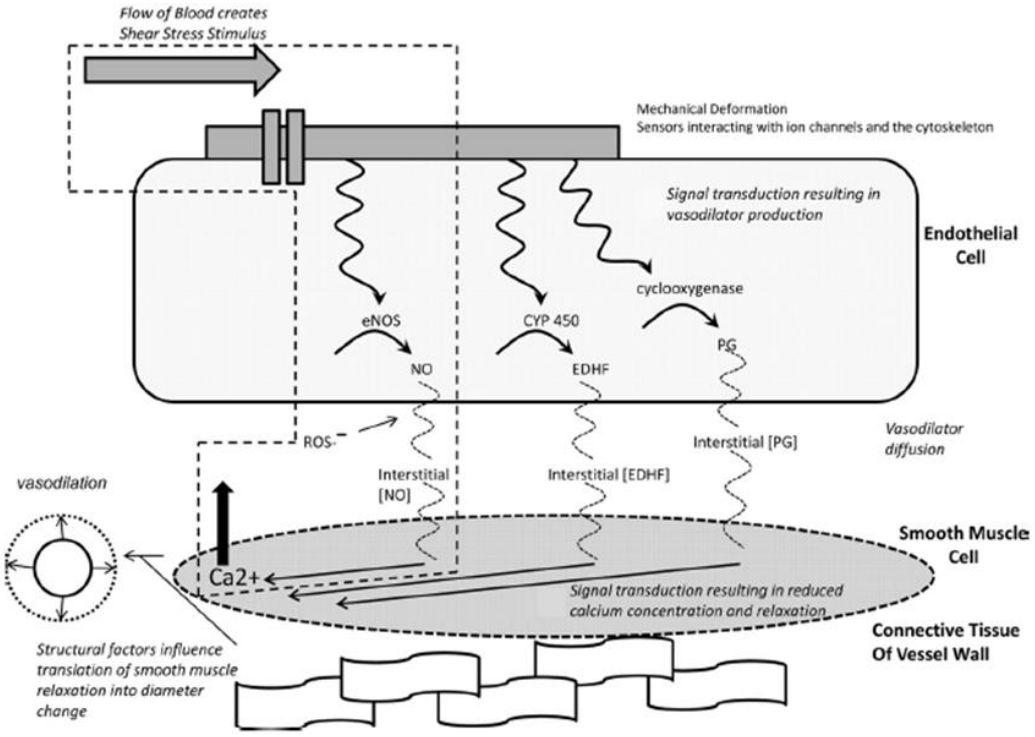

Figure 2.4 Shear-induced vasodilation. eNOS, endothelial nitric oxide synthase; NO, nitric oxide; CYP 450, cytochrome P450; EDHF, endothelium-derived hyperpolarising factor; PG, vasodilatory prostaglandins; Ca2+, calcium. Reproduced from Thijssen *et al*. (2011).

Considering that exercise induces substantial increases in blood flow and therefore shear (Thijssen et al., 2009b), it is entirely consistent that repeated episodes of augmented shear stress during exercise training are thought to be the primary signal driving adaptations in endothelial function (Tinken et al., 2009, Tinken et al., 2010). Direct evidence supporting the role of shear stress on endothelial function is provided by Tinken *et al*. (2009), who attenuated shear during cycling and handgrip exercise via forearm cuff occlusion in one arm and reported that the subsequent post-exercise increase in FMD in the contralateral arm was abolished in the cuffed limb. The same research group expanded these findings beyond the acute exercise model and demonstrated that 8 weeks of handgrip training did not alter FMD in the cuffed arm despite significant improvements in the contralateral, non-cuffed arm (Tinken et al., 2010).

Given that shear is the stimulus for subsequent improvements in vascular function, it might not be immediately apparent why these authors (Tinken et al., 2009, Tinken et al., 2010), and others (DeSouza et al., 2000, Harris et al., 2008,

Johnson et al., 2012, Linke et al., 2001) observed improvements in brachial artery FMD following lower body exercise. However, it is established that the brachial artery is exposed to increases in shear at the onset of cycling exercise (Green et al., 2002a, Green et al., 2002b, Thijssen et al., 2009b). This is due to a fall in forearm vascular resistance caused by cutaneous vasodilation for thermoregulatory purposes (Johnson and Rowell, 1975, Ooue et al., 2008), which augments shear stress in the upstream conduit arteries (Simmons et al., 2011, Tanaka et al., 2006). Data are also available indicating that 4 weeks of cycle training increase basal NO production in the forearm both between exercise sessions and 2 days after the final training bout (Kingwell et al., 1997). Therefore, exercise can promote vascular health beyond the active muscle beds (Padilla et al., 2011), and this is mediated by shear.

2.8.1 Physical activity and endothelial function in paediatric populations

Using the doubly labelled water stable isotope technique, Abbott *et al.* (2002) demonstrated that habitual physical activity is an important predictor of FMD in children, and this relationship remained significant after adjusting for age and sex. Specifically, children in the most active tertile were characterised by a significantly greater FMD than the least active (12.2% vs 7.8% respectively, P=0.04). Physical activity is also positively and significantly associated with FMD in adolescent boys (Pahkala et al., 2008). However, these authors failed to observe a similar relationship in adolescent girls, and this was hypothesised to be due to their lower overall physical activity levels. Interestingly, this study identified that an increase of ~ 10 hours per week of moderate-intensity exercise is required to increase FMD by ~ 1%. Considering that few adolescents achieve even half of this amount of daily exercise (Riddoch et al., 2007), and that interventions may only increase daily physical activity by 4 minutes in children (Metcalf et al., 2012), this is not a realistic goal.

More recently, Hopkins *et al*. (2009) reported similar differences in FMD between the most and least active tertiles as Abbott *et al*. (2002) (13.0% vs 7.0% respectively, P<0.05), however this study extended these findings by identifying that only time spent performing vigorous-intensity physical activity, but not fitness or fatness, predict FMD in children. Given that the difference in

vigorous-intensity activity time between the most and least active tertiles in this study was ~ 4 min, improvements in endothelial function should be possible in young people following interventions to increase time spent performing vigorous-intensity exercise.

2.8.2 Exercise training and endothelial function in paediatric populations

Currently, only one study has identified the efficacy of exercise training on FMD in healthy adolescents (Hopkins et al., 2012). This study analysed the change in FMD following 8 weeks of training in mono- and di-zygotic twins, and demonstrated a 1.4% increase in FMD in both groups. Whilst this improvement in FMD did not achieve statistical significance, probably due to the small sample size in each cohort (*n*=6) and large standard deviation between pairs of twins, the magnitude of this change might be considered to be meaningful (*ES*=0.47 and 0.74 for mono- and di-zygotic twins). This paper also demonstrated that the change in FMD following training was more strongly correlated in mono- (*r*=0.74) than di-zygotic twins (*r*=0.34), highlighting that the improvement in endothelial function following training is partly genetically determined.

Exercise training has also been shown to improve FMD in young people with endothelial dysfunction (Meyer et al., 2006, Watts et al., 2004a, Watts et al., 2004b, Woo et al., 2004a, Tjonna et al., 2009, Kelishadi et al., 2008, Ribeiro et al., 2005). For example, Watts *et al*. (2004a) demonstrated that 8 weeks of combined aerobic and resistance training normalised endothelial function (relative to age- and sex-matched lean control participants) in obese adolescents, despite no changes in glycaemic control, plasma lipids or blood pressure. The same group identified similar findings following 8 weeks of aerobic training in obese children, but also demonstrated that these benefits are lost following 8 weeks of detraining (Watts et al., 2004b).

Tjønna *et al*. (2009) demonstrated that aerobic interval training at 90-95% maximum heart rate may be more effectual in improving endothelial function and other CVD risk factors in obese adolescents than a combined multidisciplinary approach, consisting of 4 hours of group meetings with a physician, psychologist, psychotherapist and a clinical nutritional physiologist,

and 3 hours of supervised “activity” sessions per month for 12 months. Whilst this study provided “proof of concept” that high-intensity interval training can promote endothelial function in obese adolescents, no study has addressed how training interventions can be optimised by manipulating the exercise bouts in either at risk groups or healthy adolescents, which is essential for the primary prevention of CVD. This is a pertinent research question given the importance of vigorous-intensity exercise on endothelial function in youth (Hopkins et al., 2009) and the promising findings by Tjønna *et al*. (2009).

2.8.3 Acute exercise and shear

Acute exercise presents important challenges to the cardiovascular system, and it is thought that chronic adaptations to exercise training are a product of this repeated shear stimulus (Dawson et al., 2013). Specifically, given that the FMD response following acute exercise (Tinken et al., 2009) and exercise training (Tinken et al., 2010) has been shown to be modulated by the shear stimulus during exercise, it is pertinent to identify how the characteristics of a single exercise bout (i.e. mode and intensity) can influence shear in order to maximise the subsequent improvements in endothelial function.

The profile of the shear waveform fluctuates over the cardiac cycle, and can be separated into antegrade and retrograde shear. Changes in this oscillatory pattern play an important role in influencing endothelial phenotype (Jenkins et al., 2012). Retrograde shear is associated with a plethora of pro-atherogenic effects on the endothelium (Dai et al., 2004, Hwang et al., 2003a, Hwang et al., 2003b, O'Keeffe et al., 2009, Takabe et al., 2011) and impairs endothelial function *in vivo* (Thijssen et al., 2009c), which can be reversed with the replacement of retrograde shear with antegrade shear (Tinken et al., 2009). No study has quantified brachial artery shear during exercise in children and adolescents. Thus, our mechanistic understanding is limited to investigations with adult populations.

Data indicate that the initial increase in brachial artery retrograde shear at the onset of cycling exercise decreases with exercise duration due to downstream thermoregulatory cutaneous vasodilation (Simmons et al., 2011). However, the

influence of exercise intensity on the pattern of shear is less understood. Green *et al*. (2002b) demonstrated that brachial artery antegrade, but not retrograde, shear increased with the intensity of cycling exercise, suggesting that exercise intensity may be positively associated with endothelial function. In contrast, the same research group identified comparable increases in antegrade and retrograde shear during incremental cycling exercise (Thijssen et al., 2009b). However, the authors noted that this increase in antegrade shear likely prevents any impairment in endothelial function that would be expected with unopposed increases in retrograde shear (Thijssen et al., 2009c). Therefore, although the exercise intensity was only increased to 160 W in these studies (presumably due to difficulties in obtaining suitable ultrasound images as the exercise becomes more demanding for the participant), there is a sound rationale for identifying the influence of exercise intensity on FMD.

2.8.4 Acute exercise and endothelial function

As described in Figure 2.5, the influence of a single bout of exercise on endothelial function is the sum of a number of potential modifiers, including the duration, mode, intensity of the exercise bout, the exercise-induced shear pattern, the balance between free radical production and anti-oxidant status and the timing between exercise cessation and the FMD assessment (Dawson et al., 2013). Additionally, the post-exercise FMD response may be influenced by training status (Harris et al., 2008), age (Thijssen et al., 2009a), endothelial health (Dawson et al., 2013) and probably hereditary factors (Hopkins et al., 2012).

Currently, only one study has identified the acute influence of exercise intensity on FMD in a paediatric cohort (Mills et al., 2013). In this study, the authors reported that FMD was significantly attenuated in children immediately following a total of 30 minutes of high-intensity exergaming (i.e. playing video games which require movement) but unchanged after a similar volume of low-intensity exergaming. The authors concluded that a single bout of high-intensity exercise may promote favourable improvements in endothelial function as this acute impairment may provide the stimulus for subsequent adaptation; a concept referred to as “hormesis”.

Whilst this conclusion is speculative, as the authors did not collect any further data, evidence from adult studies support the concept that a decrease in FMD precedes a transient augmentation in endothelial function (Dawson et al., 2013), and that this biphasic response is intensity-dependent (Birk et al., 2013, Johnson et al., 2012) (Figure 2.2). Indeed, Birk *et al.* (2013) demonstrated that FMD remained unchanged following 30 minutes of cycling at 50% of maximum heart rate but was attenuated immediately after cycling at 70% and 85% of maximum heart rate. Furthermore, FMD was blunted to a greater degree in the latter condition. Additionally, the authors reported that this inverse association between exercise intensity and FMD immediately post exercise was not fully explained by changes in baseline (i.e. pre cuff occlusion) brachial artery diameter immediately after exercise cessation. Therefore, the observed change in endothelial function appears to be a physiological event, rather than a result of the ratio scaled FMD statistic.

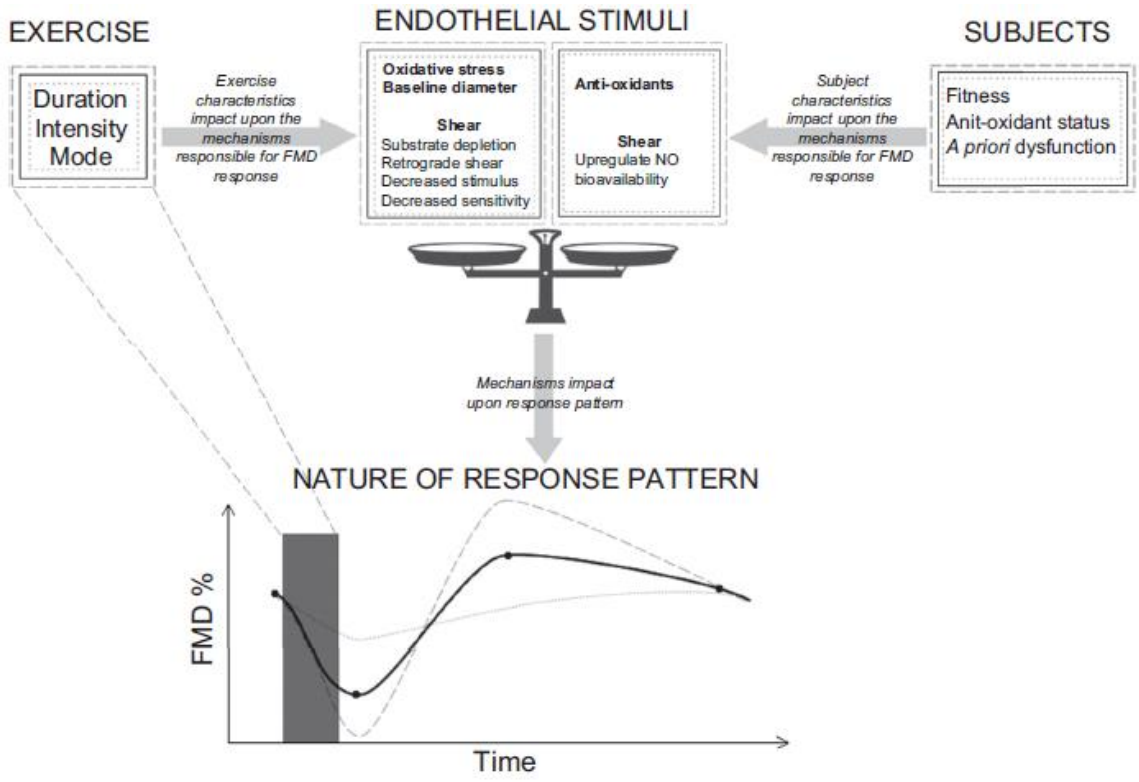

Figure 2.5 Modifiers of the acute FMD response post exercise. Reproduced from Dawson *et al*. (2013). FMD; flow mediated dilation; NO, nitric oxide.

Johnson *et al*. (2012) also demonstrated that FMD is attenuated immediately following high- but not moderate-intensity exercise, and provided evidence that this was likely due to an increase in oxidative stress. Additionally, this study confirmed that the intensity-dependent post exercise nadir does precede an augmented FMD response 1 hour later, and that this returns to pre exercise values after 2 hours. Interestingly, these authors suggested that the disparate response in post exercise FMD is a result of the exercise "dose" (the combined stimulus of exercise intensity and duration), rather than exercise intensity *per se*. However, this does not corroborate with other data in adults (Hwang et al., 2012), thus the importance of energy expenditure on post exercise FMD remains equivocal.

Further data are available which indicate that FMD is unchanged in adolescent boys the day after sprint interval cycling (Sedgwick et al., 2014), one hour of walking (Sedgwick et al., 2012), and following the accumulation of one hour of running at 70% $\dot{V}O_{2\ peak}$ (Sedgwick et al., 2013). However, given that the available evidence with adults indicates that FMD returns to pre-exercise values ~ 2 hours post exercise (Johnson et al., 2012), it is likely that the potential improvements in FMD were "missed" in these studies. Therefore, the time course of the FMD response following exercise, and how the exercise bout can be modified for endothelial health, remains to be elucidated in both adults and younger participants.

2.9 Postprandial endothelial dysfunction

The consumption of a high fat meal is known to promote a transient period of endothelial dysfunction in adults (Gaenzer et al., 2001, Vogel et al., 1997) and adolescents (Sedgwick et al., 2013, Sedgwick et al., 2014, Sedgwick et al., 2012), and endothelial dysfunction is already apparent in dyslipidaemic children (Halcox and Deanfield, 2005). Considering that an impairment in endothelial function is a prerequisite for the initiation and progression of the atherosclerotic cascade (Juonala et al., 2004), elevations in postprandial lipaemia during periods of endothelial dysfunction may work in concert to promote CVD risk. Given that much of the day may be spent in the postprandial state, it is important to identify how endothelial function can be preserved following a

meal. Thus, the inclusion of a metabolic challenge to exaggerate the postprandial endothelial response may assist researchers in extrapolating acute findings into plausible chronic benefits.

Pioneering studies in this field identified that endothelial function was impaired following an isocaloric high (50 g), but not low (0 g), fat meal (Vogel et al., 1997), thereby identifying a causal relationship between exaggerated postprandial lipaemia and endothelial dysfunction. In the same year, it was demonstrated that this deleterious effect of a high fat meal on endothelial function could be attenuated with the concomitant consumption of vitamin C, a potent antioxidant (Plotnick et al., 1997), suggesting that elevated levels of TAG-rich lipoproteins promote endothelial dysfunction via oxidative stress. Data are also available indicating that postprandial hyperglycaemia may impair endothelial function via the same mechanism (Ceriello et al., 2002).

Data are now available directly linking elevated postprandial lipaemia to transient periods (~ 4 hours) of endothelial dysfunction via oxidative stress (Anderson et al., 2001, Bae et al., 2001). A schematic overview of this process is provided in Figure 2.6. Superoxide radicals (O_2^-) generated by the mitochondria during periods of elevated lipaemia (Brownlee, 2005, Wallace et al., 2010) are preferentially scavenged by NO (Beckman and Koppenol, 1996). This both depletes NO and encourages the formation of peroxynitrite ($ONOO^-$), a potent free radical (Beckman and Koppenol, 1996). Additionally, both $ONOO^-$ and O_2^- can oxidise tetrahydrobiopterin, a key cofactor in the production of NO by endothelial nitric oxide synthase (eNOS), resulting in attenuated NO production (Wallace et al., 2010). The subsequent uncoupling of eNOS encourages the production of O_2^- instead of NO (Forstermann and Munzel, 2006). Consequently, the sum of these pathways is a reduction in the bioavailability of NO, which is considered to be a central feature of endothelial dysfunction (Harrison, 1996).

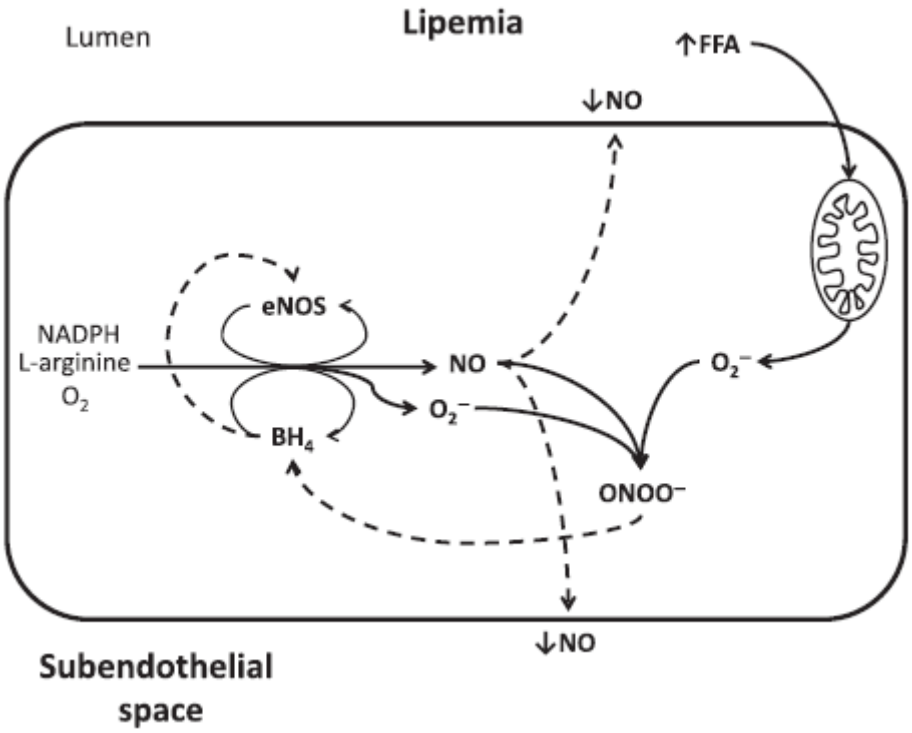

Figure 2.6 Postprandial lipaemia, oxidative stress and an impairment in endothelial function. FFA, free fatty acids; O_2^-, superoxide radicals; NO, nitric oxide; $ONOO^-$, peroxynitrite; BH_4, tetrahydrobiopterin; eNOS, endothelial nitric oxide synthase; O_2, oxygen; NADPH, the reduced form of nicotinamide adenine dinucleotide phosphate (an electron donor). Reproduced from Wallace *et al*. (2010) with permission.

In addition to the reduction in NO bioavailability, it has been demonstrated that $ONOO^-$ is a precursor for nitrogen dioxide formation (Squadrito and Pryor, 1998), which readily leads to lipid peroxidation (Byun et al., 1999). This phenomenon has also been observed in the hours following a high fat meal (Tsai et al., 2004). It has also been demonstrated that HDL concentrations protect against postprandial endothelial dysfunction (Anderson et al., 2001). Therefore, it follows that endothelial dysfunction may be exacerbated by the depletion of circulating HDL fractions (Austin et al., 1990) following repetitive episodes of postprandial lipaemia. Finally, repeated periods of elevated TAG concentrations may directly cause vascular insult and damage as postprandial lipoproteins and their remnant particles can penetrate the endothelium (Nordestgaard and Nielsen, 1994) where they are considered to be cytoxic (Chung and Segrest, 1991).

Recently, Uetani *et al.* (2012) identified that an increase in postprandial blood pressure in adults is an unfavourable event and can be considered to be a

novel risk factor for CVD. In this study, an increase in systolic blood pressure following a meal was identified as an independent determinant of insulin resistance, carotid intima-media thickness and brachial-ankle pulse wave velocity (an established marker of CVD risk (Yamashina et al., 2003)). It is plausible that a postprandial elevation in systolic blood pressure reflects transient endothelial dysfunction via systemic vasoconstriction due to the reduction in the bioavailability of NO. Indeed, elsewhere an exaggerated postprandial TAG excursion has been shown to reduce brachial artery diameter by 10% (Tyldum et al., 2009). Thus, these measures may share a common mechanistic pathway, although this was not measured in the study by Uetani *et al*. (2012) and no information regarding the macronutrient content of the meal was provided by the investigators. However, given the simplicity of the measurement, determining changes in blood pressure following a high fat meal may provide a broad insight into the systemic challenge placed upon the vasculature.

2.9.1 Postprandial endothelial function and prior exercise

Studies with adults have reported a significant relationship between postprandial TAG and the change in endothelial function, with Pearson correlation coefficients ranging from -0.31 (Bae et al., 2001) to -0.70 (Marchesi et al., 2000). Therefore, it follows that attenuating postprandial lipaemia with prior exercise may limit postprandial endothelial dysfunction.

Three studies have addressed the efficacy of prior exercise on postprandial endothelial function in adolescent boys (Sedgwick et al., 2013, Sedgwick et al., 2014, Sedgwick et al., 2012). In accordance with the majority of postprandial investigations (Peddie et al., 2012), all of these studies used a 2-day protocol, whereby the exercise bout was completed the day before a high fat breakfast and lunch. In the first of these studies, Sedgwick *et al*. (2012) demonstrated that 60 minutes of walking at 60% $\dot{V}O_{2\ peak}$ significantly limited the attenuation in FMD compared to a resting control trial following a high fat breakfast (6 vs 32%) and lunch (10% vs 24%). Furthermore, once FMD was normalised for the post-occlusion shear rate, the postprandial decline in FMD was ablated in the exercise trial. Interestingly, the change in FMD following exercise was not

related to a reduction in postprandial lipaemia (r = 0.22, P = 0.47 for total postprandial observation of breakfast and lunch). Therefore the authors were not able to provide any mechanistic insight underlying the favourable effect of prior exercise, although they were able to rule out the confounding effect of changes in baseline brachial artery diameter.

Following this initial investigation, the same research group demonstrated that accumulating 60 minutes of running at 70% $\dot{V}O_{2\ peak}$ (Sedgwick et al., 2013) and performing 40, 6 second cycle sprints (Sedgwick et al., 2014) also negated the postprandial fall in endothelial function after the same high fat meals for breakfast and lunch. In accordance with their earlier data, neither of these studies reported a significant relationship between the change in FMD and postprandial lipaemia following exercise, and the observed differences in FMD were independent to changes in baseline brachial artery diameter. Therefore, postprandial endothelial function can be preserved in adolescent boys with prior moderate- and high-intensity exercise, and this benefit is also observed when exercise is accumulated over the course of the preceding day. Furthermore, this favourable effect appears to be unrelated to changes in postprandial lipaemia.

Similar findings have been reported in studies with adults. Gill *et al.* (2003a) identified that postprandial microvascular endothelial function was the same before and after a period of detraining, despite greater lipaemia following detraining. Using a 2-day protocol, Tyldum *et al*. (2009) also documented that ~ 50 minutes of walking at 60-70% maximum heart rate attenuated the fall in postprandial endothelial dysfunction despite no change in lipaemia, and this was related to a greater total antioxidant status (TAS) following exercise. These authors also included an iso-energetic, high-intensity interval exercise bout, consisting of 4 intervals of 4 minutes of treadmill running at 85-95% maximum heart rate, and demonstrated that the subsequent attenuation in postprandial endothelial dysfunction is not only ablated, but reversed. In contrast to the findings by Sedgwick *et al*. (2014), fasted FMD was significantly elevated the day after the high-intensity interval exercise in this study, and the consumption of the high fat meal failed to modulate the postprandial response. Tyldum *et al.* attributed this benefit to a further increase in TAS as this was strongly related to FMD (r=0.9, P<0.001) (Tyldum et al., 2009).

Finally, performing exercise the day before a high fat breakfast and lunch (69 $g \cdot kg^{-1}$ BM of fat for each meal) has been demonstrated to lower postprandial SBP in young men (Miyashita et al., 2008). These authors demonstrated that SBP was 6 – 7% lower throughout the postprandial observation period (7 hours) the day after accumulating ten 3 minute walks, or a single walking bout of 30 minutes, with no differences between exercise bouts.

Collectively, these data indicate that prior exercise may protect the vasculature from the deleterious effects of a high fat meal in a manner which may be intensity dependent and at least partly related to an increase in total antioxidant capacity. Our understanding of how exercise intensity modulates the postprandial FMD response in adolescents is limited as this has yet to be addressed using a within-measures design. Furthermore, no data are available in girls despite sex differences in both the lipaemic response to a high fat test meal (Cohn et al., 1988, Couillard et al., 1999) and FMD (Juonala et al., 2008) in adults.

2.10 Microvascular function

Impaired cutaneous microvascular function in adults is associated with elevated blood pressure (Serne et al., 2001), obesity (de Jongh et al., 2004), insulin resistance (Jaap et al., 1994, Irving et al., 2002), CVD risk (Ijzerman et al., 2003) and Framingham risk scores for CVD in asymptomatic women (Vuilleumier et al., 2002). An impairment in cutaneous microvascular function is apparent even in normoglycaemic adults with metabolic syndrome (Kraemer-Aguiar et al., 2008). Additionally, an impairment in microvascular function has been identified in obese children (Schlager et al., 2011), in children and adolescents with type 1 diabetes (Khan et al., 2000) and in healthy children with clustered CVD risk factors (Khan et al., 2003).

It has been argued that the earliest changes in endothelial function due to the metabolic syndrome may be specifically linked to the capillary and arteriole beds, rather than the larger, conduit arteries (Pinkney et al., 1997). Indeed, little association has been demonstrated between reactive microvascular hyperaemia and FMD in adults (Shamim-Uzzaman et al., 2002, Dhindsa et al.,

2008). Therefore, it may not be appropriate to adopt endothelial function of the macrovasculature as a surrogate of microvascular health (Meyer et al., 2008). Thus, the direct assessment of microvascular function is an important outcome, and may provide early information regarding the development of chronic disease.

2.10.1 Assessment of microvascular function

Several non-invasive methods are available to determine microvascular function (Cracowski et al., 2006), however post-occlusive reactive hyperaemia can be determined *in vivo* using laser Doppler perfusion imaging (LDI). Currently, there is no standardised protocol for the assessment of post-occlusive reactive hyperaemia. It has been shown that post-occlusive hyperaemia increases after a 1, 2 or 3 minute ischaemic stimulus (Yvonne-Tee et al., 2004), and a 5 minute occlusion period has successfully been utilised elsewhere (Sieg-Dobrescu et al., 2001, Pellaton et al., 2002). Additionally, a range of analyses have been used to quantify the microvascular response, including peak reactive hyperaemia (PRH) and both total and incremental area under the post occlusive hyperaemic curve vs time (Cracowski et al., 2006).

It is likely that the lack of a standardised protocol and consensus regarding interpretation of the outcomes has obscured our understanding of the mechanisms involved, however they are thought to include both endothelium dependent and independent pathways (Koller and Kaley, 1990). Additionally, the role of NO in the post-occlusive hyperaemic response has been disputed (Wong et al., 2003, Zhao et al., 2004). Wong *et al*. (2003) demonstrated that PRH following 5 minutes of forearm cuff occlusion was not different after the infusion of L-NMMA, and therefore not mediated by NO. Accordingly, the processes underlying the FMD and post-occlusive microvascular response appear to be mechanistically disparate, which is consistent with the lack of association demonstrated between post-ischaemic macro- and micro-vascular reactivity in adults (Dhindsa et al., 2008). Thus, simultaneous assessment of post-occlusive microvascular hyperaemia during the FMD protocol may offer further insight of vascular health.

2.10.2 Exercise and microvascular function

There are limited data regarding exercise training and microvascular function in paediatric groups, and comparisons between studies are confounded by differences in assessment techniques. Furthermore, evidence indicates that microvascular reactivity increases throughout adolescence (Radtke et al., 2012), however many studies fail to assess maturity.

Radtke *et al.* (2012) controlled for pubertal status and demonstrated that time spent performing moderate to vigorous-intensity physical activity was not related to microvascular reactivity in 10 – 16 year old boys and girls. Roche *et al*. (2008) also controlled for maturity and demonstrated that microvascular reactivity in children and adolescents with type 1 diabetes was not related to time spent performing moderate to vigorous physical activity. Interestingly, these authors identified that $\dot{V}O_{2\ peak}$ was positively related to microvascular reactivity (R^2 = 0.29, P = 0.01), which is in line with data in healthy adults at risk of developing type 2 diabetes (Middlebrooke et al., 2005) and suggests an important role for aerobic fitness in improving microvascular function. This is consistent with data provided by Roche *et al*. (2010) who identified that trained 13-15 year old boys have enhanced microvascular function compared to an untrained, age and maturity-matched control group. Taken together, exercise training and the associated increases in aerobic fitness appear to have a favourable effect on microvascular reactivity. Given that a high-intensity exercise stimulus (> 80% heart rate max) is recommended to improve aerobic fitness in apparently healthy adolescents (Baquet et al., 2003), it is plausible that high-intensity exercise training might promote microvascular function to a greater degree than moderate-intensity training. However, the influence of the intensity of exercise training, or a single exercise bout, on microvascular function remains to be elucidated.

2.11 Autonomic function

Alterations in the activity of the autonomic nervous system, characterised by a hyperactive sympathetic and hypoactive vagus system, are associated with the pathogenesis of atherosclerosis (Thayer et al., 2010). Heightened sympathetic

and suppressed parasympathetic nervous activity impair the autonomic regulation of the cardiac system (Dekker et al., 2000) and may be linked to CVD development via an impairment in endothelial function (Harris and Matthews, 2004, Kaufman et al., 2007). Prolonged catecholamine stimulation promotes the absorption of LDL into the endothelium and a dose-dependent proliferation and migration of vascular smooth muscle (Zhang et al., 2004). Conversely, acetylcholine released by the vagus nerve has been shown to attenuate this proliferation of smooth muscle cells and subsequent increase in inflammatory cytokines (Ulloa, 2005).

Evidence in adults indicates that autonomic function is positively associated with daily physical activity (Monahan et al., 2000) and improved following exercise training (Rosenwinkel et al., 2001). Consequently, it has been proposed that favourable changes in autonomic function account for some of the "missing" ~ 40% benefit of exercise on CVD risk (Joyner and Green, 2009).

2.11.1 Assessment of autonomic function

A commonly adopted measure of autonomic function is heart rate variability (HRV), whereby the intervals between consecutive heart beats are analysed using time and frequency domain techniques (Task force of the European Society of Cardiology, 1996). Time domain indexes of HRV, including the root mean square of the squared differences between adjacent normal R-R intervals (RMSSD) reflect vagal tone and are simple to calculate using a an electro-cardiogram (Kleiger et al., 1991). Frequency domain (or "power") analysis of HRV is more complex, but yields information regarding the relative balance between the sympathetic and parasympathetic nervous systems (Stein et al., 1994). However, it has been demonstrated that that the time domain analysis of HRV is strongly correlated ($r > 0.85$) to each frequency domain variable (Kleiger et al., 1991). Therefore, it is accepted that a decreased HRV reflects an increased sympathetic activity and/or a decrease in parasympathetic tone (Stein et al., 1994), and the RSMMD outcome is used as a surrogate measure of vagus nerve (i.e. parasympathetic) activity.

2.11.2 Heart rate variability in adolescents and cardiovascular disease risk

Low levels of parasympathetic activity (i.e. a low HRV) is a powerful marker of CVD and mortality in adults (Dekker et al., 2000). Previous studies with adolescents have demonstrated that HRV is inversely related to systolic blood pressure and obesity (Zhou et al., 2012), and physical inactivity (Gutin et al., 2005). These data were recently confirmed in a cross-sectional study of 1,152 adolescent boys which demonstrated that HRV is associated with the clustering of CVD risk factors (Farah et al., 2014). Given that the clustering of traditional CVD risk factors in adolescence track into adulthood (Andersen et al., 2004), this finding is important in the absence of any longitudinal data identifying the prognostic importance of HRV in youth.

One study has identified that FMD is positively associated with parasympathetic activity (r = 0.48, P = 0.01) and negatively associated with sympathovagal balance (r = -0.51, P = 0.01) in apparently healthy children (Kaufman et al., 2007). These relationships remained moderately strong and significant following adjustment for insulin, inflammation (C-reactive protein) and maturity (Tanner stage). Therefore it appears that reduced parasympathetic activity and dysfunctional sympathovagal balance during childhood is associated with an impairment in endothelial function.

2.11.3 Exercise training and heart rate variability in young people

Exercise training has been shown to increase HRV in obese children (Gutin et al., 2000), but this outcome was not improved following 13 weeks of endurance training in apparently healthy children (Mandigout et al., 2002). Additionally, only minor improvements have been observed following 5 months of training in adolescent swimmers (Perini et al., 2006). However, time spent performing vigorous, but not moderate, physical activity is favourably related to HRV in adolescents (Buchheit et al., 2007). Furthermore, data in adults indicate that exercise intensity is a positively associated with HRV (Buchheit et al., 2005, Leicht et al., 2003). Therefore, it has been argued that training at intensities above moderate-intensity exercise is required for improvements in parasympathetic activity (Buchheit et al., 2008). Indeed, this research group

demonstrated that both 9 weeks of high-intensity training or supramaximal training improved HRV in adolescent boys (Buchheit et al., 2008), which is consistent with the recent finding that high-intensity interval training was superior to aerobic endurance training at improving cardiac autonomic function in adults (Kiviniemi et al., 2014). Thus, it appears that improvements in HRV are possible in healthy adolescents, provided that the intensity of the exercise sessions is high.

2.12 Summary and experimental aims

Zilversmit (1979) proposed that atherosclerosis was a "postprandial phenomenon" over 30 years ago. Whilst some evidence is available supporting the initial hypothesis that elevated numbers of cholesterol-rich lipoprotein remnants can penetrate the arterial wall and promote plaque formation (Mamo et al., 1998, Pal et al., 2003, Rapp et al., 1994), there are now other well-established mechanisms by which repeated episodes of postprandial lipaemia encourages the development of an atherogenic lipoprotein phenotype beyond Zilversmit's proposed pathway (Austin et al., 1990), i.e. elevated TAG-rich lipoproteins, fewer HDL fractions (particularly HDL_2) and smaller, denser LDL particles.

In addition to these unfavourable changes in lipoprotein fractions, repeat episodes of exaggerated lipaemia promote transient periods of endothelial dysfunction (Gaenzer et al., 2001, Vogel et al., 1997) via oxidative stress (Bae et al., 2001). An impairment in endothelial function is a sentinel event in the progression of atherosclerosis (Ross, 1993), preceding structural changes to the vessel wall (Zeiher et al., 1991), and a requirement for the initiation and development of fatty streaks (Juonala et al., 2004). Thus, repetitive episodes of hypertriglyceridemia likely encourage the progression of atherosclerosis via endothelial dysfunction in concert with the promotion of an atherogenic lipoprotein phenotype.

Data are available in both adult and paediatric groups that prior exercise favourably modulates postprandial lipaemia and vascular function. However, it is not known how exercise can be optimised for these parameters of health,

which is important given that few adolescents meet the recommended minimum amount of daily physical activity (Department of Health, 2008, Riddoch et al., 2007). Consequently, the purpose of thesis is to undertake a series of novel experimental studies that will identify the influence of exercise intensity on these health outcomes. The primary aims of each experimental chapter are provided below.

1. Chapter 4 is designed to isolate the effect of exercise intensity on postprandial lipaemia and SBP. This chapter also includes adolescent girls in order to assess whether the effect of sex on postprandial lipaemia in adulthood is present in youth. Furthermore, this study will determine whether an attenuation in postprandial lipaemia following exercise is related to changes in resting fat oxidation during the postprandial period.

2. Chapter 5 addresses whether the intensity of exercise accumulated over the course of the day influences postprandial lipaemia, SBP and fat oxidation in adolescent boys and girls. This is a pertinent research question given that adolescents rarely sustain exercise for longer than 10 minutes. Furthermore, the total accumulated exercise stimulus is comparable to the continuous MIE and HIIE bouts performed in Chapter 4. As such, this study provides an insight into the efficacy of accumulated compared to continuous exercise for postprandial health.

3. Chapter 6 extends the findings of this thesis beyond the outcomes measures included in Chapters 4 and 5, and is the first to isolate the influence of exercise intensity on postprandial macro- and micro-vascular function in adolescents. This study will also identify whether differences in postprandial macro- and micro-vascular function following MIE and HIIE are related to changes in postprandial lipaemia and [TAS]. Additionally, this study identifies the acute (< 4 hours) effect of exercise intensity on plasma [3-OHB] in order to provide novel insight regarding the mechanisms underlying the postprandial lipaemia response when the test meal is consumed 1 hour post exercise.

4. It is thought that the benefits of chronic exercise on vascular function may be related to the acute responses to a single bout of exercise. Accordingly, the purpose of Chapter 7 is to identify the time course of the macro- and micro-vascular response following work-matched MIE and HIIE in adolescents.

5. Chapter 8 is the first to comprehensively assess the influence of 2 weeks of high-intensity interval exercise training on traditional and novel (i.e. endothelial function and HRV) CVD risk factors in adolescent boys and girls. Given that most of the day is spent in the postprandial state, and that current evidence regarding exercise training and CVD risk factors is limited to fasted measures, this study also determines whether or not any improvements in traditional and novel CVD risk factors are apparent following a high fat and sugar test meal. Finally, this study identifies whether any changes in traditional or novel CVD risk factors remain 3 days after training.

Chapter 3

Methodology

3.1 Inclusion/exclusion criteria

All studies presented in this thesis were granted ethical approval from the Sport and Health Sciences Ethics Committee (certificates provided in the Appendix 1). Participants were recruited from years 8-10 from local schools with the consent of the Head Teacher and the Physical Education department. Details of the study were initially presented to the pupils during an assembly, who were then provided with an information pack containing further specifics regarding the study design, rationale and procedures, contact details of the research team, a parent/guardian consent form, a participant assent form and a standard health screening form (forms provided in the Appendices). Participants then returned these signed forms back to a designated member of staff at the school by a pre-arranged date. The primary investigator collected the forms and telephoned each parent/guardian to further discuss the study details. Participants were accepted onto the study if they satisfied the study recruitment criteria. Exclusion criteria included the presence of any contraindications towards maximal exercise, any relevant allergies (i.e. lactose intolerance) or the use of any supplement or medication known to influence fat or carbohydrate metabolism, or vascular function. There were no specific inclusion criteria apart from age (12-15 years).

3.2 Standardisation of testing conditions

During all studies, participants were instructed to avoid organised physical activity, and to wear an accelerometer and complete a food diary (Appendix 4) during the 48 hours preceding each laboratory visit. Participants were also asked to refrain from eating after 20:00, and replicate the same evening meal for each subsequent visit. Time was taken during the initial visit to the laboratory to ensure that each participant understood the importance of these requests. Parents/guardians and participants were verbally reminded of these conditions throughout the study by both the primary investigator and the relevant member of staff from the school's Physical Education department.

Physical activity was captured using an ActiGraph GT1M accelerometer (ActiGraph, LLC, Pensacola, USA) which was attached to an elasticated belt

and placed above the iliac crest on the right hand side in Chapter 5. This was also a requirement in Chapter 4, however adherence was low and data loss from device failure was high (complete data were only available for 5 participants). Participants were instructed to only remove the accelerometer for bathing or sleeping. A sticker was placed on the top of the unit to ensure correct orientation of the accelerometer. Data were collected using a 1 second epoch in order to best capture the sporadic pattern of physical activity which characterises paediatric groups (Ward et al., 2005) and processed using Kinesoft software (version 3.3.62; Kinesoft, New Brunswick, Canada). Non-wear time was defined as 0 counts per minute for 30 continuous minutes during daylight hours (Rowlands and Eston, 2007). Minimum wear time was set as 10 hours per day in accordance with prior work with this population (Riddoch et al., 2007). Time spent performing moderate to vigorous intensity activity was determined using established cut points for paediatric groups (Evenson et al., 2008).

Due to poor compliance and device failure, complete accelerometer data was only available for 14 out of the 20 adolescents in Chapter 5. This level of data loss is consistent with the level of non-compliance reported in other studies which use a hip-worn accelerometer (Audrey et al., 2013, Van Coevering et al., 2005). In an effort to improve the capture of physical activity data during the 48 hours preceding each laboratory visit, participants were asked to wear a water-proof tri-axial accelerometer on their wrist (GENEActiv, Activinsights Ltd, Cambridge, UK) in Chapters 6, 7 and 8 (Rowlands et al., 2014). The aforementioned criteria for non-wear and minimum wear times were adopted, and data were again captured in 1 second epochs. Data were processed using the GENEA Post Processing software (version 1.2.1), and moderate to vigorous intensity activity time was calculated using validated paediatric cut points for this device (Phillips et al., 2013).

Completed food diaries were viewed by the primary investigator during each laboratory visit and discussed with the participants in turn in order to promote compliance and ensure a satisfactory level of detail. To minimise the burden of completing the food diaries, participants were instructed to indicate unit size (e.g. one spoonful, one small glass) rather than weigh all food and drink

consumed. Food diaries were assessed for total energy and macronutrient intake (CompEat Pro, Nutrition Systems, UK) upon completion of each study.

3.3 Anthropometry

Body mass, seated height and stature were measured to the nearest 0.1 kg and 0.1 cm respectively. Percentage body fat was estimated using triceps and subscapular skinfold thickness according to Slaughter *et al*. (1988) and pubertal status was determined by a self-assessment of secondary sexual characteristics using adapted drawing (Morris and Udry, 1980) of the five Tanner stages (Tanner, 1962) of pubic hair development. Percentage body fat was estimated in Chapters 5 and 6 using the mean of the three measures of triceps and subscapular skinfold thickness (Holtain Ltd, Crymych, UK) according to Slaughter *et al*. (1988).

3.4 Maximal oxygen uptake ($\dot{V}O_{2\,max}$) and gas exchange threshold (GET)

Following an individualised habituation period, participants completed a combined ramp and supramaximal test to exhaustion to establish $\dot{V}O_{2\,max}$ (Barker et al., 2011) using an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). Participants were instructed to maintain a cadence of 70-80 revolutions per minute (rpm) throughout the warm up (20 W) and during the incremental part of the test. The ramp rate was set at 30 W min^{-1} for individuals > 50 kg, otherwise 25 W min^{-1} was used in order to elicit fatigue within 8-12 minutes (Buchfuhrer et al., 1983). Exhaustion was defined as a drop in cadence below 60 rpm for 5 consecutive seconds despite strong verbal encouragement.

Participants completed a 5 minute cool down at 20 W immediately after exhaustion, followed by a 10 minute seated rest. Participants then cycled at 20 W for 2 minutes before a “step” transition to 105% of the maximum power output achieved during the preceding ramp test. Cadence was kept constant between 70-80 rpm until exhaustion. Power output was then lowered to 20 W and the participant was monitored during a cool down which lasted at least 5

minutes. An example $\dot{V}O_2$ trace for the combined ramp and supramaximal exercise protocol is provided in Figure 3.1.

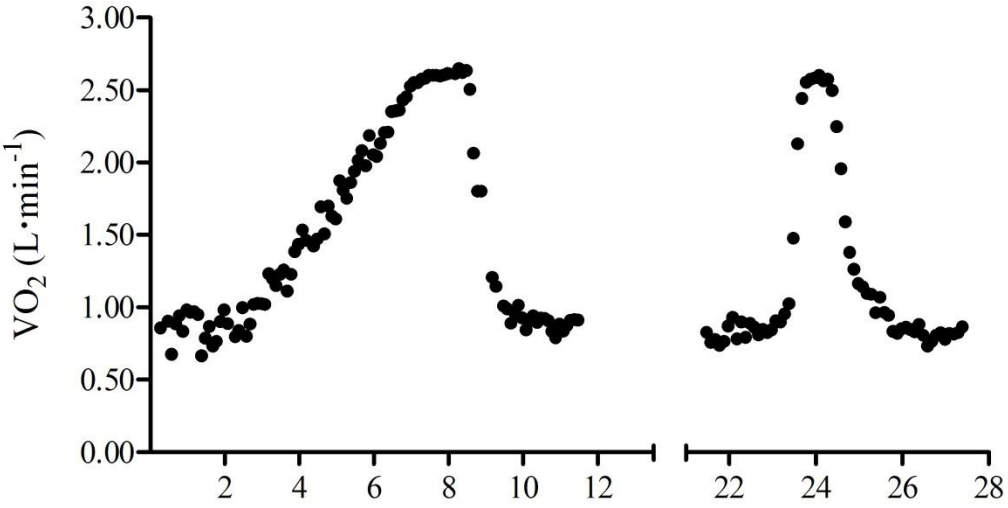

Figure 3.1 An example oxygen uptake ($\dot{V}O_2$) trace from the combined ramp and supramaximal exercise protocol to establish $\dot{V}O_{2\ max}$.

Pulmonary $\dot{V}O_2$ and carbon dioxide production ($\dot{V}CO_2$), and heart rate were assessed throughout the ramp and supramaximal protocols using a Cortex Metalyzer III B (Cortex, Leipzig, Germany), which was calibrated using gases of known volume and concentration prior to each test. The gas exchange threshold (GET) was identified as the disproportionate increase in $\dot{V}CO_2$ relative to $\dot{V}O_2$ and verified by an increase in expired ventilation ($\dot{V}E$)/ $\dot{V}O_2$ with no increase in $\dot{V}E/\dot{V}CO_2$. The mean $\dot{V}O_2$ response time was taken into account when determining the power output which equated to the GET, i.e. two-thirds of the ramp rate was subtracted from the initial work rate at the GET (Whipp et al., 1981). $\dot{V}O_{2\ max}$ was determined as the highest 10 second average in $\dot{V}O_2$ elicited either during the ramp test or supramaximal bout (Barker et al., 2011). When calculated in this manner, the typical error in $\dot{V}O_{2\ max}$ across three ramp tests is ~ 4% in children (Welsman et al., 2005).

3.5 Moderate-intensity and high-intensity interval exercise protocols

In Chapters 4, 6 and 7, participants completed two exercise bouts using the Lode Excalibur Sport (Groningen, the Netherlands) in a randomised order; moderate-intensity exercise (MIE) and high-intensity interval exercise (HIIE). These exercise bouts were replicated in Chapter 5, however the total exercise stimulus was accumulated in four separate bouts over the course of the day, each separated by 2 hours.

Moderate-intensity exercise encompasses work performed below the GET, which is analogous to the lactate threshold (Wasserman et al., 1973), and represents the highest work rate achievable without an appreciable rise in blood lactate concentrations from rest or the development of a $\dot{V}O_2$ slow component (Whipp et al., 2005). As such, the physiological and perceptual stress of exercise is profoundly different when exercising above and below an individual's GET. Given that the GET may vary from ~ 40 to 70% $\dot{V}O_{2\ peak}$ in adolescents (Reybrouck et al., 1985), and that any error in determining the GET may be exacerbated by the smaller absolute differences in power output between exercise domains in paediatric groups, the MIE was prescribed at 90% of the GET in all studies. A 3 minute warm up and 2 minute cool down at 20 W was also included in the MIE bout.

The duration of the MIE was work-matched to the total mechanical work performed during the high-intensity interval exercise (HIIE) using Equation 3.1:

$$kJ\ min^{-1} = \frac{power\ output \times 60}{1000}$$

Equation 3.1 Calculating mechanical work performed from power output

Repeated sprint interval training is unlikely to provide a feasible model of exercise for public health promotion. Consequently, we adopted a "practical" alternative which is similar to previous work with adult groups (Little et al., 2010). The high-intensity interval exercise (HIIE) consisted of a 3 minute warm up at 20 W, followed by 8 intervals at 90% of the peak power output achieved

during the prior incremental ramp test to exhaustion interspersed with 75 seconds of active recovery at 20 W. A 2 minute cool down at 20 W was also included. Therefore, the duration of the HIIE in Chapter 4, 6 and 7 was 23 minutes.

In Chapter 5, participants performed a 75 second warm up at 20 W, and then two 1 minute intervals at 90% peak power, interspersed by 75 seconds at 20 W. A 75 second cool down at 20 W was also included. Thus, the total HIIE stimulus accumulated over the course of the day was the same as Chapters 4, 6 and 7.

In Chapter 8, the HIIE sessions were completed at school using a friction-braked cycle ergometer (Monark 827e, Monark exercise AB, Sweden). Accordingly, participants completed a 3 minute warm up of unloaded (~ 20 W) pedalling before the weight of the cradle was lowered for each 1 minute interval, and raised for each 75 second unloaded active recovery interval and 3 minute unloaded cool down. Given that power output using a friction-braked cycle ergometer is a function of cadence and load, participants individually selected their preferred cadence between 70 – 80 rpm and the required load was calculated accordingly.

Chapter 8 required the completion of 6 HIIE bouts over 2 weeks. The first 2 training sessions included eight 1 minute intervals, which increased to nine 1 minute intervals for sessions 3 and 4, and ten 1 minute intervals for the final 2 training sessions in accordance with the progressive nature of other HIIE training studies (Little et al., 2010, Whyte et al., 2010).

Participants were encouraged to maintain a constant cadence between 70-80 rpm and remain seated in both MIE and HIIE trials. Apart from Chapter 8, $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$ and heart rate were assessed (Cortex Metalyzer III B, Leipzig, Germany) throughout both exercise protocols. Total energy expenditure and the contributions of fat and carbohydrate oxidation to MIE were estimated using the mean exercise $\dot{V}O_2$ and respiratory exchange ratio (RER) as described in Equation 3.2 (Frayn, 1983). Protein oxidation was assumed to be negligible. The assumptions underlying indirect calorimetry are violated when the RER > 1 (Frayn, 1983). Furthermore, indirect calorimetry is only reliable at intensities < 80% $\dot{V}O_{2\ max}$ due to the confounding effect of non-metabolic carbon dioxide

production on the RER outcome (Romijn et al., 1992). Therefore, it was not possible to determine substrate oxidation during HIIE.

Participants provided a rating of perceived exertion (RPE) immediately before the cool down for both MIE and HIIE using the 1-10 Pictorial Children's Effort Rating Table (Yelling et al., 2002). Immediately following exercise, participants completed the 16-point Physical Activity Enjoyment Scale (PACES) which has been validated for use in adolescents (Motl et al., 2001). Additionally, participants were asked which trial they preferred upon completion of both exercise bouts.

3.6 Resting metabolic rate (RMR)

Resting metabolic rate was assessed via indirect calorimetry (Cortex Metalyzer II, Leipzig, Germany) for 15 min in order to determine total energy expenditure and substrate oxidation (fat and carbohydrate) throughout the day in Chapters 4 and 5. The same metabolic analyser was used for all tests. Data indicate that RMR determined from 15 minutes provide a valid surrogate of longer (≤ 30 minutes) assessment periods in 7 – 12 year olds (Mellecker and McManus, 2009) and is in accordance with current adult guidelines (Compher et al., 2006).

Resting metabolic rate was identified as the average $\dot{V}O_2$ after the removal of errant $\dot{V}O_2$ values lying more than 4 standard deviations (SD) from the local mean. Average substrate oxidation was subsequently calculated using the Frayn equation (Frayn, 1983) under the assumption that protein oxidation was negligible (Equation 3.2). The energy liberated from fat and carbohydrate oxidation was calculated using the Atwater factors, which were summed to determine total RMR.

$$\text{Fat oxidation } (g \cdot min^{-1}) = (1.67 \times VO_2) - (1.67 \times VCO_2)$$

$$\text{Carbohydrate oxidation } (g \cdot min^{-1}) = (4.55 \times VCO_2) - (3.21 \times VO_2)$$

Equation 3.2 Estimation of substrate oxidation and energy expenditure using indirect calorimetry (Frayn, 1983).

In chapters 4 and 5, postprandial fat oxidation was determined by summing the amount of fat oxidized during each postprandial RMR assessment. When calculated in this manner, the TAUC for postprandial fat oxidation is comparable to total fat oxidation determined from the TAUC for postprandial $\dot{V}O_2$ and $\dot{V}CO_2$.

Whilst a ventilated hood system is recommended for the assessment of RMR (Forse, 1993), pooled data from Chapters 4 and 5 report a between-day coefficient of variation (CV) for RMR of 14.7%. Given that the day to day variation in RMR is thought to be ~ 5% (Cooper et al., 2009, Compher et al., 2006), it appears that our method to determine RMR in this group is consistent with a satisfactory level of reliability.

3.7 Test meals

A cereal breakfast was included in Chapters 4, 6 and 7 in order to address concerns raised by the institutional ethics committee regarding exercise in a fasted state. This breakfast (30 g Kelloggs® Corn Flakes and 130 mL skimmed milk) is unlikely to have influenced endothelial function (Padilla et al., 2006, Vogel et al., 1997), but may have modulated the postprandial lipaemic response observed in Chapters 4 and 6 (Grant et al., 1994, Pedersen et al., 1999).

A high fat meal (HFM) was consumed in Chapters 4, 5 and 6. This was a milkshake comprised of 3 parts Cornish ice cream and 1 part double cream, which provided ~ 1.50 g of fat (70% total energy), 1.20 g carbohydrate (25%) and 0.21 g of protein (5%) per kilogram of body mass (80 $kJ \cdot kg^{-1}$) in accordance with previous postprandial investigations in this population (Tolfrey et al., 2012, Tolfrey et al., 2013). This meal was also included in Study 6 as it provided ~ 85 g of fat, and similar fat loads have been shown to impair endothelial function in adults (Padilla et al., 2006, Tyldum et al., 2009, Vogel et al., 1997) and adolescents (Sedgwick et al., 2013, Sedgwick et al., 2014, Sedgwick et al., 2012).

A second, identical HFM was consumed 4 hours after the first in Chapter 5 as the total duration of each visit was 9 hours. A similar fat load for both breakfast and lunch has also been used in postprandial investigations with adolescents

(Sedgwick et al., 2013, Sedgwick et al., 2014, Sedgwick et al., 2012, Thackray et al., 2013, Thackray et al., 2014).

Study 5 included a high fat and sugar mixed breakfast meal, consisting of a chocolate croissant with added chocolate spread, a chocolate muffin and a 300 mL commercially available fruit smoothie with 50 mL added double cream. This meal provided approximately 68 g of fat, 80 g of sugar and 7134 kJ which is broadly in line with the amount of fat provided in Chapters 4, 5 and 6. The high sugar content may have lowered the lipaemic response to this test meal (Cohen and Berger, 1990, Cohen and Schall, 1988), however postprandial hyperglycaemia has been demonstrated to impair endothelial function via oxidative stress (Ceriello et al., 2002), i.e. the same mechanism as elevated plasma [TAG] (Bae et al., 2001).

Every effort was made to ensure that all test meals were consumed within 15 minutes.

3.8 Blood pressure

Systolic and diastolic blood pressure (SBP and DBP, respectively) were measured in the seated position using a validated (Chang et al., 2003) automated oscillometric device (Dinamap Pro 100V2, GE Medical Systems Information Technologies, Florida, USA). Participants were instructed to sit quietly with their backs supported for 10 minutes and remain silent during each measure. SBP and DBP were determined from the mean of 3 assessments, with at least 30 seconds between measures. Data pooled from Chapters 4 and 5 demonstrate a between-day coefficient of variation for SBP and DBP of 5.4% and 5.5%, respectively. Postprandial SBP in Chapters 4, 5 and 8 was calculated as the total area under the SBP curve versus time using the time point immediately before the first HFM.

3.9 Blood sampling and analyses

Capillary blood samples were collected in Chapters 4, 5, 6 and 8 for the determination of plasma [triacylglycerol] ([TAG]), [glucose], [total antioxidant

status] ([TAS]), [3-hydroxubutyrate] ([3-OHB]) and [glutathione peroxidase] ([GTP]), as described in Table 3.1. Please refer to the specific chapters regarding the timings of each capillary sample.

Table 3.1 Capillary plasma analyses in each experimental chapter

	Chapter 4	Chapter 5	Chapter 6	Chapter 7	Chapter 8
TAG	X	X	X		X
Glucose	X	X			X
TAS			X		X
3-OHB			X		X
GTP					X

TAG, triacylglycerol; TAS, total antioxidant status; 3-OHB, 3-hydroxybutyrate; GTP, glutathione peroxidase.

The participants' hand was warmed for 5 minutes using a 40°C water bath and then dried prior to each sample. Participants elected which finger or thumb to be sampled, which was then sterilised (Steret) and punctured (Lancet). The initial drop of blood was discarded in order to prevent contamination of the sample with debris, before ~ 600 µL of capillary blood was collected into lithium-heparin coated ([TAG], [TAS], [3-OHB]), ([GTP]) and heparin-fluoride coated ([glucose], Microvette CB 300 tubes (Sarstedt Ltd, Leicester, UK) and centrifuged immediately at 13,000 g for 15 min. Plasma was then removed and either stored at -80°C for analysis, or analysed immediately ([glucose]).

All participants were familiarised with the blood sampling procedure during their initial visit the laboratory. Parents were also instructed of this practice during the initial telephone consultation, and the school Physical Education staff member was informed. All participants consented to the capillary blood sampling procedure, although one boy in Chapter 6 felt faint so no further samples were collected for this individual.

Plasma [TAG], [TAS], [3-OHB] and [GTP] were quantified in duplicate by enzymatic, colorimetric methods using an assay kit according to the manufacturer's guidelines (Cayman Chemical Company, MI, USA). The within-batch coefficients of variation for these analyses are provided in the experimental chapters.

In Chapters 4 and 5, haematocrit and haemoglobin values were determined from the fasted and final capillary blood samples in order to calculate changes in plasma volume (Dill and Costill, 1974). Haematocrit was visually determined using a capillary tube reader (Hawksley and Sons, England) following centrifugation. Haemoglobin was assessed using a portable photometric system (HemoCue Ltd, Angelholm, Sweden) which has been validated for use in paediatric groups (Cohen and Seidl-Friedman, 1988) and is known not be influenced by hyperlipidaemia (von Schenck et al., 1986). Haematocrit and haemoglobin were also determined before and after training in Chapter 8.

In chapter 8, total [cholesterol], [HDL] and [LDL] were determined in whole capillary blood using a validated (Panz et al., 2005) portable system (CardioChek, Polymer Technology Systems, IN, USA).

3.10 Vascular function

Macro- and micro-vascular function were simultaneously assessed *in vivo* in Chapters 6 and 7. Participants were familiarised to these procedures during their initial laboratory visit for the determination of anthropometric data and $\dot{V}O_{2\,max}$, GET and peak power output. All vascular measures were performed in darkened, temperature controlled (~ 24°C) room. Apart from the immediate assessment of macro- and micro-vascular function following exercise in Chapter 7, participants were asked to lie prone for 10 minutes in the vascular laboratory before these measures.

3.10.1 Macrovascular function

Macrovascular function was assessed via FMD in accordance with recent guidelines (Harris et al., 2010, Thijssen et al., 2011). When determined in this manner, it is generally accepted (Green, 2005) that FMD is NO-mediated (Doshi et al., 2001, Mullen et al., 2001), and may provide a valid surrogate measure of coronary artery endothelial function (Anderson et al., 1995, Takase et al., 1998), although this has recently been questioned (Atkinson and Batterham, 2015).

Brachial artery diameter was measured using high resolution ultrasonography (Sequoia 512, Acuson, Siemens Corp, Aspen, USA) in duplex mode with a 13 MHz linear array transducer. B mode images were obtained at a reproducible point in the upper arm with the brachial artery in longitudinal section. The linear array transducer was clamped in place once the luminal-arterial wall interface image was optimised, and the position of the probe was marked on the arm in pen. Simultaneous assessment of Doppler velocity was obtained using an insonation angle of 60° with the sample volume as wide as possible without encompassing the vessel walls. The width of the Doppler sample gate was noted and subsequently replicated in all further assessments (Figure 3.2).

Baseline arterial diameter was measured for 1.5 minutes, before the rapid inflation of a pneumatic cuff (Hokanson, Bellevue, USA) placed distal to the humeral epicondyles (Figure 3.3). To minimise movement of the ultrasound probe, the cuff was initially inflated to 150 mmHg, and was then increased to 220 mmHg within 5 seconds. The cuff was rapidly deflated following 5 minutes of occlusion. Brachial artery diameter and blood flow were continuously determined during the last 30 seconds of occlusion and for 5 minutes post deflation.

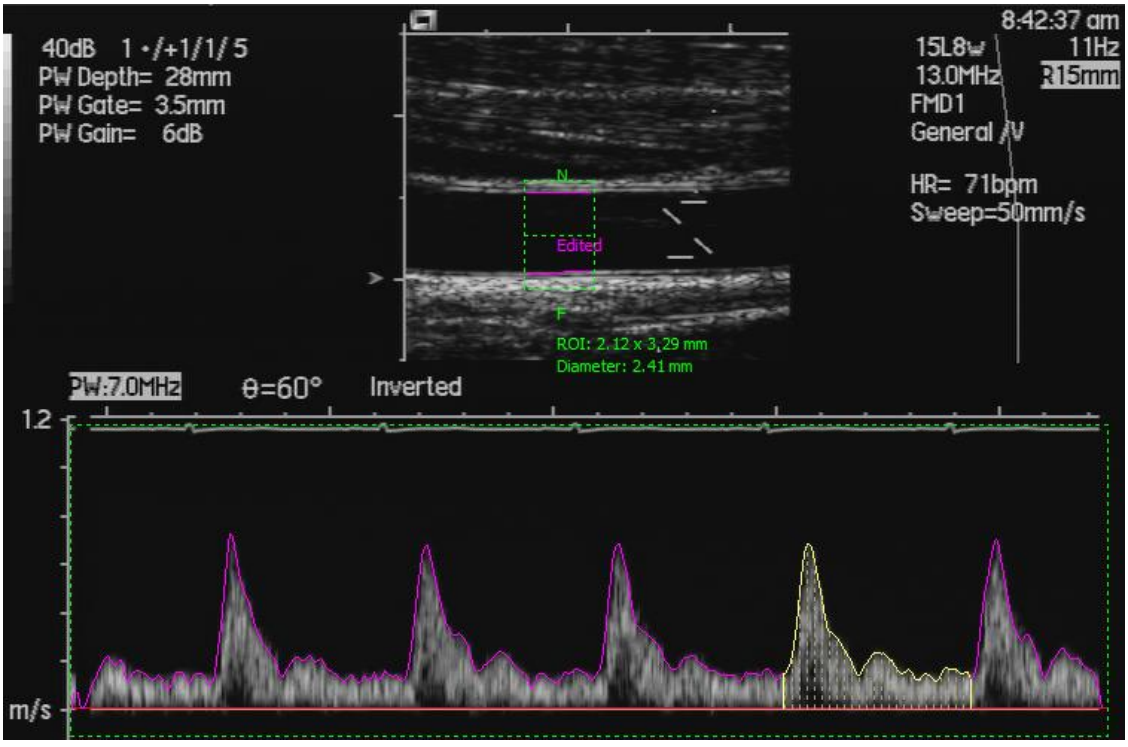

Figure 3.2 Longitudinal B mode images of the brachial artery (top) and continuous Doppler velocity (bottom).

Baseline arterial diameter was measured for 1.5 min, before the rapid inflation of a pneumatic cuff (Hokanson, Bellevue, USA) placed distal to the humeral epicondyles (Figure 3.3). To minimise movement of the ultrasound probe, the cuff was initially inflated to 150 mmHg, and was then increased to 220 mmHg within 5 seconds. The cuff was rapidly deflated following 5 minutes of occlusion. Brachial artery diameter and blood flow were continuously determined during the last 30 seconds of occlusion and for 5 minutes post deflation.

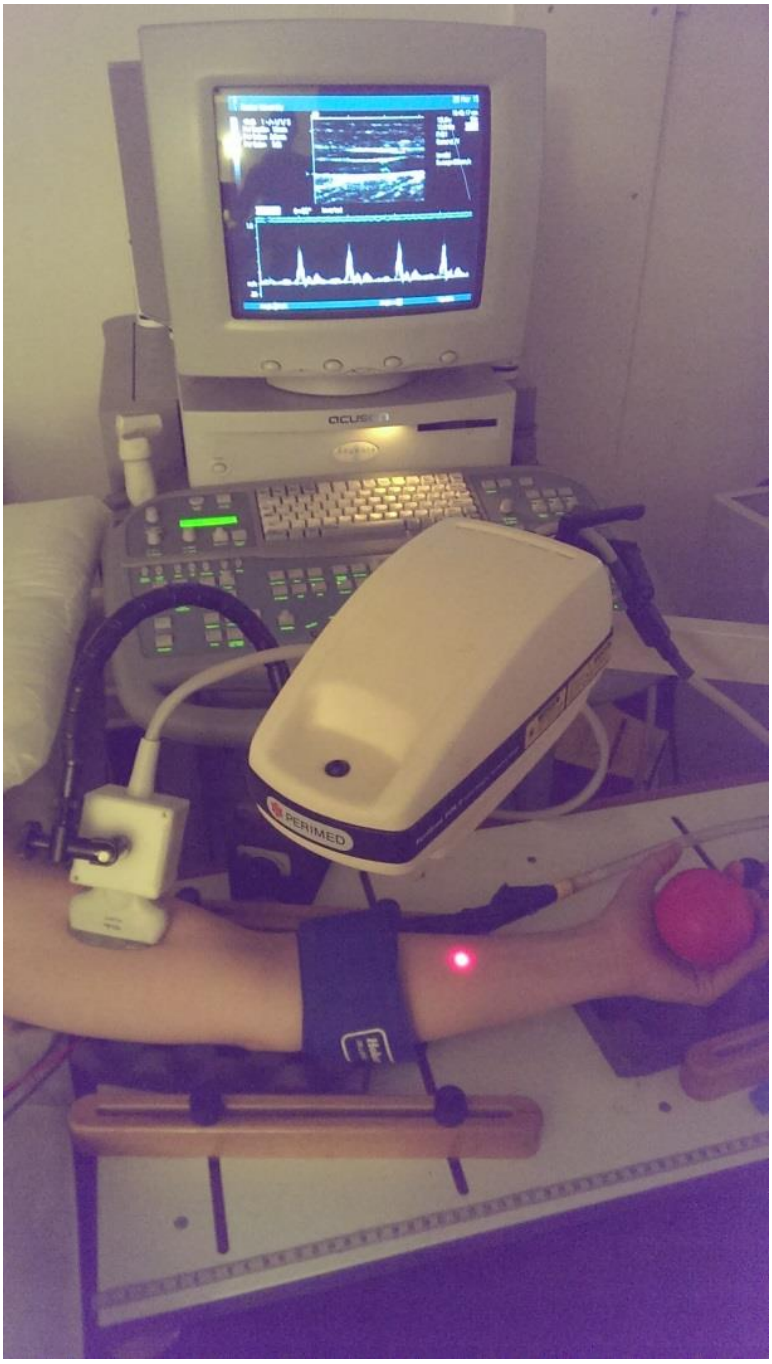

Figure 3.3 Simultaneous acquisition of macro- and micro-vascular function.

All FMD files were labelled in a coded form according to visit number, rather than trial. Thus the primary investigator was blind to the condition (but not time) for all analyses. Brachial artery diameter was assessed during end diastole using validated ECG-gating software (Medical Imaging Applications LLC, Coralville, USA) (Mancini et al., 2002) which is supported for use by current FMD guidelines (Harris et al., 2010, Thijssen et al., 2011) and frequently used in paediatric investigations (Sedgwick et al., 2013, Sedgwick et al., 2014, Sedgwick et al., 2012).

In order to account for the laminar flow of blood through an artery, shear was determined as described in Equation 3.3 as recommended in the current FMD guidelines (Harris et al., 2010).

$$\text{Shear rate} = \frac{(8 \times \text{mean blood velocity})}{\text{artery diameter}}$$

Equation 3.3 Calculation of shear rate.

The total shear stimulus (SR_{AUC}) was calculated as the area under the shear curve versus time from the final 30 seconds of occlusion until peak arterial diameter (Harris et al., 2010, Pyke and Tschakovsky, 2007, Thijssen et al., 2011). Fasted FMD was weakly related to SR_{AUC} in Study 5 (r=0.39, P=0.02). However, no relationship was apparent between fasted FMD and SR_{AUC} in Studies 3 and 4, nor in the post-exercise or postprandial state in any study (r<0.40, P>0.30 for all). Consequently, FMD was not normalised for SR_{AUC}, and the shear stimulus is provided separately (Thijssen et al., 2011).

The assumption of proportionality of the ratio-scaled FMD statistic was checked by determining the slope of the regression line between the logarithmically transformed baseline and peak diameters. Data were allometrically adjusted to baseline diameter in Studies 3, 4 and 5 as the 95% confidence interval around this relationship did not span unity (Atkinson and Batterham, 2013, Atkinson et al., 2013). Pooled pre-exercise FMD data from Chapters 6 and 7, and the fasted, pre-training data from Chapter 8 revealed a regression slope of 0.96, 95% confidence interval 0.94 - 0.99 (n = 49).

Allometric scaling requires the construction of a power function ratio (y/x^{b}), whereby baseline diameter is raised to the common group exponent from the log-linear regression model (*b*) in order to partition out the confounding effect of vessel calibre. This can be checked by confirming the absence of a significant correlation between baseline diameter and the power function ratio (Tanner, 1949). The allometrically-adjusted FMD was then interpreted in the usual manner.

Data from the resting control condition of Chapter 6 indicate that the within-day coefficient of variation for baseline arterial diameter, peak diameter, percentage increase in diameter (FMD), and SR_{AUC} were 1.7%, 1.8%, 2.7% and 14.2%. The between-day coefficients of variation for these outcomes for Study 3 and 4 are provided in table 3.2.

3.10.2 Microvascular function

Microvascular function was simultaneously assessed during the FMD protocol via laser Doppler perfusion imaging (Periscan PIM II, Perimed, Järfalla, Sweden) of the forearm cutaneous circulation (Figure 3.3). The scanner emits a laser beam (wavelength: 670 nm), which becomes Doppler-shifted by interference with moving red blood cells. This Doppler-shifted light is reflected and received by the Doppler imager, and then interpreted as “flux” which is expressed in arbitrary units commonly reported as “perfusion units” (PU). High resolution data were collected at 4.33 Hz, and then interpolated to 1 s averages before being smoothed using a 5 s moving average.

The distance between the scanner and the forearm was 20 cm, in order to determine any changes in cutaneous perfusion prior to occlusion. Freckles, scars or hairs were avoided and the area around the region of interest was marked in pen to promote reproducibility. Participants were also acclimatised to the temperature-controlled room for ~ 10 minutes before each scan (apart from the immediate post exercise vascular assessments in Chapter 7).

Resting flux was determined as the average perfusion during the 2 minutes prior to cuff inflation. Any signal detected during the 5 minutes of occlusion was interpreted as the “biological zero” (Cracowski et al., 2006). Peak reactive

hyperaemia (PRH) was defined as the highest point after occlusion. Cutaneous perfusion was assessed for 5 minutes post occlusion. The total hyperaemic response following occlusion was calculated as the total area under the post-occlusive hyperaemic response, minus the resting flux which was multiplied by the time taken for blood flow to return to baseline values in accordance with Wong *et al*. (2003). When determined in this manner, the total hyperaemic response is known not be NO-mediated (Wong et al., 2003). These values are identified in Figure 3.4.

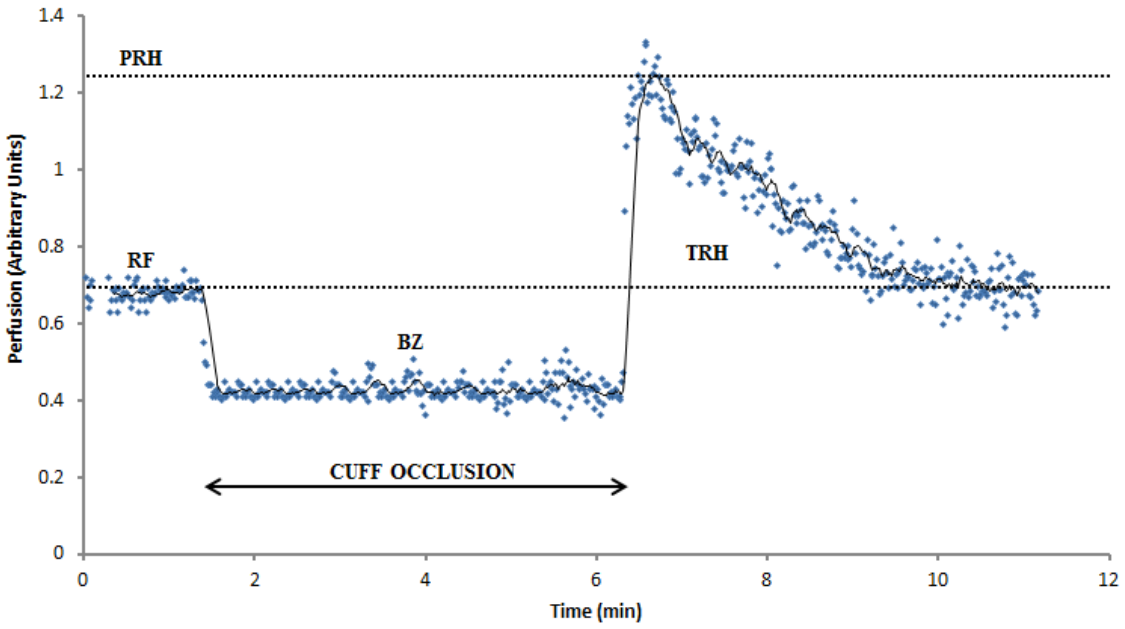

Figure 3.4 Example laser Doppler imaging trace of the post occlusive reactive hyperaemia technique. RF, resting flux; PRH, peak reactive hyperaemia; BZ, biological zero; THR, total hyperaemic response as defined by Wong *et al*. (2003). The data points represent the raw perfusion values. The line represents the 5 second moving average.

The within-day coefficients of variation for PRH and total hyperaemic response (from the resting control condition of Chapter 6) were 11.0% and 21.6%, respectively. The between-day coefficients of variation for PRH and total hyperaemic response from Chapters 6 and 7 are provided in Table 3.2. No microvascular data were recorded in Chapter 8 due to equipment failure.

Table 3.2 Between-day reproducibility of different parameters of macro- and micro-vascular function

	Chapter 6	Chapter 7
Baseline diameter	2.1	6.0
Peak diameter	2.2	5.9
FMD	10.5	9.7
SR_{AUC}	20.3	19.4
PRH	16.2	13.3
Total hyperaemic response	26.1	21.7

FMD, flow mediated dilation statistic; SR_{AUC}, area under the curve for shear; PRH, peak reactive hyperaemia. Values are the coefficient of determination (%).

3.11 Heart rate variability

Heart rate variabiltiy was simultaneously assessed during the FMD protocol in Chapters 6, 7 and 8 using the time intervals between each ECG-gated image of the brachial artery (i.e. the R-R interval) during the 1.5 minutes prior to cuff occlusion. In accordance with current guidelines, data were screened for ectopic beats (defined as a variation of > 20% from the previous beat) and these artefacts were removed and replaced by the mean of the adjacent beats (Task force of the European Society of Cardiology, 1996). The root mean square of the squared differences between adjacent normal R-R intervals (RMSSD) was calculated using the Kubios HRV software (Biosignal Analysis and Medical Imaging Group, Joensuu, Finland), which has recently been used to establish reference values in adolescent boys (Farah et al., 2014). Based upon the resting control condition in Chapter 6, the within-day coefficient of variation for the RMSSD statistic was 17.6%. The between-day coefficients of variation were 21.9% and 18.3% for Chapters 6 and 7, respectively.

The aforementioned guidelines recommend that HRV is analysed from at least 5 minutes of data, preceded by a 5 minute stabilisation period in the supine position (Task force of the European Society of Cardiology, 1996). However, it has been demonstrated that 1 minute of data is an appropriate surrogate of longer assessment periods for the time domain analysis of HRV, with the

intraclass correlations between these two approaches ranging from 0.92 to 0.97 (Esco and Flatt, 2014, Flatt and Esco, 2015). Additionally, the RMSSD outcome has been shown to be uninfluenced by ventilatory rate (Saboul et al., 2013), which is important as no attempt was made to control for this confounder during data acquisition. Given that participants were acclimatising to the temperature-controlled (~ 24°C) room for 10 minutes prior to the capturing of brachial artery diameter, it is likely that this is a robust method to determine HRV. However, 1 minute of data is insufficient for the analysis of frequency domains (Task force of the European Society of Cardiology, 1996), so these analyses were not performed.

3.12 Statistical analyses

All statistical analyses were performed using SPSS (version 19.0, Chicago, USA) apart from the calculation of AUC analyses which were performed using GraphPad (Prism, GraphPad Software, San Diego, California, USA). Unless otherwise stated, all data are presented as means ± standard deviation throughout this thesis.

Due to the limitations of hypothesis testing and the accompanying *P* value, comparisons between means were also interpreted using 95% confidence intervals (95% CI) in accordance with statistical recommendations (Hopkins et al., 2009, Nakagawa and Cuthill, 2007, Wilkinson, 2014). Standardised effect sizes (*ES*), determined as the difference in means divided by the pooled standard deviation (i.e. Cohen's *d*), are included in order to determine whether the mean change in a variable was meaningful using the following thresholds: small (0.2), moderate (0.5), and large (0.8) (Cohen, 1988).

Chapter 4

Exercise intensity and postprandial health outcomes in adolescents

4.1 Introduction

Postprandial lipaemia has been implicated in the progression of atherosclerosis (Zilversmit, 1979), and is an independent predictor of CVD in adults (Nordestgaard et al., 2007). Although the clinical significance of atherosclerosis is not apparent until later life, the atherosclerotic process has its origins in childhood (Stary, 1989) and the progression of which is associated with CVD risk factors in childhood and adulthood (Berenson et al., 1998). SBP during adolescence is also associated with future CVD risk (Berenson et al., 1998), and postprandial hypertension has been purported as a novel atherosclerotic risk factor in adults (Uetani et al., 2012). Considering that up to two thirds of the day may be spent in the postprandial state, interventions that attenuate postprandial lipaemia and SBP in young people may offer primary prevention against the development of atherosclerosis.

It is known that 60 minutes of intermittent or continuous exercise at a moderate to vigorous intensity (50-75% $\dot{V}O_{2\ peak}$) can reduce postprandial lipaemia by ~ 20-30% in male and female adolescents (Tolfrey et al., 2012, Tolfrey et al., 2008, Tolfrey et al., 2014a). Furthermore, 30 minutes of MIE, expending <1 MJ, was similarly effective at reducing postprandial lipaemia as 60 minutes in 13 year old boys (Tolfrey et al., 2012), indicating that even a small volume of exercise may have a beneficial effect on postprandial lipaemia. However, the same authors failed to observe a meaningful reduction in postprandial lipaemia after 30 minutes of moderate exercise in 10-14 year old girls (Tolfrey et al., 2014a). Currently, the optimal exercise interventions to modulate postprandial lipaemia in adolescent boys and girls are currently unknown, and no study has investigated the effect of exercise on postprandial SBP in young people.

Recent evidence has identified that low volume, HIIE can lower postprandial lipaemia in healthy young adults (Freese et al., 2011), and may be more effective than MIE (Trombold et al., 2013). Furthermore, an increase in postprandial resting fat oxidation was related to the beneficial effects of HIIE on postprandial lipaemia (Trombold et al., 2013). It has recently been demonstrated that HIIE can lower postprandial lipaemia in 12-13 year old boys (Thackray et al., 2013), but this study did not compare the efficacy of HIIE to a bout of isoenergetic MIE to isolate the effect of exercise intensity. As many

children fail to achieve the recommended 60 minutes of daily moderate intensity physical activity (Riddoch et al., 2007), it is important to identify whether low volume HIIE offers either similar or superior benefits to postprandial health outcomes compared to traditional MIE.

Surprisingly, no data are available regarding the influence of an acute bout of exercise on postprandial lipaemia in adolescents when exercise is performed immediately before a high fat meal. Reductions in postprandial lipaemia are possible in adult males when exercise is performed 60 minutes before the test meal (Katsanos et al., 2004), however this acute response may be sex dependent (Henderson et al., 2010). Recent evidence in youth suggests that exercise performed during the postprandial period does not influence postprandial lipaemia (Sisson et al., 2013), but it is currently unknown whether exercise performed on the same day prior to the test meal can modulate postprandial lipaemia in young people.

The purpose of this investigation was to test the hypothesis that exercise performed one hour before a high fat meal attenuates postprandial lipaemia and SBP, and augments postprandial fat oxidation, in an intensity-dependent manner. Specifically, HIIE would provide superior benefits than MIE on these outcomes, and the larger reduction in lipaemia after HIIE is related to a greater increase in fat oxidation during the postprandial period.

4.2 Methodology

4.2.1 Participants

Twenty 13 to 14 year old adolescents (10 girls) volunteered to take part in this study. Participant assent and parental consent were obtained before participation in the project, which was approved by the institutional ethics committee. Participants showed no contraindications to exercise and were not using any medication or substance known to influence carbohydrate or fat metabolism.

Body mass and stature were measured to the nearest 0.1 kg and 0.1 cm respectively. Percentage body fat was estimated using triceps and subscapular

skinfold thickness according to Slaughter *et al*. (1988) and pubertal status was determined by a self-assessment of secondary sexual characteristics using adapted drawing of the five Tanner stages of pubic hair development (Morris, 1980).

4.2.2 Experimental protocol

This study required four visits to the laboratory, separated by approximately 1 week, and incorporated a within measures design. All exercise tests were performed using an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands).

4.2.3 Visit 1: Maximal oxygen uptake and gas exchange threshold

Participants were habituated to exercise on the cycle ergometer before completing a combined ramp and supramaximal test to exhaustion to establish $\dot{V}O_{2\,max}$ (Barker et al., 2011). Pulmonary $\dot{V}O_2$ was monitored throughout (Cortex Metalyzer III B, Leipzig, Germany). The GET and $\dot{V}O_{2\,max}$ were determined as described in Section 3.4.

4.2.4 Visits 2-4: Exercise and postprandial measures

An overview of the study protocol is illustrated in Figure 4.1. Following a ~ 12 hours overnight fast, participants arrived at the laboratory at 07:45 and rested for 10 minutes before providing a fasting capillary blood sample for plasma [TAG] and [glucose]. Blood pressure was determined as the mean of three measures using an automated inflation cuff (Dinamap Carescape V100, GE Healthcare, USA). RMR was assessed at 08:15 via indirect calorimetry (Cortex Metalyzer II, Leipzig, Germany) for 15 minutes as described in Section 3.6. Between 08:30 and 08:45 participants consumed a standard breakfast cereal with 125 mL semi skimmed milk (2.5 g fat, 31 g carbohydrates, 6 g protein, 732 kJ energy intake).

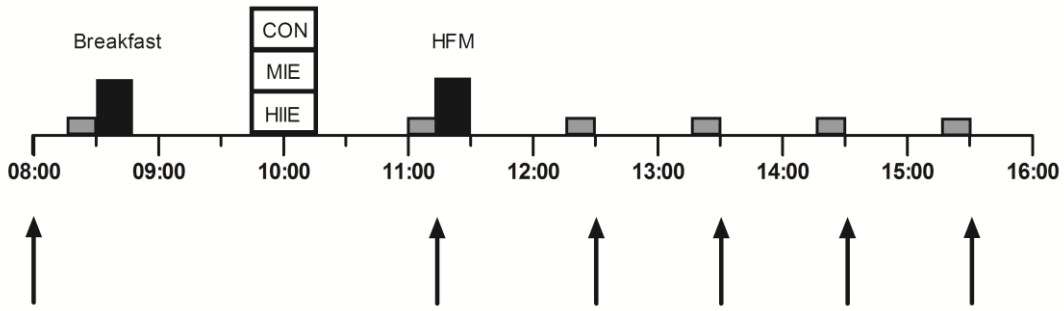

Figure 4.1 Protocol schematic. 1 = rest; 2 = moderate-intensity exercise; 3 = high-intensity interval exercise. Arrows represent capillary blood samples for plasma triacylglycerol and glucose; grey boxes represent the assessment of resting metabolic rate, fat oxidation and blood pressure; HFM = high fat meal.

At 09:45, 1 hour after breakfast, participants completed on separate days and in a randomised order: 1) 23 minutes of HIIE; 2) ~ 30 minutes of continuous moderate intensity cycling at 90% GET (MIE); or 3) rested in the laboratory for 30 minutes. The HIIE bout consisted of a 3 minute warm up at 20 W, followed by 8 x 1 minute intervals at 90% of the peak power determined from the ramp test to exhaustion, interspersed with 75 seconds of recovery at 20 W, before a 2 minute cool down at 20 W. The duration of the MIE trial was calculated to match the total external work performed during the HIIE bout for each participant. Heart rate, $\dot{V}O_2$ and $\dot{V}CO_2$ were monitored throughout both trials. For both MIE and HIIE the participants provided a RPE using the 1-10 Pictorial Children's Effort Rating Table (Yelling et al., 2002) in the final 10 s of exercise, and then completed the 16-point PACES (Motl et al., 2001) on completion of the exercise. After their final exercise trial, each participant was asked to identify which exercise bout they preferred. Exercise was completed by 10:15.

One hour after the completion of the rest/exercise condition, post exercise plasma [TAG], [glucose] and blood pressure were assessed. Participants then consumed a milkshake of 3 parts Cornish ice cream and 1 part double cream between 11:15 and 11:30, which provided ~ 1.50 g of fat (70% total energy), 1.20 g carbohydrate (25%) and 0.21 g of protein (5%) per kilogram of body mass (80 $kJ \cdot kg^{-1}$) in accordance with previous postprandial investigations in this population (Tolfrey et al., 2012, Tolfrey et al., 2014a). Plasma [TAG], [glucose], blood pressure, RMR and substrate oxidation were assessed at hourly intervals during the 4 hour postprandial period. A 4 hour postprandial observational

period was employed as this has been shown to provide a valid estimate of the postprandial lipaemic response compared to an 8 hour observational period (Weiss et al., 2008). No other food was consumed during the postprandial period, although water was available *ad libitum* and subsequently replicated for each trial. Participants remained in the laboratory and inactive throughout the postprandial period, by reading, completing homework, watching DVDs or playing computer games.

4.2.5 Blood sampling and analyses

For each blood sample, ~ 600 µL of capillary blood was collected into lithium-heparin coated ([TAG]) and heparin-fluoride coated ([glucose]) Microvette CB 300 tubes (Sarstedt Ltd, Leicester, UK) and centrifuged immediately at 13,000 *g* for 15 minutes. Plasma was then removed and either stored at -80°C for 1 month for [TAG] analysis, or analysed immediately for [glucose] (YS1 2300 Stat Plus Glucose and L-Lactate Analyzer, YSI Inc., Yellow Springs, USA). Plasma [TAG] was quantified in duplicate by enzymatic, colorimetric methods using an assay kit according to the manufacturer's guidelines (Cayman Chemical Company, MI, USA). The within-batch coefficients of variation for plasma [TAG] and [glucose] were 2.9 and 1.0% respectively. Haematocrit and haemoglobin values were determined from the fasted and final capillary blood samples in order to calculate plasma volume. Changes in plasma volume were small across each trial (-2.6 to 1.7%).

4.2.6 Resting metabolic rate and substrate oxidation

Total EE and the contributions of fat and carbohydrate oxidation to MIE were estimated using the mean exercise $\dot{V}O_2$ and RER for each 15 minute measurement period (Frayn, 1983). Protein oxidation was assumed to be negligible, and an RER >1 during exercise was taken to represent 100% carbohydrate oxidation.

4.2.7 Standardisation of diet

With parental supervision, participants were asked to complete a food diary during the 48 hours period immediately preceding each laboratory visit. Participants were asked to replicate their diet prior to each laboratory visit and were verbally reminded of this requirement. The food diaries were subsequently assessed for total energy and macronutrient intake (CompEat Pro, Nutrition Systems, UK). Participants were also asked to avoid strenuous exercise during this period.

4.2.8 Statistical analyses

Area under the curve analyses were performed using the trapezium rule (GraphPad Prism, GraphPad Software, San Diego, CA) to describe the changes in plasma [TAG], [glucose], SBP, RMR and fat oxidation over the 4 hour period following the high fat meal. Both TAUC and IAUC analyses were performed to characterise the magnitude of the response and the changes over time respectively. It has previously been shown that changes in TAUC-TAG are largely attributable to differences in baseline [TAG] after exercise (Kolifa et al., 2004), and that IAUC-TAG more accurately describes the [TAG] response after a test meal (Carstensen et al., 2003). Consequently, the IAUC analysis was used to account for changes in baseline plasma [TAG] across the experimental trials prior to the high fat meal and adopted as the primary outcome measure which is in line with previous studies (Trombold et al., 2013, Petitt et al., 2003). All AUC analyses were calculated using the time point immediately before the high fat meal (baseline).

Descriptive statistics were calculated using SPSS (version 19.0, Chicago, USA) and presented as mean ± SD. Mean differences in descriptive statistics between boys and girls were analysed using independent samples *t* tests. The mean differences in the physiological and perceptual responses of the boys and girls during HIIE and MIE were analysed using paired samples *t* tests. Analysis of fasting TAG and glucose, and AUC analyses for TAG, glucose, fat oxidation and SBP were performed using a mixed model ANOVA with trial (CON, MIE, HIIE) and sex (male, female) as the main effects. Normality of distribution was

checked using the Shapiro-Wilk test, and data were log transformed if this assumption was violated. Homogeneity of variance was determined using Mauchly's test of sphericity and the degrees of freedom were adjusted using the Greenhouse-Geisser correction if required. To facilitate comparison with recent studies examining exercise and postprandial lipaemia in adolescents (Thackray et al., 2013, Tolfrey et al., 2014a) pairwise comparisons between means were interpreted using the *P* value, 95% CI and standardised *ES* (Cohen, 1988, Hopkins et al., 2009). The null hypothesis was rejected at an alpha level of 0.05, and an *ES* of 0.20 was considered to be a small change between means, with 0.50 and 0.80 interpreted as a moderate and large change respectively (Cohen, 1988). Relationships between changes in AUC outcomes for TAG and potentially mechanistically important variables (e.g. postprandial resting fat oxidation) were explored using Pearson's correlation coefficients and their associated 95% CI.

4.2.9 Power calculation

The calculation of sample size includes an alpha value of 0.05 and a power value of 0.8. Calculating the level of reduction in postprandial TAG which can be considered clinically significant is problematic, as postprandial lipaemia does not have an established clinical end point in paediatric populations, and that this field of research is characterized by a lack of data in adolescents. Consequently, the level of change deemed to be mechanistically significant is based upon the mean difference between the TAUC-TAG response to a similar high fat meal in the control group and exercise condition reported by Tolfrey *et al*. 2008 in 13 year old boys (1.25 $mmol \cdot L^{-1}$).

$$N = \frac{2\,(1.5)^2\,(1.96 + 0.84)^2}{1.25^2}$$

$$N = 9.33$$

The work by Tolfrey *et al*. 2008 reported that moderate exercise reduced the total area under the plasma TAG curve for the 6 hour postprandial period by 24% (95% CI -42% to 0%, *P* = 0.05), despite a sample size of 8. By design, the current project is deliberately replicating several key elements of this study and thereby controlling for many potential confounding methodological issues, including the age of participants, the length of the fasting period, the macronutrient and energetic content of the high fat meal and the frequency of the postprandial TAG measures. Consequently, a sample size of 10 boys and 10 girls is deemed to elicit sufficient statistical power for this study.

4.3 Results

Baseline participant characteristics are presented in Table 4.1. Girls and boys were matched for age and body mass, but boys were taller, had a lower percentage of body fat and higher $\dot{V}O_2$ max compared to girls. The sexual maturation status for boys and girls was as follows; Tanner stage 2, *n*=2 and *n*=0; Tanner stage 3, *n*=0 and *n*=2; Tanner stage, 4 *n*=6 and *n*=5; Tanner stage 5, *n*=2 and *n*=3. No differences in energy intake (main effect for trial, *P*=0.98; main effect for sex, *P*=0.17; trial by sex interaction, *P*=0.99) or individual macronutrient contributions (main effects for trials all *P*>0.70; main effects for sex all *P*>0.71; trial by sex interaction, *P*>0.58) were apparent for boys or girls during the 48 hours preceding each laboratory visit (Table 4.2).

Table 4.1 Participant characteristics

	Boys (*n* = 10)	Girls (*n* = 10)	95% CI	*ES*
Age (y)	14.3 ± 0.3	14.3 ± 0.3	-0.4 to 0.2	0.00
Body mass (kg)	55.7 ± 10.5	56.1 ± 8.8	-8.7 to 9.6	0.04
Stature (m)	1.70 ± 0.09	1.63 ± 0.04	0.01 to 0.14	1.12
Body fat (%)	15 ± 2	25 ± 4	-14 to -8	3.89
$\dot{V}O_{2\,max}$ ($L \cdot min^{-1}$)	2.66 ± 0.56	1.89 ± 0.27	0.36 to 1.18	1.75
$\dot{V}O_{2\,max}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	47.7 ± 4.0	34.1 ± 5.0	9.3 to 17.7	3.04
GET ($L \cdot min^{-1}$)	1.34 ± 0.28	1.08 ± 0.13	0.06 to 0.47	1.19
GET (% $\dot{V}O_{2\,max}$)	49 ± 7	57 ± 8	-14.5 to -1.3	1.06
SBP (mmHg)	107 ± 6	104 ± 3	-1 to 8	0.64
TAG ($mmol \cdot L^{-1}$)	0.37 ± 0.15	0.44 ± 0.17	-0.22 to 0.08	0.43
Glucose ($mmol \cdot L^{-1}$)	5.07 ± 0.26	4.94 ± 0.23	-0.11 to 0.35	0.51

$\dot{V}O_2$, oxygen uptake; GET, gas exchange threshold; SBP, systolic blood pressure; TAG, triacylglycerol; 95% CI, 95% confidence interval for the true difference; *ES*, effect size. Data presented as mean ± SD. Plasma [TAG] and [glucose] are measured in the fasted state.

4.3.1 Exercise trials

Table 4.3 presents the physiological and perceptual data from the exercise trials. An example $\dot{V}O_2$ profile during the MIE and HIIE trials for one participant is provided in Figure 4.2. Despite no differences in work done, HIIE elicited a higher $\dot{V}O_2$, heart rate and RPE compared to MIE for both boys and girls. The highest $\dot{V}O_2$ achieved during the HIIE condition equated to 93 ± 5% and 96 ± 4% $\dot{V}O_{2\,max}$ for boys and girls respectively. Average length of the MIE trial was 27.1 ± 3.5 min. Enjoyment, as measured using PACES was higher for HIIE for boys and girls, and seven boys and seven girls indicated that they preferred the HIIE exercise bout.

Table 4.2 Food diary data during the 48 hours preceding each trial

	CON	MIE	HIIE	MIE vs. CON 95% CI	HIIE vs. CON 95% CI	HIIE vs. MIE 95% CI
Total energy intake (kcal day^{-1})	2207 ± 668	2200 ± 691	2236 ± 687	-418 to 402	-313 to 369	-284 to 356
Energy from carbohydrates (%)	50 ± 5	50 ± 7	50 ± 4	-4 to 3	-2 to 3	-3 to 4
Energy from fat (%)	35 ± 6	35 ± 6	35 ± 6	-3 to 4	-3 to 3	-4 to 4
Energy from protein (%)	15 ± 4	15 ± 3	14 ± 3	-2 to 2	-2 to 2	-3 to 2

CON, control trial; MIE, moderate-intensity exercise trial; HIIE, high-intensity interval exercise trial. 95% CI = 95% confidence limits for the true difference. Data have been pooled as ANOVA analysis revealed no main effect for sex

Table 4.3 Physiological and perceptual responses to MIE and HIIE

	MIE	HIIE	95% CI	ES
*Boys**				
Mean HR ($b \cdot min^{-1}$)	122 ± 9	152 ± 9	21 to 39	3.33
Mean HR (% HR_{max})	61 ± 4	76 ± 3	10 to 19	4.24
Mean $\dot{V}O_2$ ($L \cdot min^{-1}$)	1.17 ± 0.19	1.50 ± 0.25	0.21 to 0.45	1.50
Mean $\dot{V}O_2$ (% $\dot{V}O_{2\,max}$)	45 ± 6	58 ± 5	9 to 17	2.57
RER	0.92 ± 0.02	1.10 ± 0.02	0.15 to 0.20	9.00
RPE	3 ± 1	6 ± 2	2 to 5	1.90
PACES	59 ± 9	64 ± 8	-3 to 12	0.59
Work performed (kJ)	134 ± 21	134 ± 21	-	-
Energy Expenditure (kJ)	656 ± 113	-	-	-
Girls†				
Mean HR ($b \cdot min^{-1}$)	133 ± 12	155 ± 7	15 to 29	2.24
Mean HR (% HR_{max})	68 ± 6	78 ± 3	8 to 15	2.35
Mean $\dot{V}O_2$ ($L \cdot min^{-1}$)	0.99 ± 0.09	1.24 ± 0.13	0.18 to 0.32	2.24
Mean $\dot{V}O_2$ (% $\dot{V}O_{2\,max}$)	53 ± 6	66 ± 5	10 to 16	2.25
RER	0.94 ± 0.03	1.06 ± 0.6	0.08 to 0.17	2.53
RPE	4 ± 1	7 ± 1	3 to 5	3.00
PACES	58 ± 5	64 ± 5	1 to 12	1.20
Work performed (kJ)	106 ± 12	106 ± 12	-	-
Energy Expenditure (kJ)	545 ± 68	-	-	-

HR, heart rate; $\dot{V}O_2$, oxygen uptake; RER, respiratory exchange ratio; RPE, rate of perceived exertion; PACES, physical activity questionnaire; MIE, moderate-intensity exercise trial; HIIE, high-intensity exercise trial; 95% CI, 95% confidence interval for the true difference; *ES* = effect size. Data presented as mean ± SD for MIE and HIIE. * *n* = 10 apart from mean HR where *n* = 9 due to data loss. † *n* = 10 apart from mean HR where *n* = 8 due to data loss.

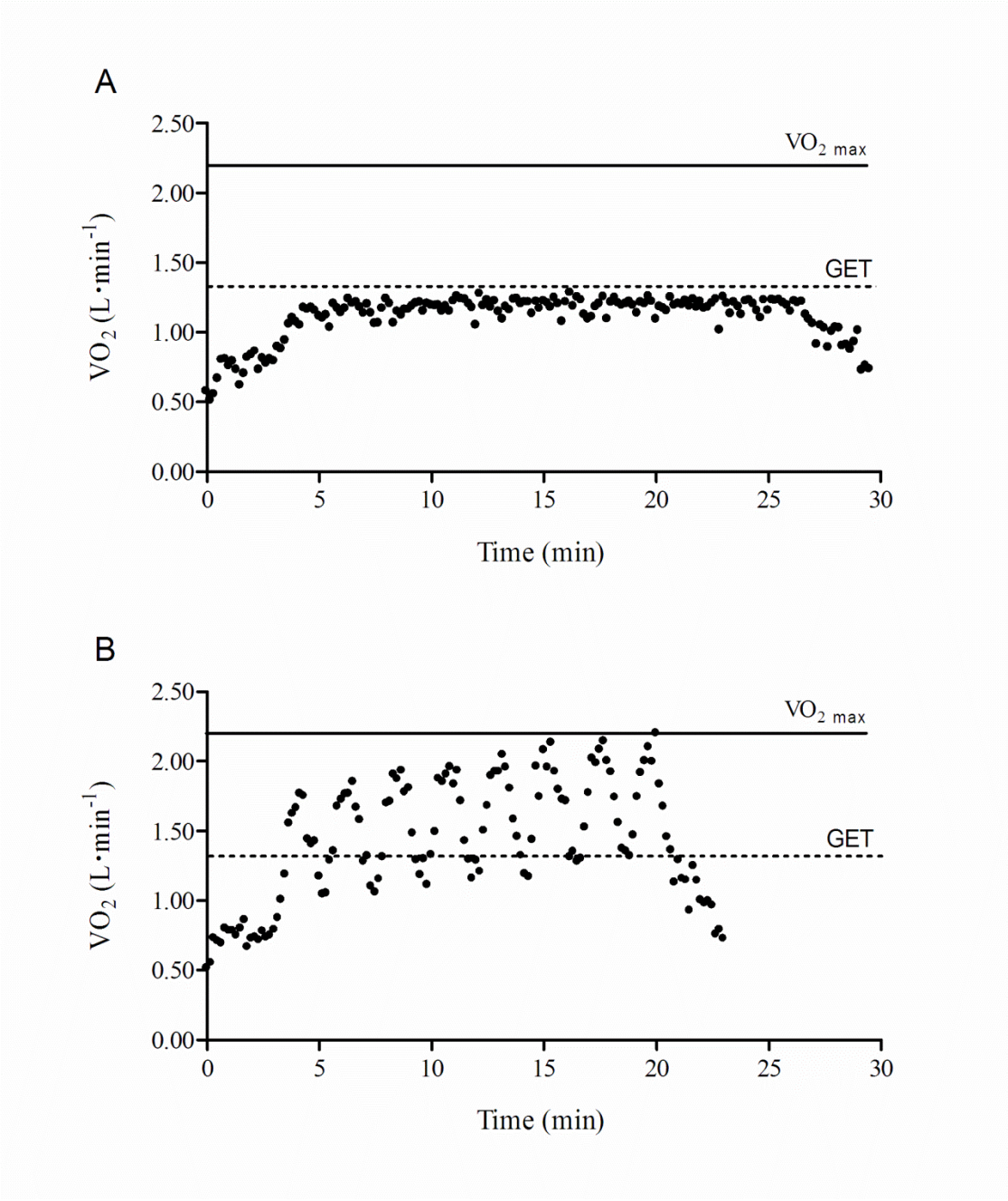

Figure 4.2 An example oxygen uptake ($\dot{V}O_2$) profile for one participant during the moderate-intensity exercise (A) and high-intensity interval exercise (B) bout. The dotted and solid lines demarcate the gas exchange threshold (GET) and maximal oxygen uptake ($\dot{V}O_{2\,max}$) respectively.

4.3.2 Plasma [triacylglycerol]

There were no differences in fasted plasma [TAG] between trials for boys or girls (main effect for trial, *P*=0.86; main effect for sex, *P*=0.59; trial by sex interaction, *P*=0.70). Changes in plasma [TAG] during the postprandial period are illustrated in

Figure 4.3 and the AUC analyses are described in Table 4.4. No differences were apparent in TAUC-TAG (main effect for trial, *P*=0.50; main effect for sex, *P*=0.82; trial by sex interaction, *P*=0.47). However, there was a significant trial by sex interaction for the IAUC-TAG (*P*=0.02). For boys, IAUC-TAG was not significantly different in HIIE compared with CON (*P*=0.22, 95% CI -0.14 to 0.56, *ES*=0.24) or MIE (*P*=0.34, 95% CI -0.19 to 0.54, *ES*=0.20), or for MIE compared with CON (*P*=0.65, 95% CI -0.30 to 0.37, *ES*=0.04). For girls, IAUC-TAG was 34% lower after MIE compared with CON (*P*=0.02, 95% CI -1.43 to -0.17, *ES*=0.58) and a strong trend for a 38% reduction in IAUC-TAG was observed after HIIE compared to CON (*P*=0.09, 95% CI -2.13 to 0.31, *ES*=0.73). There was no difference between HIIE and MIE for IAUC-TAG (*P*=0.74, 95% CI -0.77 to 0.57, *ES*=0.14). There were no differences in IAUC-TAG between boys and girls for CON (*P*=0.52, 95% CI -0.91 to 1.69, *ES*=0.29) or MIE (*P*=0.28, 95% CI -1.28 to 0.40, *ES*=0.50), however IAUC-TAG was lower in girls for HIIE (*P*=0.03, 95% CI -1.36 to -0.08, *ES*=1.10). Pearson's correlations did not reveal any relationships between percentage body fat, EE or mean $\dot{V}O_2$ and IAUC-TAG for boys or girls (all *r*<0.2, *P*>0.05)).

4.3.3 Plasma [glucose]

No differences in fasted plasma [glucose] were apparent for boys or girls between trials (main effect for trial, *P*=0.76; main effect for sex, *P*=0.28; sex by trial interaction, *P*=0.72). Changes in plasma [glucose] during the postprandial period are illustrated in Figure 4.3 and the AUC analyses are presented in Table 4.4. No differences were present between trials for boys or girls in TAUC-glucose (main effect of trial, *P*=0.22; main effect of sex, *P*=0.36; age by sex interaction, *P*=0.44) or IAUC-glucose (main effect of trial, *P*=0.89; main effect of sex, *P*=0.56; age by sex interaction, *P*=0.90).

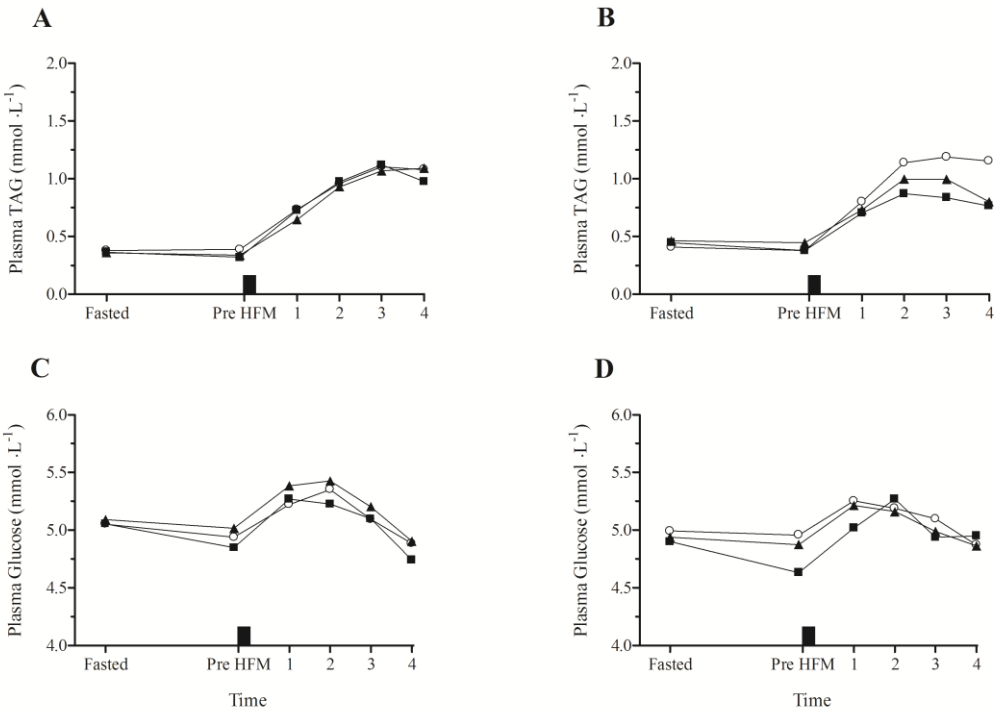

Figure 4.3 Mean plasma triacylglycerol and glucose concentrations for the control (○), moderate-(▲) and high-(■) intensity exercise trials for boys (A, C) and girls (B, D). Error bars are omitted for clarity. The high fat meal (HFM) is represented by the black rectangle.

Table 4.4 Postprandial plasma [TAG] and [glucose]

	CON	MIE	HIIE
Boys			
TAUC-TAG (mmol·L^{-1}·4 h)	3.53 ± 1.54	3.36 ± 1.16	3.46 ± 1.38
IAUC-TAG (mmol·L^{-1}·4 h)	1.98 ± 0.83	2.01 ± 0.80	2.18 ± 0.86
TAUC-glucose (mmol·L^{-1}·4 h)	20.59 ± 1.00	20.98 ± 1.29	20.39 ± 1.22
IAUC-glucose (mmol·L^{-1}·4 h)	0.74 ± 0.92	0.91 ± 1.80	0.92 ± 1.43
Girls			
TAUC-TAG (mmol·L^{-1}·4 h)	3.89 ± 2.46	3.35 ± 1.46	2.99 ± 0.82
IAUC-TAG (mmol·L^{-1}·4 h)	2.37 ± 1.71	1.57 ± 0.97 *	1.47 ± 0.36 †
TAUC-glucose (mmol·L^{-1}·4 h)	20.46 ± 0.80	20.23 ± 1.12	20.02 ± 1.44
IAUC-glucose (mmol·L^{-1}·4 h)	0.55 ± 0.76	0.67 ± 1.29	0.46 ± 3.05

TAUC, total area under the curve; IAUC, incremental area under the curve; [TAG], plasma triacylglycerol; MIE, moderate-intensity exercise trial; HIIE, high-intensity interval exercise trial. * = P<0.05 for MIE vs CON. † = P<0.05 for HIIE boys vs girls.

4.3.4 Resting metabolic rate and fat oxidation

There was no effect of trial (*P*=0.27) or trial by sex interaction (*P*=0.73) on TAUC-RMR, but there was a main effect of sex, which was lower in girls (*P*=0.04, 95% CI -228 to -5, *ES*=0.85, data not presented). There was a main effect for trial on postprandial TAUC-Fat oxidation (*P*<0.001), but not sex (*P*=0.55) or a trial by sex interaction (*P*=0.63). Data were subsequently pooled for further analysis of the TAUC-Fat oxidation main effect (*n*=20) and are presented in Figure 4.4. TAUC-Fat oxidation increased in HIIE by 23% compared to CON (*P*<0.001, 95% CI 0.04 to 0.12, *ES*=0.88) and by 16% compared to MIE (*P*=0.001, 95% CI 0.03 to 0.09, *ES*=0.66). TAUC-Fat oxidation was not different in MIE compared to CON (*P*=0.20, 95% CI -0.01 to 0.06, *ES*=0.28). Changes in TAUC-TAG analyses during MIE and HIIE were not related to postprandial TAUC-fat oxidation (all $r < 0.2$).

4.3.5 Blood pressure and heart rate

Changes in TAUC-SBP and heart rate over time are presented in Figure 4.4. Compared to the initial fasting measure, SBP was attenuated following HIIE (*P*=0.003, 95% CI -8 to -2, *ES*=0.72) and MIE (*P*=0.04, 95% CI -5 to 0, *ES*=0.33).

There was a main effect for trial on the postprandial TAUC-SBP (*P*=0.01), but not sex (*P*=0.17) or a trial by sex interaction (*P*=0.39). Data were subsequently pooled for further analysis of the TAUC-SBP main effect (*n*=20). Postprandial TAUC-SBP was 3% lower in HIIE compared to CON (*P*=0.01, 95% CI -19 to -3, *ES*=0.68) and 3% lower compared to MIE (*P*=0.02, 95% CI -15 to -2, *ES*=0.60). Postprandial TAUC-SBP was not different between MIE and CON (*P*=0.45, 95% CI -8 to 4, *ES*=0.14). Resting heart rate was elevated 1 hour post exercise compared to pre exercise values in HIIE, but not MIE, for boys (*P*=0.01, 95% CI 3 to 14, *ES*=1.13) and girls (*P*<0.001, 95% CI 7 to 16, *ES*=1.83).

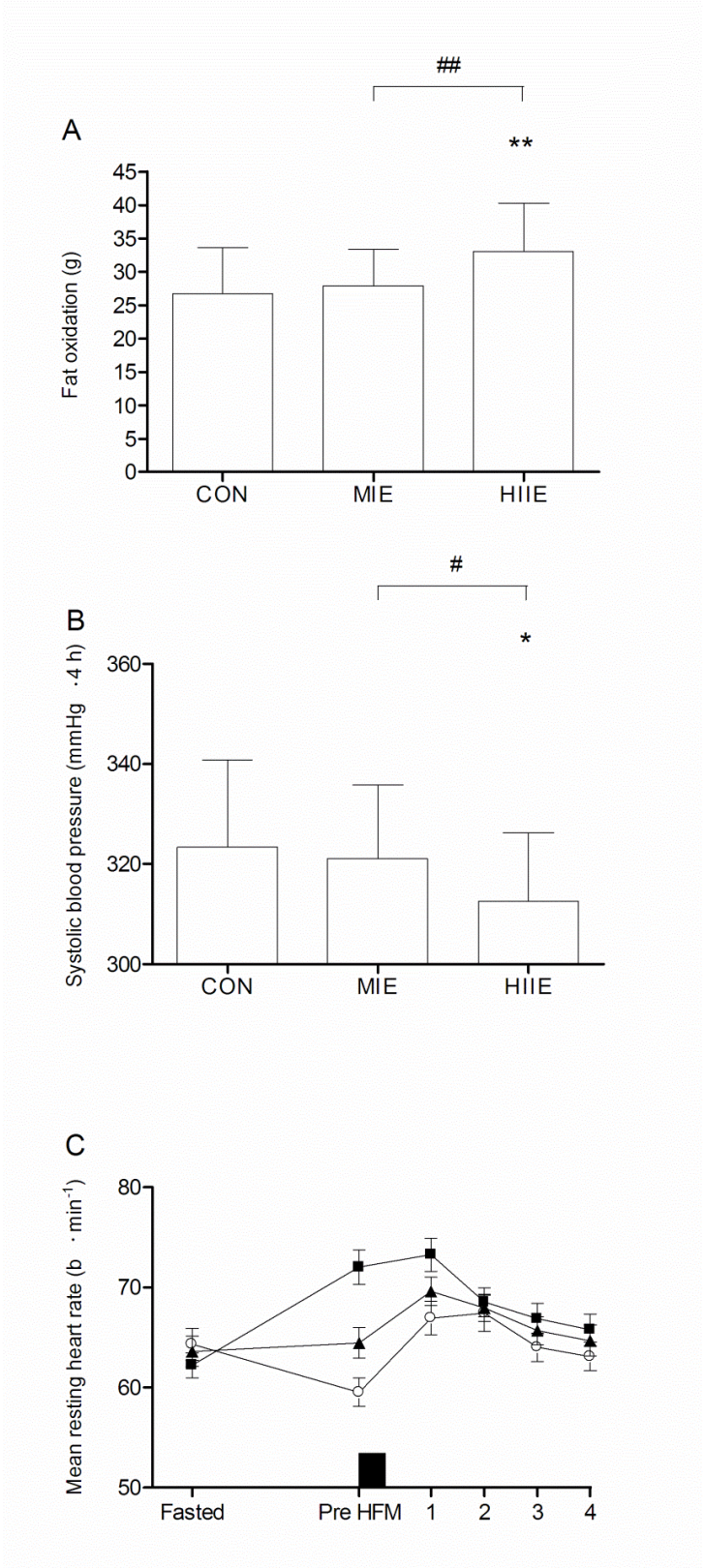

Figure 4.4 Mean total area under the curves for postprandial fat oxidation (A) and systolic blood pressure vs time (4 hours; B), and heart rate (C) collapsed for the boys and girls (*n*=20). CON, control trial (○); MIE, moderate-intensity exercise trial (▲); HIIE, high-intensity interval exercise trial (■). ** = *P*<0.001 for HIIE vs CON; * = *P*<0.05 for HIIE vs CON; ## = *P*<0.001 for HIIE vs MIE; # = *P*<0.05 for HIIE vs MIE. The high fat meal (HFM) is represented by the black rectangle. Error bars describe the standard deviation.

4.4 Discussion

The novel findings of the present study are: 1) based on the IAUC-TAG analyses, both HIIE and MIE reduced postprandial lipaemia (moderate *ES*) in girls. In contrast, HIIE and MIE did not attenuate postprandial lipaemia in boys; 2) resting postprandial fat oxidation was increased after HIIE compared to CON (large *ES*) and MIE (moderate *ES*) for both boys and girls, but was not related to changes in postprandial lipaemia; 3) HIIE reduced postprandial SBP compared to CON and MIE (moderate *ES*) in boys and girls; and 4) PACES score was greater in HIE compared to MIE for boys and girls (moderate and large *ES*, respectively). These data, therefore, show for the first time that exercise intensity and sex play an important role in modulating different postprandial health outcomes in adolescents when the test meal is consumed one hour after exercise cessation.

4.4.1 Postprandial lipaemia

There is consistent evidence showing that performing 30-60 minutes of moderate to vigorous exercise (50-75% $\dot{V}O_{2\ peak}$) ~ 12-16 hours before a high fat meal can reduce postprandial lipaemia in healthy adolescents (Tolfrey et al., 2014b). Furthermore, an acute bout of low volume HIIE running performed 15.5 hours before a high fat meal can reduce TAUC-TAG (~11%, *ES*=0.50) and IAUC-TAG (~15%, *ES*=0.39) over a 6.5 hours postprandial period in healthy 11-12 year old boys (Thackray et al., 2013). Given recent data in healthy adult men showing HIIE to be more effectual at attenuating postprandial lipaemia compared to an isoenergetic bout of MIE (Trombold et al., 2013), it was hypothesised that HIIE would offer either a similar or superior attenuation of postprandial lipaemia in adolescents compared to a work-matched bout of MIE. In contrast, the present study indicates that reductions in TAUC-TAG are not apparent when a high fat meal is consumed 1 hour after an exercise bout. However, interpreting the TAUC-TAG has been criticised due to its dependency on changes in fasting plasma [TAG] (Kolifa et al., 2004). Therefore the IAUC-TAG was used to quantify the effect of exercise on plasma [TAG] following the high fat meal, as recommended elsewhere (Carstensen et al., 2003) and in accordance with other studies (Petitt et al., 2003, Trombold et al., 2013).

There were no significant changes in IAUC-TAG following HIIE and MIE in the adolescent boys. In addition, the *ES* for this finding was either small (HIIE) or trivial (MIE), suggesting no meaningful effect on the IAUC-TAG outcome for boys. This result is surprising given the well documented beneficial effect of MIE and HIIE exercise on plasma [TAG] following a high fat meal in adolescent boys (*ES* range from 0.39 to 1.40) (Thackray et al., 2013, Tolfrey et al., 2008). A possible explanation for this lack of effect in boys in the present study could reside in the adoption of a single day protocol, as postprandial lipaemia may be attenuated to a greater degree when exercise is performed 12 hours compared to 1 hour after exercise in adult males (Zhang et al., 1998), possibly due to a delayed increase in activity of lipoprotein lipase (Seip and Semenkovich, 1998). Furthermore, it has been shown that 135 minutes of light walking during a 3 hour postprandial period does not attenuate postprandial lipaemia in adolescents (Sisson et al., 2013). The EE of exercise may be important in modulating the lipaemic response (Gill et al., 2002) and may explain the findings in adolescent boys. The current study induced a lower EE (~ 650 kJ) than previous investigations in adolescent boys (~ 1-2.5 MJ (Tolfrey et al., 2012, Tolfrey et al., 2008)), and adult studies which reported a reduction in postprandial lipaemia using a similar 1 day protocol to that adopted in the present study (4.6 MJ (Katsanos et al., 2004)). Interestingly, the EE in present study was similar to that used by Pfeiffer *et al*. (630 kJ), who failed to observe a reduction in postprandial lipaemia in healthy young men (Pfeiffer et al., 2006), and may indicate that the EE in the present study was insufficient to reduce postprandial lipaemia in boys. The EE explanation, however, cannot account for the work of Thackray *et al*. (2013) who found low volume HIIE running exercise to attenuate postprandial lipaemia in 11-12 year old boys over a 2 day protocol. This may suggest that the delayed increase in lipoprotein lipase activity is a key determinant of the attenuation in postprandial lipaemia after HIIE, and may account for the findings in adolescent boys.

4.4.2 Postprandial lipaemia and sex

A novel finding of the present study was that the effect of exercise on postprandial lipaemia was dependent on sex with both MIE and HIIE eliciting moderate reductions

(34% and 38% respectively) in IAUC-TAG in the adolescent girls only. There were no sex differences in postprandial lipaemia for CON, suggesting that this sexual dimorphism may be mechanistically linked to the exercise bout performed 1 hour before the high fat meal. These data are consistent with the work of Henderson *et al*. (2010) who found that exercise at 45% and 65% $\dot{V}O_{2\ peak}$ reduced plasma [TAG] 3 hours post exercise in young healthy women but not men. While this study cannot offer any insight into the mechanistic basis of this sex difference in postprandial lipaemia as no meaningful relationship was observed for EE, exercise intensity, RMR or substrate utilisation during or after MIE and HIIE exercise, previous adult studies have suggested an important role for body fat distribution (Couillard et al., 1999), the rate of [TAG] uptake by muscle (Horton et al., 2002), and/or hepatic VLDL output (Mittendorfer et al., 2003) and metabolism (Magkos et al., 2007).

There was no meaningful difference between work-matched MIE and HIE to attenuate postprandial lipaemia in adolescent girls. Thus, exercise intensity *per se* does not appear to determine the magnitude of the reduction in postprandial lipaemia in this population. This finding is not consistent with the recent work of Trombold *et al*. (2013) who found HIIE (repeated bouts of exercise at ~90% $\dot{V}O_{2\ max}$) to be more effectual at reducing the IAUC-TAG compared to 60 minutes of moderate intensity exercise (~50% $\dot{V}O_{2\ max}$) in healthy men using a 2 day protocol. However, a direct comparison between studies is limited due to the confounding effect of sex (Henderson et al., 2010), and the probable disparity in mechanisms underlying the postprandial response after exercise between a 1- and 2-day protocol (Zhang et al., 1998).

4.4.3 Postprandial fat oxidation

Both HIIE and MIE increased postprandial fat oxidation despite no change in RMR in adolescent boys and girls, with HIIE being more effectual than MIE. This is of importance given the relationship between elevated resting fat oxidation and exercise-induced fat loss (Barwell et al., 2009). Similar changes in resting fat oxidation have been reported 24 hours after a single bout of HIIE in overweight and obese men (Whyte et al., 2012), and it has been shown that ~2 MJ of exercise at an

intensity corresponding to peak fat oxidation (~63% $\dot{V}O_{2\ peak}$) increases postprandial fat oxidation in normal weight 12 year old girls on the subsequent day (Zakrzewski and Tolfrey, 2012). Given the greater increase in postprandial fat oxidation following HIIE in the current study, exercise intensity appears to be an important mediator of this response and offers a low volume alternative to MIE.

4.4.4 Postprandial blood pressure

Recent evidence in adults implicates postprandial hypertension as a novel risk factor for atherosclerosis (Uetani et al., 2012). In the present study the high fat meal promoted a transient increase (~ 4 mmHg) in SBP in the CON trial in both boys and girls, which may be indicative of the endothelial dysfunction and arterial stiffness that has been reported following a high fat meal (Vogel et al., 1997). Importantly, a significant reduction in postprandial SBP was present after HIIE compared to both CON (*ES*=0.68) and MIE (*ES*=0.60), highlighting for the first time, the role that HIIE can play in modulating postprandial SBP even in normotensive youth. A protective effect afforded by exercise on endothelial function after a high fat meal has been demonstrated in adolescents (Sedgwick et al., 2012), suggesting that the exercise performed in the current study may have preserved endothelial function. In addition, there was an increase in resting heart rate after both exercise trials, suggesting the reduction in SBP may be related to a fall in peripheral vascular resistance via an attenuated sympathetic drive. While it cannot be ruled out that the effect of exercise on SBP in the current study was related, in part, to the hypotensive response observed after exercise cessation, adult data indicates that postprandial SBP remains lower the day after exercise (Miyashita et al., 2008).

4.4.5 Translational perspective

Few UK adolescents meet the minimum daily guideline of 60 minutes of moderate to vigorous physical activity (Riddoch et al., 2007), and school-based interventions to promote physical activity in youth typically only increase physical activity by a small (~ 4 minutes) amount (Metcalf et al., 2012). Thus, it is important to either address how small volumes of exercise can be optimised for health. This study provides

novel data which demonstrate that exercise intensity is positively associated with favourable changes in postprandial fat oxidation and SBP in adolescents, whilst HIIE and MIIE provided comparable reductions in postprandial lipaemia in girls. It is also encouraging that the adolescents enjoyed the HIIE more than the MIE. Therefore, this data tentatively suggest that interventions which promote HIIE several times per week may be more effectual than traditional MIE interventions for postprandial health, and more popular.

4.4.6 Limitations and considerations

The data presented in the current study should be viewed in the light of a number of methodological considerations. Firstly, given the inherent problems in calculating EE via indirect calorimetry during HIIE, the exercise trials were matched based on the mechanical work done. Consequently, substrate oxidation and EE were only determined during MIE in the present study. Indirect calorimetry is also not appropriate immediately following high-intensity exercise. However, disturbances to the bicarbonate pool have been reported to return to baseline 30 minutes after 6 minutes of high-intensity excise in adult males (Stringer et al., 1992), which broadly corresponds with the HIIE stimulus in the present study. Furthermore, paediatric populations may be characterised by a better acid/base regulation than adults (Ratel et al., 2002). Thus, the determination of postprandial RMR and fat oxidation in the present study is probably appropriate. Secondly, this study design included a high carbohydrate breakfast which was not standardised to body mass and may have altered the postprandial response to the high fat meal (Pedersen et al., 1999). However, no differences in mean body mass were apparent between boys and girls indicating the caloric intake relative to size was equivalent across the groups. Thirdly, postprandial lipaemia in adolescents is known to be influenced by exercise performed up to 16 hours before the high fat meal (Tolfrey et al., 2014b). Whilst it was not possible to provide objective measurements of the participants' physical activity, all participants were asked not to undertake formal exercise 48 hours before each laboratory visit. Finally, the PACES scale has been validated for use with adolescent girls (Motl et al., 2001), but no study has addressed whether it is appropriate to use with adolescent boys.

4.5 Conclusion

This study demonstrates that different postprandial health outcomes are dependent on exercise intensity and sex in healthy adolescents when exercise is performed 1 hour before a high fat meal. Specifically, reductions in postprandial lipaemia were achieved after a single bout of MIE and HIE in girls but not boys, and not dependent on exercise intensity. In contrast, favourable changes in postprandial SBP and lipid oxidation are possible after HIIE, but not MIE, even in normotensive adolescents. Given that postprandial lipaemia is implicated in the atherosclerotic process (Zilversmit, 1979), which starts in childhood (Stary, 1989), and that SBP is associated with future CVD risk (Berenson et al., 1998), these findings may have clinical significance. Finally, given that HIIE was perceived to be more enjoyable than MIE, despite the greater physiological stress, these findings support the use of HIIE as an attractive, feasible and effective strategy to improve postprandial health outcomes in adolescents.

Chapter 5

Accumulating exercise and postprandial health in adolescents

5.1 Introduction

Repeat exposure to elevated postprandial plasma [TAG] and [glucose] has been implicated in the pathogenesis of atherosclerosis (Zilversmit, 1979) and type two diabetes (Reaven, 2005), which have their origins in youth (Stary, 1989, McGill et al., 2008). Elevated non-fasting plasma [TAG], [glucose], and SBP in adolescence are independently associated with fatty streaks in the coronary arteries and future cardiovascular risk (McGill et al., 1995, Morrison et al., 2009, Raitakari et al., 2003). Furthermore, postprandial hypertension has been purported as a novel risk factor for atherosclerosis in adults (Uetani et al., 2012). Considering that most of the day may be spent in the postprandial state, it is important to identify feasible interventions to modulate these risks factors for cardiovascular disease in youth.

Chapter 4 demonstrated that a single bout of exercise (~ 30 minutes) can improve postprandial health outcomes in adolescents in an intensity-dependent manner. However, it is known that adolescents rarely sustain exercise for longer than 10 minutes (Riddoch et al., 2007). Therefore it is important to address whether accumulating short bouts of exercise over the course of the day can favourably modulate postprandial health in this group.

It has been demonstrated that performing brief (3-10 minutes) bouts of low to moderate intensity exercise throughout the day may reduce postprandial plasma [TAG] to the same extent (Miyashita et al., 2008), or greater than (Altena et al., 2004), an equivalent volume of continuous exercise in adults. Similar exercise patterns have also been shown to lower SBP in normotensive adults (Miyashita et al., 2008). Accumulating MIE the day before a high fat meal has been shown to lower postprandial plasma [glucose] and [TAG], and improve endothelial function in adolescent boys (Sedgwick et al., 2013). However, the timing of the exercise stimulus in relation to a high fat meal is known to effect the subsequent lipaemia (Zhang et al., 1998), and no study with adolescents has addressed the impact of exercise accumulated on the same day as the test meal, or if the intensity of accumulated exercise influences the postprandial response. The latter point is important to consider as there is evidence showing that performing high-intensity exercise is superior than MIE at modifying cardiometabolic risk factors in youth (Hay

et al., 2012, Hopkins et al., 2009), even when the total amount of high-intensity exercise performed is small (~ 4 minutes) (Carson et al., 2014).

Given the above, the purpose of this investigation was to test the hypothesis that accumulating short bouts of work matched HIIE and MIE would improve parameters of postprandial health in youth (e.g. plasma [glucose] and [TAG], fat oxidation and SBP), but the benefits would be superior in HIIE compared to MIE. The present study also builds on the findings of Chapter 4 by identifying whether comparable benefits are achievable when the same exercise stimulus is accumulated in smaller bouts rather than performed in a single session.

5.2 Methodology

5.2.1 Participants

Twenty one 13 to 14 year old adolescents (11 boys) initially volunteered to take part in this study. Participant assent and parental consent were provided before participation in the project, which was approved by the institutional ethics committee (reference number 2012/391). Exclusion criteria included any contraindications to exercise, the presence of disease or musculoskeletal injury and the use of any medication or substance known to influence carbohydrate or fat metabolism. These criteria precluded two boys from taking part (diagnosed asthma *n*=1; use of medication *n*=1), thus only 19 adolescents (9 boys) completed this investigation.

5.2.2 Experimental protocol

This study required four visits to the laboratory over a period of three weeks, each separated by at least 4 days, and incorporated a within measures design. All exercise tests were performed using an electronically braked cycle ergometer (Lode Excallibur Sport, Groningen, the Netherlands).

5.2.3 Visit 1: Maximal oxygen uptake and gas exchange threshold

Body mass, stature, percentage body fat and pubertal status were determined as described in Section 3.3. Body mass index was interpreted using established cut points for this population (Cole et al., 2000). Participants were habituated to exercise on the cycle ergometer before completing a validated combined ramp and supramaximal test to exhaustion to establish the GET and $\dot{V}O_{2\ max}$ (Barker et al., 2011) as described in Section 3.4. Aerobic fitness was interpreted using current thresholds for metabolic health (Adegboye et al., 2011).

5.2.4 Visits 2-4: Experimental trials

A schematic of each trial is provided in Figure 5.1. Following a ~ 12 hour overnight fast, participants arrived at the laboratory at 07:45 and rested for 10 minutes before providing a fasting capillary blood sample for plasma [TAG] and [glucose]. At 08:00 SBP was recorded after spending ~ 10 minutes in a seated position (Dinamap Carescape V100, GE Healthcare, USA). RMR was then assessed via indirect calorimetry (Cortex Metalyzer 3B, Leipzig, Germany) for 15 minutes in order to determine total resting EE and substrate oxidation (fat and carbohydrate). These measures were repeated 45 minutes after the cessation of each exercise bout or rest.

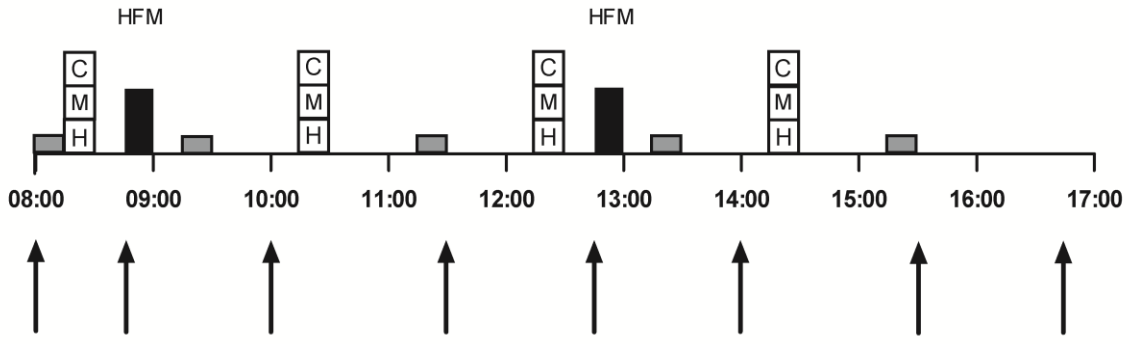

Figure 5.1 Protocol schematic. C, control (rest); M, moderate-intensity exercise; H, high-intensity interval exercise. Arrows represent capillary blood samples for plasma [triacylglycerol] and [glucose]; grey boxes represent the assessment of resting metabolic rate, fat oxidation and systolic blood pressure; HFM = high fat meal.

On three separate occasions, approximately one week apart, participants completed in a randomised order: 1) two, 1 minute intervals at 90% of the peak power determined from the ramp test, separated by 75 seconds at 20 W (HIIE); 2) ~ 6 minutes of cycling at 90% GET (MIE); or 3) remained seated and watched films in the laboratory (control; CON). The exercise bouts were repeated four times, each separated by two hours (see Figure 5.1). A warm up and cool down of 75 seconds at 20 W was included for each HIIE and MIE. The duration of the MIE trial was calculated to match the total work performed during the HIIE bout for each participant, and the total accrued exercise stimulus was equivalent to the exercise bouts in Chapter 4. Total EE and the macronutrient energetic contributions to MIE were determined using the mean exercise $\dot{V}O_2$ and RER values (Frayn, 1983). Protein oxidation was assumed to be negligible, and an RER >1 was taken to represent 100% carbohydrate oxidation.

Participants were asked to provide a RPE using the 1-10 Pictorial Children's Effort Rating Table (Yelling et al., 2002) in the final 10 seconds of exercise. Participants also completed the PACES questionnaire (Kendzierski, 1991) and identified which exercise trial they preferred upon immediate completion of the final exercise bout.

5.2.5 High fat meal and postprandial observation

Participants consumed a milkshake of three parts Cornish ice cream and one part double cream between 08:45 and 09:00. An identical milkshake was consumed between 12:45 and 13:00. The milkshake provided approximately 1.50 g of fat (70% total energy), 1.20 g carbohydrate (25%) and 0.21 g of protein (5%) per kilogram of body mass (80 kJ·kg BM^{-1}) in accordance with Chapter 4 and other postprandial investigations with adolescents (Tolfrey et al., 2012, Tolfrey et al., 2014). No other food was consumed during the postprandial period, although water was available *ad libitum* and subsequently replicated for each trial.

5.2.6 Blood sampling and analyses

For each blood collection, ~ 600 µL of capillary blood was collected into lithium-heparin coated (TAG) and heparin-fluoride coated (glucose) Microvette CB 300

tubes (Sarstedt Ltd, Leicester, UK) and centrifuged immediately at 13,000 g for 15 min. Plasma was then removed and either stored at -80°C for one month for TAG analysis, or analysed immediately using a YS1 2300 Stat Plus Glucose and L-Lactate Analyzer (YSI Inc., Yellow Springs, USA) for glucose. Plasma [TAG] was quantified in duplicate by enzymatic, colorimetric methods using an assay kit according to the manufacturer's guidelines (Cayman Chemical Company, MI, USA). The within-batch coefficients of variation for plasma [TAG] and [glucose] were 1.8% and 0.6% respectively.

5.2.7 Standardisation of diet and physical activity

With parental supervision, participants were asked to wear an ActiGraph GT1M accelerometer (ActiGraph, LLC, Pensacola, USA) and complete a food diary during the 48 hour period immediately preceding each laboratory visit. Participants were asked to replicate their diet prior to each laboratory visit and were verbally reminded of this requirement. The food diaries were subsequently assessed for total energy and macronutrient intakes (CompEat Pro, Nutrition Systems, UK). Time spent performing moderate to vigorous activity was determined using established cut points for paediatric groups (Evenson et al., 2008).

5.2.8 Statistical analyses

The TAUC analysis was performed using the trapezium rule (GraphPad Prism, GraphPad Software, San Diego, CA) to describe the changes in plasma [TAG], [glucose], RMR, fat oxidation and SBP. The IAUC was also calculated for plasma [TAG] and [glucose] in order to characterise the magnitude of the response and the changes over time. All plasma TAG and glucose area under the curve analyses were calculated using the time point immediately before the first high fat meal.

Descriptive statistics were calculated using SPSS (version 19.0, Chicago, USA) and presented as mean ± SD. Mean differences in descriptive characteristics between boys and girls were analysed using independent samples *t* tests. The mean differences in the physiological and perceptual responses of the boys and girls

during HIIE and MIE were analysed using paired samples *t* tests. Analysis of fasting plasma [TAG] and [glucose], and AUC analyses for plasma [TAG], plasma [glucose], fat oxidation and SBP were performed using a mixed model ANOVA with trial (CON, MIE, HIIE) and sex (male, female) as the main effects. A main effect for sex was only present for plasma [TAG]. Thus, all other variables are reported with boys and girls combined into a single ANOVA model. Normality of distribution was checked using the Shapiro-Wilk test, and data were log transformed if this assumption was violated. Homogeneity of variance was determined using Mauchly's test of sphericity and the degrees of freedom were adjusted using the Greenhouse-Geisser correction if required. Pairwise comparisons between means were interpreted using the *P* value, 95% CI and standardised *ES*. The null hypothesis was rejected at an alpha level of 0.05, and an *ES* of 0.20, 0.50 and 0.80 was considered to represent a small, moderate and large change between means (Cohen, 1988). Relationships between changes in AUC outcomes for TAG and potentially mechanistically important variables (e.g. postprandial resting fat oxidation) were explored using Pearson's correlation coefficients and their associated *P* value.

5.2.9 Power calculation

This study was designed before any paediatric data were available regarding the efficacy of accumulated exercise on postprandial lipaemia (i.e. before the work by Sedgwick *et al.* 2013 was published). Therefore, a power calculation was based upon data provided by Miyashita *et al*. (2008), who demonstrated that 30 minutes of accumulated moderate-intensity exercise significantly lowered postprandial lipaemia in adult men by ~1.00 $mmol \cdot L^{-1} \cdot 7$ h (n=15). As with all other experimental chapters presented in this thesis, this power calculation was performed using an alpha value of 0.05 and a power value of 0.8.

$$N = \frac{2\,(0.8)^2\,(1.96 + 0.84)^2}{1.00^2}$$

$$N = 10$$

5.3 Results

Baseline participant characteristics are presented in Table 5.1. The sexual maturation status for boys and girls was as follows: Tanner stage 3, *n*=5 and *n*=4; Tanner stage, 4 *n*=3 and *n*=6; Tanner stage 5, *n*=1 and *n*=0. Two boys and 2 girls were overweight, and 5 boys and 2 girls did not achieve the recommended aerobic fitness level for metabolic health. No differences in energy intake, individual macronutrient contributions, or time spent performing moderate to vigorous physical activity were apparent for boys or girls during the 48 hours preceding each laboratory visit (*P*>0.74, *ES*<0.20; Table 5.2). Only 2 boys and 1 girl achieved the recommended minimum of > 60 minutes of moderate to vigorous physical activity per day.

Table 5.1 Participant characteristics

	Boys (*n* = 9)	Girls (*n* = 10)	95% CI	*ES*
Age (y)	13.5 ± 0.3	13.9 ± 0.5	-0.8 to 0.0	0.96
Body mass (kg)	59.4 ± 18.1	55.2 ± 7.7	-9.0 to 17.4	0.31
Stature (m)	1.68 ± 0.14	1.63 ± 0.05	-5.3 to 16.4	0.49
Percentage body fat (%)	18 ± 5	23 ± 7	-12 to 0	0.96
$\dot{V}O_{2\,max}$ ($L \cdot min^{-1}$)	2.60 ± 0.87	2.02 ± 0.27	-0.03 to 1.19	0.92
$\dot{V}O_{2\,max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	43.7 ± 6.0	36.8 ± 3.9	2.0 to 11.7	1.39
GET ($L \cdot min^{-1}$)	1.29 ± 0.40	1.09 ± 0.18	-0.12 to 0.52	0.66
GET (% $\dot{V}O_{2\,max}$)	50 ± 7	55 ± 9	-13 to 4	0.52
SBP (mmHg)	108 ± 14	111 ± 10	-15 to 7	0.32
TAG ($mmol \cdot L^{-1}$)	0.23 ± 0.10	0.16 ± 0.09	-0.01 to 0.14	0.73
Glucose ($mmol \cdot L^{-1}$)	5.17 ± 0.36	5.11 ± 0.32	-0.25 to 0.36	0.15

$\dot{V}O_2$, oxygen uptake; GET, gas exchange threshold; SBP, systolic blood pressure; TAG, plasma triacylglycerol;95% CI, 95% confidence interval for the true difference; *ES*, effect size. SBP, HR, plasma [TAG] and [glucose] are measured in the fasted state. Data presented as mean ± SD for boys and girls.

Table 5.2 Accelerometer and food diary data during the 48 hours preceding each trial

	CON	MIE	HIIE	MIE vs. CON 95% CI	HIIE vs. CON 95% CI	HIIE vs. MIE 95% CI
Moderate-vigorous activity (min day^{-1})	45 ± 16	44 ± 11	47 ± 20	-9 to 7	-7 to 12	-7 to 13
Total energy intake (kcal day^{-1})	1873 ± 520	1884 ± 487	1942 ± 498	-231 to 274	-397 to 452	-209 to 221
Energy from carbohydrates (%)	49 ± 5	47 ± 5	49 ± 5	-8 to 4	-5 to 4	-3 to 7
Energy from fat (%)	35 ± 6	35 ± 6	37 ± 5	-6 to 7	-4 to 8	-3 to 6
Energy from protein (%)	16 ± 4	17 ± 4	15 ± 3	-1 to 4	-5 to 2	-5 to -1

CON, control trial; MIE, moderate-intensity exercise trial; HIIE, high-intensity interval exercise trial; 95% CI, 95% confidence interval for the true difference. Data have been pooled as ANOVA analysis revealed no main effect for sex

5.3.1 Exercise trials

Table 5.3 presents the physiological and perceptual data from the exercise trials. The average length of each MIE bout was 6.8 ± 1.2 minutes for boys and 5.5 ± 1.2 minutes for girls. The total accumulated exercise time in the MIE trial was 29.6 ± 4.9 minutes and 23.6 ± 4.3 minutes for boys and girls respectively. Eight boys but only 3 girls indicated that they preferred the HIIE exercise bout.

5.3.2 Plasma [triacylglycerol]

There were no differences in fasted plasma [TAG] between trials for boys or girls (main effect for trial, *P*=0.74; main effect for sex, *P*=0.07; trial by sex interaction, *P*=0.33). Mean plasma [TAG] during the postprandial period are illustrated in Figure 5.2 and the AUC analyses are described in Table 5.4. There was an effect of sex, but not trial, on TAUC-TAG (main effect for trial, *P*=0.87; main effect for sex, *P*=0.01; trial by sex interaction, *P*=0.29), with mean TAUC-TAG lower for girls than boys across all trials (*P*=0.01, 95% CI -2.96 to -0.50, *ES*=1.25). There was also an effect of sex, but not trial, on IAUC-TAG (main effect for trial, *P*=0.61; main effect for sex, *P*=0.01; trial by sex interaction, *P*=0.18), with mean IAUC-TAG across all trials lower for girls than boys (*P*=0.01, 95% CI -2.39 to -0.35, *ES*=1.21). There were no effects of trial on TAUC-TAG or IAUC-TAG during the 4 hours after the first (*P*=0.74 and *P*=0.24) or second (*P*=0.95 and *P*=0.17) high fat meal. Relationships between $\dot{V}O_2$ max, body fat, EE, fat oxidation or mean $\dot{V}O_2$ and IAUC-TAG were not statistically significant for boys or girls ($r < 0.2$, $P>0.16$ for all).

Table 5.3 Physiological and perceptual responses to MIE and HIIE

	MIE	HIIE	95% CI	*ES*
*Boys**				
Mean HR ($b \cdot min^{-1}$)	130 ± 14	141 ± 9	4 to 16	0.81
Mean HR (% HR_{max})	67 ± 5	73 ± 3	2 to 9	0.81
Mean $\dot{V}O_2$ ($L \cdot min^{-1}$)	1.21 ± 0.25	1.45 ± 0.34	0.14 to 0.35	0.88
Mean $\dot{V}O_2$ (% $\dot{V}O_{2\,max}$)	48 ± 7	58 ± 7	6 to 12	0.93
RER	0.86 ± 0.04	1.01 ± 0.95	0.11 to 0.19	0.95
RPE	3 ± 1	6 ± 1	2 to 4	0.90
PACES	61 ± 14	66 ± 9	-1 to 11	0.54
Work performed (kJ)	120 ± 28	120 ± 28	-	-
Energy Expenditure (kJ)	220 ± 46	-	-	-
Girls†				
Mean HR ($b \cdot min^{-1}$)	135 ± 13	148 ± 10	4 to 20	0.83
Mean HR (% HR_{max})	71 ± 5	76 ± 5	2 to 8	0.91
Mean $\dot{V}O_2$ ($L \cdot min^{-1}$)	1.09 ± 0.14	1.25 ± 0.15	0.07 to 0.24	0.81
Mean $\dot{V}O_2$ (% $\dot{V}O_{2\,max}$)	54 ± 6	63 ± 7	3 to 13	0.81
RER	0.89 ± 0.03	1.04 ± 0.05	0.11 to 0.19	0.95
RPE	3 ± 1	5 ± 1	1 to 3	0.92
PACES	62 ± 11	58 ± 8	-14 to 7	0.24
Work performed (kJ)	103 ± 9	103 ± 9	-	-
Energy Expenditure (kJ)	172 ± 36	-	-	-

HR, heart rate; $\dot{V}O_2$, oxygen uptake; RER, respiratory exchange ratio; RPE, rate of perceived exertion; PACES, physical activity enjoyment scale; MIE, moderate-intensity exercise trial; HIIE, high-intensity interval exercise trial; *ES*, effect size. Data presented as mean ± SD for MIE and HIIE. * n = 10 apart from mean HR where n = 9 due to loss of telemetric data. † n = 10 apart from mean HR where n = 8 due to loss of telemetric data.

5.3.3 Plasma [glucose]

Mean plasma [glucose] are depicted in Figure 5.2 and the AUC analyses are provided in Table 5.4. Fasted plasma [glucose] was not different between trials (P=0.46). There was a strong trend for a main effect of trial on TAUC-glucose

(*P*=0.05), and pairwise comparisons revealed that TAUC-glucose was lower in HIIE compared to CON (*P*=0.03, 95% CI -1.64 to -0.08, *ES*=0.42) and MIE (*P*=0.04, 95% CI -1.57 to -0.04, *ES*=0.41), with no difference between MIE and CON (*P*=0.89, 95% CI -0.93 to 0.82, *ES*=0.04). There was no effect of trial on the 4 hour TAUC-glucose after the first high fat meal (*P*=0.16), but there was a difference after the second high fat meal (*P*=0.03) with TAUC-glucose lower in HIIE compared to CON (*P*=0.01, 95% CI -1.02 to -0.15, *ES*=0.61) and MIE (*P*=0.02, 95% CI -0.89 to -0.10, *ES*=0.55), but no difference between MIE and CON (*P*=0.75, 95% CI -0.67 to 0.49, *ES*=0.09). There was no main effect of trial for IAUC-glucose (*P*=0.49), or for the IAUC-glucose during the 4 hours after the first (*P*=0.90) and second (*P*=0.55) high fat meal.

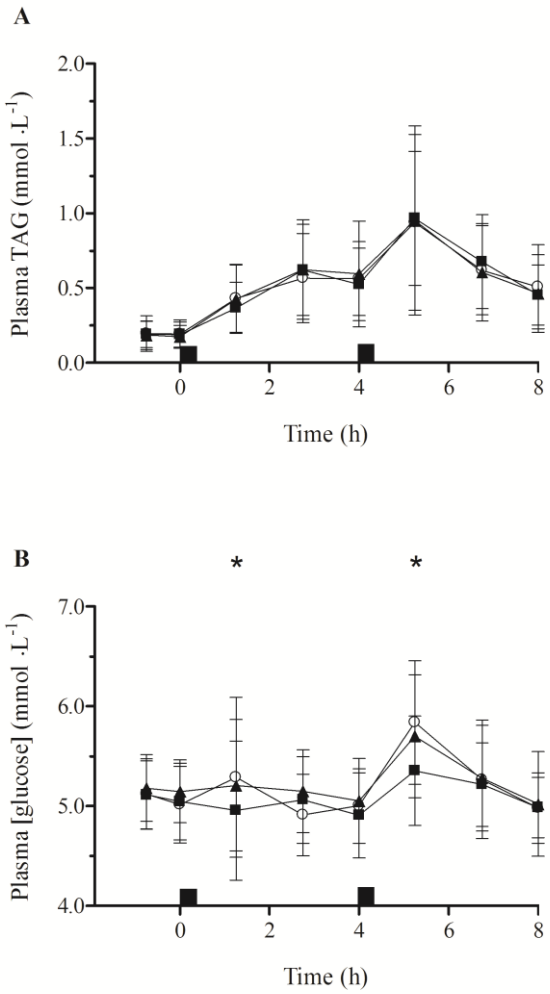

Figure 5.2 Mean plasma [triacylglycerol] (A) and [glucose] (B) in the control (○), moderate-intensity exercise (▲) and high-intensity interval exercise (■) trials. Data are pooled for sex (*n*=19). The high fat meals are represented by the black rectangles. * = *P*<0.05 for HIIE vs CON. Error bars describe the standard deviation.

Table 5.4 Postprandial plasma [TAG] and [glucose]

	CON	MIE	HIIE	MIE vs. CON 95% CI	HIIE vs. CON 95% CI	HIIE vs. MIE 95% CI
Boys (n = 9)						
TAUC-TAG (mmol · L^{-1} 6 h)	4.27 ± 1.53	4.54 ± 2.07	4.22 ± 1.11	-0.72 to 1.82	-0.69 to 0.88	-1.26 to 0.35
IAUC-TAG (mmol · L^{-1} 6 h)	2.89 ± 1.40	3.28 ± 1.79	2.96 ± 0.99	-0.31 to 1.57	-0.49 to 0.83	-1.03 to 0.11
Girls (n = 10)						
TAUC-TAG (mmol · L^{-1} 6 h)	2.94 ± 1.58	2.55 ± 0.98	2.79 ± 1.26	-1.53 to 0.74	-0.85 to 0.56	-0.48 to 0.96
IAUC-TAG (mmol · L^{-1} 6 h)	1.94 ± 1.17	1.67 ± 0.78	1.77 ± 0.72	-1.11 to 0.57	-0.75 to 0.43	-0.40 to 0.61
All (n = 19)						
TAUC-glucose (mmol · L^{-1} 6 h)	31.34 ± 1.93	31.26 ± 1.65	30.54 ± 1.83	-0.93 to 0.82	-1.64 to -0.08*	-1.57 to -0.04*
IAUC-glucose (mmol · L^{-1} 6 h)	0.53 ± 1.88	0.54 ± 1.51	0.04 ± 1.71	-1.11 to 1.06	-1.50 to 0.49	-1.47 to 0.50

TAUC, total area under the curve; IAUC, incremental area under the curve; TAG, triacylglycerol; CON, control trial; MIE, moderate-intensity exercise trial; HIIE, high-intensity interval exercise trial. 95% CI = 95% confidence limits for the true difference. Glucose data have been pooled as ANOVA analysis revealed no main effect for sex.* = $P < 0.05$

5.3.4 Resting metabolic rate and fat oxidation

There was no main effect of trial on TAUC-RMR (*P*=0.29, data not presented). There was a main effect for trial on postprandial TAUC-Fat (*P*=0.01; Figure 5.3). TAUC-Fat oxidation increased in HIIE by 17% compared to CON (*P*=0.01, 95% CI 3 to 13, *ES*=0.74) and by 11% compared to MIE (*P*=0.048, 95% CI 0 to 11, *ES*=0.51). There was no difference in TAUC-Fat oxidation between MIE and CON (*P*=0.37, 95% CI -3 to 8, *ES*=0.24). Changes in TAUC-TAG and IAUC-TAG were not related to postprandial TAUC-fat oxidation (all *r* < 0.2).

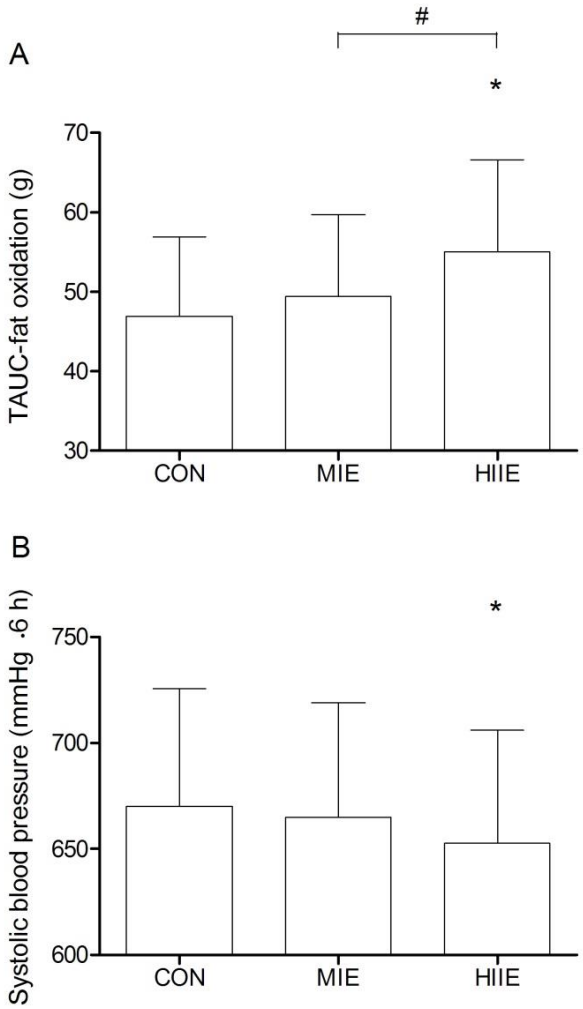

Figure 5.3 Total area under the curves for postprandial fat oxidation (A) and systolic blood pressure (B) vs time (6 hours) collapsed for the boys and girls (*n*=20). CON, control; MIE, moderate-intensity exercise; HIIE, high-intensity interval exercise. * = *P*<0.05 for HIIE vs CON; # = *P*<0.05 for HIIE vs MIE. Error bars describe the standard deviation.

5.3.5 Postprandial blood pressure

There was a strong trend for a main effect for trial on postprandial TAUC-SBP (*P*=0.05; Figure 5.3). TAUC-SBP was lower in HIIE compared to CON (*P*=0.02, 95% CI -31 to -3, *ES*=0.31) but not different to MIE (*P*=0.10, 95% CI -28 to 3, *ES*=0.23). TAUC-SBP was not different between MIE and CON (*P*=0.54, 95% CI -18 to 10, *ES*=0.09).

5.4 Discussion

This study is the first to isolate the effect of exercise intensity on postprandial health outcomes in adolescent girls and boys when exercise is accumulated on the same day as two high fat meals. The novel findings of this study are: 1) accumulating small volumes of HIIE and MIE throughout the day did not attenuate postprandial lipaemia; and 2) repeating brief HIIE, but not MIE, lowered postprandial plasma [glucose] and SBP, and increased resting fat oxidation in adolescent boys and girls. This study demonstrates that even small volumes (8 min) of exercise performed throughout the day may favourably modulate postprandial health outcomes such as plasma [glucose], fat oxidation and SBP in a manner which is intensity-dependent.

5.4.1 Postprandial lipaemia

Chapter 4 demonstrated that the same total MIE and HIIE stimulus performed in a single session 1 hour before an identical high fat meal can attenuate postprandial lipaemia in girls but not boys. However, postprandial plasma [TAG] was not attenuated in the present study. Only two studies have investigated the efficacy of accumulating exercise on the same day as a series of high fat meals (Murphy et al., 2000, Miyashita et al., 2009), and these authors reported only a ~ 10% reduction in TAUC-TAG was achievable in adults when accruing a total of 30 minutes of exercise at 60-70% $\dot{V}O_{2\ max}$. In agreement with the presented findings, a recent study has shown that performing 135 minutes of walking during the postprandial period did not reduce plasma [TAG] in adolescent boys and girls (Sisson et al., 2013). Furthermore, Zhang *et al*. (1998) have previously

demonstrated in adults that postprandial lipaemia was only attenuated when exercise was performed 1 hour before a high fat meal, compared to when identical exercise was performed during the postprandial period. Therefore, accumulating exercise during the postprandial period may only have a limited effect on postprandial lipaemia. Interestingly, 75% of the exercise stimulus in the present study was completed before the consumption of the second high fat meal, however there were no differences between trials in the 4 hour TAUC-TAG after the first or the second test meal. Accumulating exercise has been shown to lower postprandial lipaemia the following day in adults (Altena et al., 2004, Miyashita et al., 2008) and adolescents (Sedgwick et al., 2013), although the latter did not reach statistical significance. Therefore it is likely that repeating brief bouts of exercise may have some utility in attenuating postprandial lipaemia several hours after the final exercise bout, but further work is needed to establish whether this effect can be modulated by exercise intensity.

5.4.2 Postprandial glycaemia

An interesting finding of the present study is the reduction in TAUC-glucose in the HIIE trial, but not MIE, compared to CON. It is acknowledged that the absolute differences in TAUC-glucose between CON and MIE was small (*ES*=0.42 and 0.41, respectively), and this probably reflects the high fat content of the test meal and that the participants were normoglycaemic. It is also not known how the magnitude of this reduction in TAUC-glucose affects cardiometabolic risk if repeated on a daily basis. However, this is the first study to identify that an independent effect of exercise intensity exists when exercise bouts are brief, which may be important considering that young people rarely exercise for longer than 10 minutes (Riddoch et al., 2007). Consequently, future studies exploring this interaction between intensity and accumulated exercise are warranted.

It is important to note that Chapter 4 failed to demonstrate an effect of exercise on plasma [glucose] after a single bout of MIE or HIIE (equating to the total exercise stimulus accrued in the present study) performed one hour before an identical test meal. Whilst it is not possible to provide any mechanistic insight regarding why accumulating HIIE exercise may be more efficacious than

performing the same total exercise stimulus in a single bout, studies with adults have shown that repeatedly interrupting sitting time can lower postprandial plasma [glucose] (Dunstan et al., 2012), and that minimising continuous sedentary behaviour is important for metabolic health independent of physical activity (Healy et al., 2008). This suggests that the repetitive pattern of exercise in the present study may play an important role in promoting glycaemic control, although this does not explain the absence of an effect in MIE. Interestingly, our laboratory recently demonstrated that a similar bout of MIE delivered in a single session offered comparable reductions in TAUC-glucose as HIIE one hour before an oral glucose tolerance test (Cockcroft et al., 2014). Therefore, exercise intensity may be an important factor regarding glycaemic control when the duration of exercise is short. This finding is consistent with existing literature identifying that accumulating even small volumes (4-8 minutes) of high-intensity (but not moderate-intensity) exercise is associated with a reduction in cardiometabolic risk in adolescents (Carson et al., 2014, Hay et al., 2012). Given that adolescents often fail to achieve the recommended 60 minutes of physical activity (Janssen and Leblanc, 2010), and the duration of exercise bouts performed by young people rarely last longer than 10 minutes (Riddoch et al., 2007), it would appear that HIIE has some utility in promoting glycaemic control in adolescents.

5.4.3 Postprandial fat oxidation

Data presented in Chapter 4 demonstrate that HIIE, but not MIE, increases postprandial fat oxidation during the immediate hours after exercise in adolescent boys and girls. The findings of the current study indicate that this favourable shift in resting substrate utilisation may also occur when small volumes of HIIE, but not MIE, are accumulated throughout the day in adolescents. Additionally, the effect size for this outcome in the present study is broadly comparable with the magnitude of change observed in Chapter 4 (*ES*=0.74 and 0.88, respectively). This finding may have clinical importance as repeating 2 minute bouts of HIIE is likely to be achievable for most adolescents, and an elevation in resting fat oxidation is an important predictor of the

magnitude of exercise-induced fat loss (Barwell et al., 2009), and may be linked to insulin sensitivity (Kelley et al., 1999).

5.4.4 Postprandial blood pressure

Postprandial hypertension is purported to be a novel risk factor for atherosclerosis in adults (Uetani et al., 2012), and elevated SBP during adolescence is associated with future cardiovascular disease (Raitakari et al., 2003). These data indicate that reductions in postprandial SBP are achievable when only two minutes of HIIE, but not MIE, are performed on four occasions over the course of the day. Whilst it is not possible to shed light on the mechanism(s) underlying this intensity-dependent response in postprandial SBP, it has been demonstrated that 2 x 15 minutes of high, but not moderate, intensity exergaming influences vascular function in children (Mills et al., 2013). Therefore, changes in SBP in HIIE may be related to an improvement in endothelial function and/or a reduction in vascular resistance. Further work is needed to identify the acute influence of exercise intensity on vascular function in this group, but it has been shown that accumulating exercise is equally effective in lowering SBP the following day as an equivalent bout of continuous exercise in adults (Miyashita et al., 2008). In contrast, the magnitude of the reduction in postprandial SBP observed in the accumulated HIIE trial in the present study is lower than reported in Chapter 4 when HIIE is performed in a single bout (*ES*=0.31 vs 0.68). However, the present findings are promising considering the very brief nature of each HIIE bout, and these data add to a growing body of literature indicating that low volumes of HIIE may be effectual in lowering SBP even in normotensive adolescents (Buchan et al., 2011, Burns et al., 2012).

5.4.5 Translational perspective

This study is the first to demonstrate that accumulating exercise may provide small benefits in postprandial health in a manner which is intensity-dependent. Whilst this investigation is unable to provide any mechanistic insight regarding the independent effect of exercise intensity, or translate these effects into

clinical relevance, these findings are conceptually important as adolescents rarely perform exercise for longer than 10 minutes (Riddoch et al., 2007), and most of the day may be spent in the postprandial state. Thus, investigations which identify how this pattern of exercise can be optimized are warranted. Cross-sectional data are available indicating that achieving approximately 7 minutes of vigorous intensity physical activity per day (but not ~ 110 minutes of light or ~ 46 minutes of moderate intensity) reduces cardiometabolic risk in adolescents (Hay et al., 2012). Furthermore, longitudinal evidence in young people indicates that performing ~ 4 minutes of vigorous activity per day may lower cardiovascular disease risk over time (Carson et al., 2014). Therefore, it is plausible that the small effects observed in the HIIE trial may provide meaningful health benefits if performed on a regular basis. It is also of interest that the PACES score for HIIE and MIE were comparable for both boys and girls. Thus, accumulating HIIE may be a feasible alternative to MIE for health promotion in adolescents.

5.4.6 Limitations and considerations

The strengths of this study include the isolation of exercise intensity, the adoption of multiple meals and the comparison between boys and girls. Furthermore, the brief exercise bouts in this investigation more accurately reflect the pattern of physical activity performed by this age group than continuous exercise of a longer (>10 minutes) duration (Riddoch et al., 2007). Therefore, identifying that the intensity of accumulated exercise intensity is important regarding postprandial health is likely to be an important health message. However, these data should be considered in light of a number of limitations. Firstly, the test meals adopted in this study bear little resemblance to a typical diet. It is therefore important to see if these findings can be replicated following the consumption of more representative meals in a larger sample size. Secondly, it is not possible to extrapolate these findings beyond the sample population. Further work is required to establish the influence of the intensity of accumulated exercise on these outcomes in adolescents with cardiometabolic risk factors. Finally, we were not able to assess the influence of the MIE and HIIE bouts on these parameters the following day. Given that exercise

accumulated the day beforehand has been shown to lower postprandial lipaemia in adolescents (Sedgwick et al., 2013) and blood pressure in adults (Miyashita et al., 2008), it is pertinent to identify whether the favourable changes in glycaemic control, SBP and fat oxidation observed in the HIIE trial remain the following day.

5.5 Conclusion

This study investigated the influence of accumulating work-matched exercise of different intensities on postprandial plasma [TAG], [glucose], fat oxidation and SBP in male and female adolescents. We have demonstrated that repeating very brief bouts of HIIE, but not MIE, is effectual in lowering postprandial plasma [glucose] and SBP, and increasing resting fat oxidation on the same day in adolescents. These observations are encouraging, and add to a growing body of literature which identifies that accumulating short bouts of high-intensity exercise may be more important for cardiometabolic health than MIE (Carson et al., 2014, Hay et al., 2012, Hopkins et al., 2009). Further work is now needed to establish the efficacy of accumulating HIIE on parameters of postprandial health in adolescents with established cardiometabolic risk factors, and whether this translates into a clinically meaningful outcome with time.

Chapter 6

Exercise intensity and the protection from postprandial vascular dysfunction in adolescents

6.1 Introduction

It is well established that the atherosclerotic process originates in childhood (Stary, 1989), and that CVD risk factors in youth are associated with the progression of atherosclerosis during adulthood (Mahoney et al., 1996). Endothelial dysfunction is a sentinel event in the progression of atherosclerosis, preceding the development of fatty streaks, and holds prognostic value in predicting CVD end points and patient mortality (Suwaidi et al., 2000). Conduit artery endothelial function has been shown to be impaired in asymptomatic adolescents with CVD risk factors (Celermajer et al., 1992), whilst microvascular function is also impaired in children with clustered CVD risk (Khan et al., 2003). The ingestion of a high fat meal causes a transient period of macro- and micro-vascular dysfunction (Bae et al., 2001, Sedgwick et al., 2012, Tyldum et al., 2009), and given the central role endothelial dysfunction plays in the atherosclerotic process (Bonetti et al., 2003), it is likely that repeat exposure of the vasculature to this environment has long-term implications for vascular health.

In adults, acute moderate and high-intensity exercise have transient benefits on macrovascular endothelial function in the fasted and postprandial state (Harris et al., 2008, Tyldum et al., 2009), with the benefits more pronounced following high-intensity exercise possibly due to favourable changes in [TAS] (Tyldum et al., 2009). Prior exercise has also been shown to protect the microvasculature from the deleterious effects of a high fat meal in adults (Gill et al., 2004). In children, cross-sectional evidence suggests that high-intensity exercise may have a positive effect on fasting vascular function (Hopkins et al., 2009). Additionally, a single bout of MIE (Sedgwick et al., 2012) and sprint interval exercise (Sedgwick et al., 2014) has been shown to preserve postprandial macrovascular function the following day in adolescent boys. However, the total exercise stimulus in these two studies was not equivalent, and the authors did not include a measure of microvascular function. Therefore, it is currently unknown whether exercise intensity modulates the postprandial macro- and micro-vascular dysfunction observed after a high fat meal in adolescents, which may have important public health implications as much of the day may be spent in the postprandial state. Furthermore, it has recently been shown that performing even small amounts (~ 4 minutes) of high-intensity exercise is

superior than MIE at modifying cardiometabolic risk factors in youth (Carson et al., 2014). Considering that few adolescents meet the current recommended minimum of 60 minutes of moderate to vigorous-intensity physical activity per day (Riddoch et al., 2007), and that habitual physical activity levels decline during adolescence (Kimm et al., 2005, Trost et al., 2002), it is pertinent to identify how small volumes of exercise can be optimised for vascular health in this group.

Given the above, the purpose of this investigation was to test the hypothesis that the intensity of prior exercise is positively associated with postprandial vascular function, and that these postprandial changes are related to [TAS].

6.2 Methodology

6.2.1 Participants

Twenty 12 to 15 year old adolescents (10 males) volunteered to take part in this study. Participant assent and parental consent were obtained before participation in the project, which was approved by the institutional ethics committee. Exclusion criteria included the use of any medication or substance known to influence fat metabolism or vascular function.

6.2.2 Visit 1: Maximal oxygen uptake and gas exchange threshold

Anthropometric measures and somatic maturity were assessed as described in Section 3.3. The GET and $\dot{V}O_{2\,max}$ were determined using a validated combined ramp and supramaximal test (Barker et al., 2011) as described in Section 3.4. All exercise was performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands).

6.2.3 Visits 2-4: Exercise and postprandial measures

Participants completed three experimental trials, separated by approximately one week (Figure 6.1). Following a ~ 12 hour overnight fast, participants were

transported to the laboratory at 07:45 and rested for 15 minutes before providing a fasting fingertip capillary blood sample for plasma [TAG]. Participants then consumed 30 g of commercially available Corn Flakes with 130 mL of skimmed milk, which is unlikely to have influenced endothelial function (Vogel et al., 1997).

At 08:45, participants rested in a darkened, temperature-controlled (24°C) room for 10 minutes before the simultaneous assessment of macrovascular (FMD) and microvascular (PRH and the total hyperaemic response) function. Immediately afterwards, capillary blood samples were obtained for plasma [TAG], [3-OHB] and [TAS]. These measurements were repeated one hour after exercise (but before the high fat meal) and three hours after the high fat meal in order to coincide with peak plasma [TAG] observed in Chapter 4.

At 09:45, one hour after breakfast, participants either: 1) remained seated in the laboratory (CON); 2) performed ~30 minutes of continuous MIE at 90% of the GET; or 3) completed 23 minutes of HIIE. These trials were completed on separate days and in a randomised order. The HIIE bout consisted of a 3 minute warm up at 20 W, followed by 8 x 1 minute intervals at 90% of the peak power determined from the ramp test to exhaustion, interspersed with 75 seconds of recovery at 20 W, before a 2 minute cool down at 20 W. The duration of the MIE trial was calculated to match the total work performed during the HIIE bout for each participant. Thus, the exercise trials were identical in design to the MIE and HIIE bouts performed in Chapter 4. RPE was measured in the final 10 seconds of exercise (Yelling et al., 2002), and participants completed the PACES questionnaire (Motl et al., 2001) immediately after exercise cessation. After their final exercise trial, each participant was asked to identify which exercise bout they preferred. Plasma [TAG] and [TAS] were assessed one hour after the exercise/rest condition. Plasma [3-OHB] was also assessed as a marker of hepatic fatty acid oxidation and VLDL secretion (Gill et al., 2007). Participants then consumed a milkshake of 3 parts Cornish ice cream and one part double cream between 10:45 and 11:00, which provided ~ 1.50 $g \cdot kg^{-1}$ (80 $kJ \cdot kg^{-1}$) of fat in accordance with Chapters 4 and 5, and other postprandial investigations in this group (Tolfrey et al., 2008, Tolfrey et al., 2014, Sedgwick et al., 2012). Plasma [TAG] was assessed at hourly intervals

during the three hour postprandial period. Participants remained seated in the laboratory throughout the postprandial period.

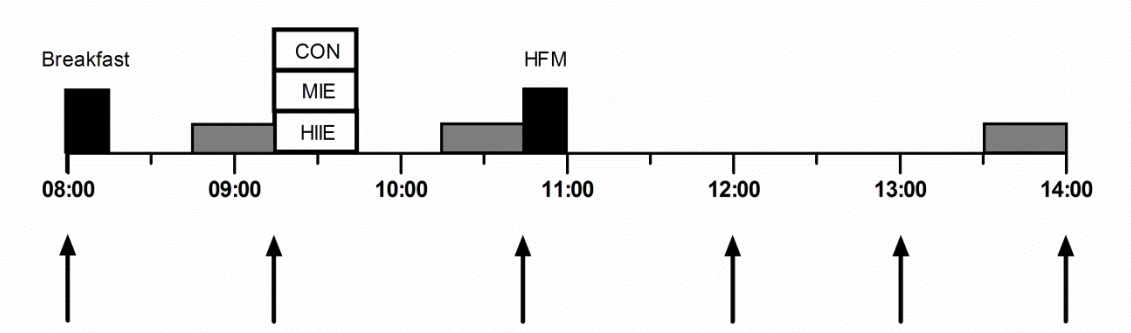

Figure 6.1 Protocol schematic. CON, control (rest); MIE, moderate-intensity exercise; HIIE, high-intensity interval exercise. Arrows represent capillary blood samples for plasma [triacylglycerol]; grey boxes represent the assessment of macro- and micro-vascular function and capillary blood samples for plasma [3-hydroxybutyrate] and [total antioxidant status]; HFM, high fat meal.

6.2.4 Measures of vascular function

Flow mediated dilation and SR_{AUC} were measured as described in Section 3.10.1 and in accordance with recent guidelines (Corretti et al., 2002, Thijssen et al., 2011). All FMD analyses were performed by primary investigator who was blinded to the condition. To address concerns about the ratio-scaled FMD statistic (Atkinson et al., 2009), FMD was also allometrically scaled according to published guidelines (Atkinson and Batterham, 2013). The between-day coefficient of variation for FMD was 10.5%.

During the FMD protocol, microvascular function was simultaneously assessed using a laser Doppler perfusion imager (Periscan PIM II, Perimed, Järfälla, Sweden) at a reproducible point on the distal third of the forearm (Cracowski et al., 2006). Methodological details are provided in Section 3.10.2. PRH was defined as the highest point after occlusion. The total hyperaemic response was calculated in by determining the area under the post-occlusive hyperaemic curve minus the baseline (pre-occlusion) blood flow (expressed as a percentage of PRH), multiplied by the time taken for reactive hyperaemia to return to baseline (Wong et al., 2003). When calculated in this manner, the post-occlusive hyperaemic response is known to be NO independent (Wong et al.,

2003), and accounts for differences in baseline skin perfusion. The between-day coefficients of variation for PRH and the total hyperaemic response were 16.2% and 26.1% respectively.

6.2.5 Blood sampling and analyses

For each blood sample, ~ 600 µL of capillary blood was collected and centrifuged immediately at 13,000 g for 15 minutes at 4°C. Plasma was then removed and stored at -80°C for no more than one month. Plasma [TAG], [3-OHB] and [TAS] were quantified in duplicate by enzymatic, colorimetric methods using an assay kit according to the manufacturer's guidelines (Cayman Chemical Company, MI, USA). The within-batch coefficients of variation for plasma [TAG], [3-OHB] and [TAS] were 2.9, 3.8 and 4.2% respectively. The TAUC and IAUC analyses were performed using the time point immediately before the high fat meal for plasma [TAG], and the time point immediately before exercise for plasma [3-OHB] and [TAS].

6.2.6 Standardisation of diet and physical activity

With parental supervision, participants were asked to replicate their evening meal prior to each laboratory visit. Participants also completed a food diary during the 48 hour period immediately preceding each visit, which were subsequently assessed for total energy and macronutrient intake (CompEat Pro, Nutrition Systems, UK). Participants were instructed to avoid strenuous exercise and wear a tri-axial accelerometer on their wrist (GENEActiv, Activinsights Ltd, Cambridge, UK) during the 48 hour prior to each visit. Time spent performing moderate to vigorous physical activity was determined using established cut points for paediatric groups (Phillips et al., 2013).

6.2.7 Statistical analyses

Descriptive statistics were calculated using SPSS (version 19.0, Chicago, USA) and presented as mean ± SD. Mean differences in descriptive statistics

between boys and girls were analysed using independent samples *t* tests. The mean differences in the physiological and perceptual responses of the boys and girls during HIIE and MIE were analysed using paired samples *t* tests. Analysis of plasma [TAG], [3-OHB] and [TAS], and parameters of macro- and micro-vascular function were performed using a mixed model ANOVA with trial (CON, MIE, HIIE) and sex (male, female) as the main effects. For clarity, the main effects for time and condition are not discussed if the ANOVA output revealed a significant interaction effect. The inclusion of sex into the ANOVA model did not reveal a significant interaction effect for plasma [3-OHB] and [TAS] or parameters of macro- and micro-vascular function. Data were subsequently pooled for these outcomes. Pairwise comparisons between means were interpreted using the *P* value, 95% CI and standardised *ES* to document the magnitude of the effect using the thresholds: small (0.2), moderate (0.5) and large (0.8) (Cohen, 1988). Relationships between changes in vascular outcomes and mechanistically important variables were explored using Pearson's correlations.

6.2.8 Power calculation

No study has identified the influence of exercise intensity on postprandial FMD in paediatric populations. Thus, the sample size for this study was calculated using data provided by Tyldum *et al*. 2009, who demonstrated in adult males (*n*=8) that postprandial FMD is preserved and improved the day after a single bout of equivalent moderate- and high-intensity exercise, respectively. The magnitude of the mean change in FMD following the high-intensity interval exercise was 2.8%, with a standard deviation of 1.9% at baseline.

$$N = \frac{2\ (1.7)^2\ (1.96 + 0.84)^2}{2.8^2}$$

$$N = 7$$

6.3 Results

Baseline participant characteristics are presented in Table 6.1. The maturation status for boys and girls was as follows; Tanner stage 3, *n*=4 and *n*=1; stage, 4 *n*=4 and *n*=8; stage 5, *n*=2 and *n*=1. No differences in energy intake, individual macronutrient contributions, or time spent performing moderate to vigorous physical activity were apparent for boys or girls during the 48 hour preceding each laboratory visit (*P*>0.14, *ES*<0.20; Table 6.2).

Table 6.1 Participant characteristics

	Boys (*n* = 10)	Girls (*n* = 10)	95% CI	*ES*
Age (y)	14.8 ± 0.2	14.1 ± 0.9	0.0 to 1.3	1.07
Body mass (kg)	61.1 ± 11.9	54.5 ± 9.3	-3.4 to 16.6	0.62
Stature (m)	1.69 ± 0.07	1.61 ± 0.09	0.00 to 0.16	0.99
Body fat (%)	13 ± 9	20 ± 4	-14 to 0	0.97
$\dot{V}O_{2\,max}$ ($L \cdot min^{-1}$)	2.76 ± 0.54	2.03 ± 0.27	0.31 to 1.14	1.71
$\dot{V}O_{2\,max}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	45.5 ± 6.4	37.8 ± 4.5	2.4 to 12.9	1.39
GET ($L \cdot min^{-1}$)	1.40 ± 0.25	1.09 ± 0.20	0.10 to 0.52	1.37
GET (% $\dot{V}O_{2\,max}$)	51 ± 6	54 ± 7	-9 to 4	0.46
TAG ($mmol \cdot L^{-1}$)	0.20 ± 0.09	0.13 ± 0.04	0.00 to 0.14	1.05

$\dot{V}O_2$, oxygen uptake; GET, gas exchange threshold; TAG, plasma [triacylglycerol]; 95% CI, 95% confidence interval for the true difference; *ES*, effect size. Data presented as mean ± SD.

Table 6.2 Accelerometer and food diary data during the 48 hours preceding each trial

	CON	MIE	HIIE	MIE vs. CON 95% CI	HIIE vs. CON 95% CI	HIIE vs. MIE 95% CI
Moderate-vigorous activity (min day^{-1})	75 ± 30	73 ± 36	75 ± 27	-35 to 19	-37 to 23	-18 to 24
Total energy intake (kcal day^{-1})	1862 ± 427	1980 ± 388	2027 ± 551	-122 to 245	-134 to 455	-171 to 369
Energy from carbohydrates (%)	46 ± 5	47 ± 5	45 ± 5	-1 to 5	-3 to 3	-5 to 2
Energy from fat (%)	37 ± 6	36 ± 4	37 ± 6	-5 to 2	-5 to 2	-4 to 4
Energy from protein (%)	17 ± 4	17 ± 3	18 ± 3	-4 to 2	-1 to 3	0 to 4

CON, control trial; MIE, moderate-intensity exercise trial; HIIE, high-intensity interval exercise trial. 95% CI = 95% confidence limits for the true difference. Data have been pooled as ANOVA analysis revealed no main effect for sex

6.3.1 Exercise trials

Table 6.3 presents the physiological and perceptual data from the exercise trials. The highest $\dot{V}O_2$ achieved during the HIIE condition equated to 93 ± 5% and 96 ± 5% $\dot{V}O_{2\,max}$ for boys and girls respectively. Average length of the MIE trial was 24.9 ± 2.3 min. Nine boys and nine girls indicated that they preferred the HIIE exercise bout.

Table 6.3 Physiological and perceptual responses to MIE and HIIE

	MIE	HIIE	95% CI	*ES*
Boys				
Mean HR ($b \cdot min^{-1}$)	117 ± 7	144 ± 4	22 to 33	4.74
Mean HR (% HR_{max})	63 ± 4	77 ± 3	12 to 18	3.96
Mean $\dot{V}O_2$ ($L \cdot min^{-1}$)	1.25 ± 0.19	1.59 ± 0.25	0.22 to 0.47	1.53
Mean $\dot{V}O_2$ (% $\dot{V}O_{2\,max}$)	46 ± 7	58 ± 4	8 to 16	2.10
RER	0.89 ± 0.04	1.05 ± 0.04	0.12 to 0.19	4.00
RPE	4 ± 1	8 ± 1	3 to 4	4.00
PACES	53 ± 15	64 ± 7	-1 to 22	0.94
Work performed (kJ)	136 ± 24	136 ± 24	-	-
Energy Expenditure (kJ)	635 ± 100	-	-	-
Girls				
Mean HR ($b \cdot min^{-1}$)	144 ± 13	158 ± 12	5 to 22	1.12
Mean HR (% HR_{max})	74 ± 6	81 ± 5	3 to 11	1.27
Mean $\dot{V}O_2$ ($L \cdot min^{-1}$)	1.10 ± 0.09	1.26 ± 0.11	0.10 to 0.22	1.59
Mean $\dot{V}O_2$ (% $\dot{V}O_{2\,max}$)	55 ± 4	62 ± 5	5 to 10	1.55
RER	0.89 ± 0.05	1.04 ± 0.02	0.12 to 0.18	3.94
RPE	5 ± 2	7 ± 1	1 to 4	1.26
PACES	54 ± 10	59 ± 7	-3 to 12	0.58
Work performed (kJ)	109 ± 11	109 ± 11	-	-
Energy Expenditure (kJ)	700 ± 82	-	-	-

HR, heart rate; $\dot{V}O_2$, oxygen uptake; RER, respiratory exchange ratio; RPE, rate of perceived exertion; PACES, physical activity enjoyment questionnaire; MIE, moderate-intensity exercise trial; HIIE, high-intensity exercise trial; 95% CI, 95% confidence interval for the true difference; *ES*, effect size. Data presented as mean ± SD. *n* = 10 for boys and girls apart from mean HR where *n* = 8 due to loss of telemetric data.

6.3.2 Blood analyses

Mean differences in plasma [TAG], [3-OHB] and [TAS] during the postprandial period are illustrated in Figure 6.2. Mean fasted plasma [TAG] was lower across all trials in girls (*P*=0.03, *ES*=0.96). There was no trial by sex interaction (*P*=0.44) for TAUC-TAG, but there was a trend for TAUC-TAG to be lower in girls across all trials (*P*=0.05). There was no trial by sex interaction (*P*=0.58) for IAUC-TAG.

A time by trial interaction (*P*=0.04) was apparent for plasma [3-OHB], which was elevated three hours after the high fat meal in HIIE compared to CON (*P*=0.01, *ES*=0.59), with no differences between MIE and CON (*P*=0.16, *ES*=0.26) or HIIE and MIE (*P*=0.13, *ES*=0.29). An increase in TAUC plasma [3-OHB] in HIIE was associated with lower TAUC-TAG (*P*=0.01, *r*=0.61) but not for MIE (*P*=0.22, *r*=0.30).

There was no time by trial interaction (*P*=0.53) or effect of trial (*P*=0.88), but there was a main effect of time for [TAS] (*P*=0.04). Mean [TAS] across trials was lower after the high fat meal compared to baseline (*P*=0.02, *ES*=0.39). Changes in [TAS] were not related to parameters of vascular function (*P*>0.05 and *r*<0.2).

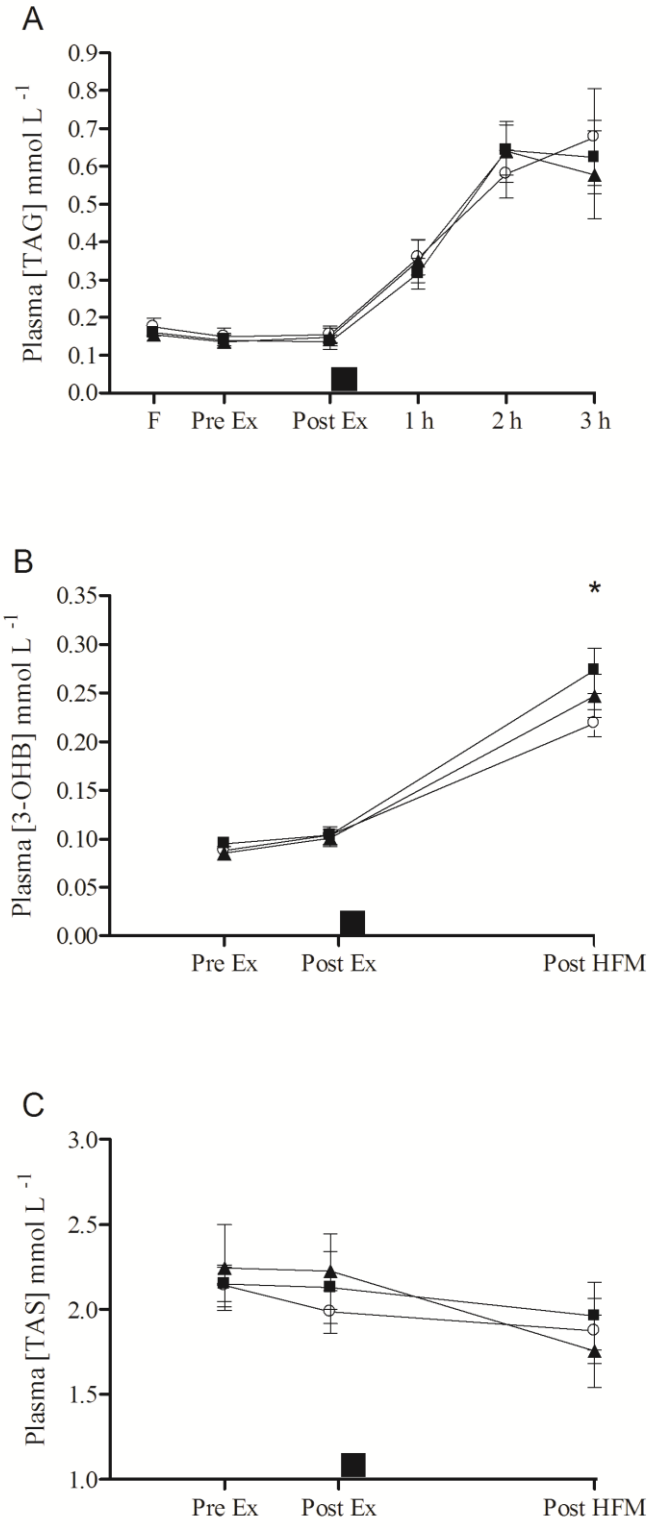

Figure 6.2 Mean plasma triacylglycerol (TAG; A) and 3-hydroxybutyrate (3-OHB; B) concentrations, and total antioxidant status (TAS; C) for the control (○), moderate-(▲) and high-(■) intensity exercise trials. Error bars represent the standard deviation. The high fat meal is represented by the black rectangle. *P<0.05 for HIIE vs CON.

6.3.3 Macrovascular function

Differences in baseline arterial diameter, SR_{AUC} and FMD between trials are provided in Figure 6.3. Changes in FMD were not related to SR_{AUC} in any trial. Consequently, FMD was not normalised for SR_{AUC}. There was no time by trial interaction for SR_{AUC} (*P*=0.25), resting arterial diameter (*P*=0.11), or SR_{AUC} (*P*=0.25).

There was a time by trial interaction (*P*<0.001) for FMD. FMD was greater one hour after HIIE (*P*<0.001, *ES*=1.20), but unchanged after MIE (*P*=0.22, *ES*=0.09) and CON (*P*=0.99, *ES*<0.01) compared to before exercise. Consequently, FMD was greater after HIIE compared to MIE (*P*=0.002, *ES*=1.14) and CON (*P*=0.002, *ES*=1.15), with no difference between MIE and CON (*P*=0.59, *ES*=0.15) one hour after exercise.

FMD was greater three hours after the high fat meal in HIIE compared to MIE (*P*<0.001, *ES*=1.47) and CON (*P*<0.001, *ES*=2.54), and in MIE compared to CON (*P*<0.001, *ES*=1.40). FMD was attenuated after the high fat meal in CON (*P*<0.001, *ES*=1.78) compared to before the meal. FMD remained elevated after the high fat meal compared to baseline in HIIE (*P*<0.001, *ES*=1.56). Postprandial FMD was not different compared to baseline in MIE (*P*=0.46, *ES*=0.16).

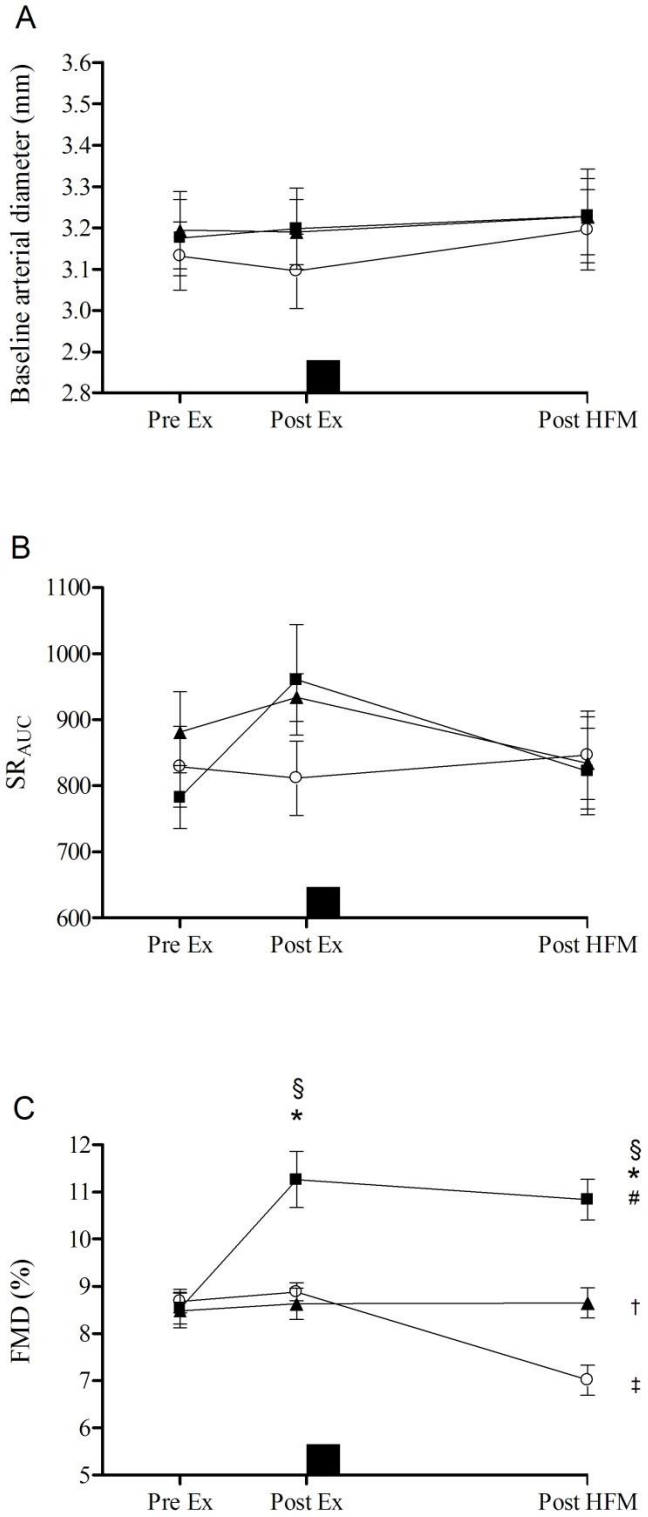

Figure 6.3 Mean baseline arterial diameter (A), area under the curve for shear versus time (SR_{AUC}; B) and flow mediated dilation (FMD; C), for the control (○), moderate-(▲) and high-(■) intensity exercise trials. Error bars represent the standard deviation. The high fat meal is represented by the black rectangle. Statistical significance between trials at the same time point are described as follows: * HIIE vs CON; # HIIE vs MIE; † MIE vs CON. Within-trial significant difference from baseline: § HIIE; ‡ CON. Refer to text for specific *P* values.

6.3.4 Microvascular function

Differences in parameters of microvascular function between trials are presented in Figure 6.4. There was a time by trial interaction (*P*=0.002) for PRH. PRH was greater one hour after HIIE (*P*=0.004, *ES*=0.82) but unchanged after MIE (*P*=0.22, *ES*=0.26) and CON (*P*=0.27, *ES*=0.26). PRH was attenuated three hours after the high fat meal in CON (*P*=0.02, *ES*=0.59). Compared to baseline, postprandial PRH was preserved in MIE (*P*=0.27, *ES*=0.23) and HIIE (*P*=0.08, *ES*=0.49). Compared to CON, PRH was greater three hours after the high fat meal in HIIE (*P*=0.02, *ES*=0.71) and MIE (*P*=0.02, *ES*=0.84), with no difference between HIIE and MIE (*P*=0.72, *ES*=0.16). There was no effect of trial (*P*=0.15), time (*P*=0.40), or a trial by time interaction (*P*=0.27) for time taken to achieve PRH.

There was no time by trial interaction (*P*=0.08), or main effect of time (*P*=0.06) for this outcome, but there was a main effect of trial (*P*=0.002). Total reactive hyperaemia was greater one hour after HIIE (*P*=0.04, 95% CI 7 to 175, *ES*=0.76) but unchanged after MIE (*P*=0.08, 95% CI -4 to 66, *ES*=0.42) and CON (*P*=0.61, 95% CI -38 to 23, *ES*=0.11). The total reactive hyperaemic response was not attenuated three hours after the high fat meal in CON (*P*=0.37, 95% CI -59 to 23, *ES*=0.25). Compared to CON, total reactive hyperaemia was greater three hours after the high fat meal in HIIE (*P*=0.02, 95% CI 10 to 115, *ES*=0.71), with a trend to be greater after MIE (*P*=0.06, 95% CI -3 to 106, *ES*=0.59). There was no difference between HIIE and MIE three hours after the high fat meal (*P*=0.55, 95% CI -28 to 50, *ES*=0.11).

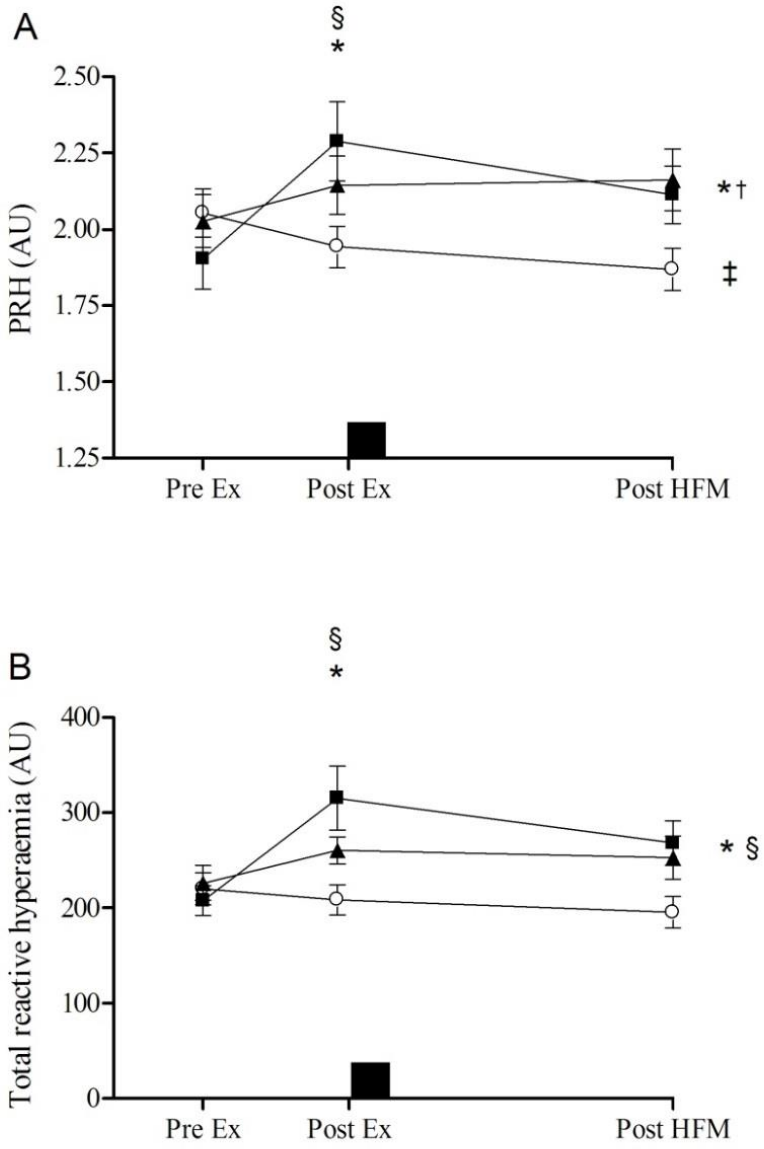

Figure 6.4 Mean peak reactive hyperaemia (PRH; A), and total reactive hyperaemia (B) for the control (○), moderate-(▲) and high-(■) intensity exercise trials. Error bars represent the standard deviation. The high fat meal is represented by the black rectangle. Statistical significance between trials at the same time point are described as follows: * HIIE vs CON; # HIIE vs MIE; † MIE vs CON. Within-condition significant difference from baseline: § HIIE; ‡ CON. Refer to text for specific *P* values.

6.4 Discussion

The novel findings from this study are: 1) macro- and micro-vascular function were enhanced one hour after HIIE compared to CON and MIE, and remained elevated three hours after a high fat meal; 2) a single bout of MIE did not alter macro- or micro-vascular function one hour after exercise, but prevented the

decline in function observed three hours after a high fat meal; and 3) the interactions between exercise intensity and vascular function were independent of changes in plasma [TAG] or [TAS]. These data show for the first time that the effect of exercise on postprandial vascular function is dependent on exercise intensity. Specifically, macrovascular function after a high fat meal is preserved by MIE, and augmented by HIIE. These findings may have a clinically important public health message as a significant proportion of time is spent in the postprandial state, and endothelial function predicts cardiovascular events independently of conventional CVD risk factors (Bonetti et al., 2003).

6.4.1 Macrovascular function

The high fat meal reduced FMD by 21% in CON, which is consistent with other adolescent (Sedgwick et al., 2012) and adult (Bae et al., 2001, Tyldum et al., 2009, Vogel et al., 1997) data. For the first time in adolescents, this study provides evidence that a single bout of MIE performed one hour before a high fat meal may preserve endothelial function, and that an equivalent bout of HIIE not only prevents this attenuation but improves endothelial function despite no reduction in plasma [TAG]. Whilst the benefits of prior moderate-intensity (Sedgwick et al., 2012) and sprint interval (Sedgwick et al., 2014) exercise on postprandial macrovascular function have been shown to be unrelated to changes in plasma [TAG] in adolescents, this investigation is the first to identify an independent effect of exercise intensity. These findings concur with those reported by Tyldum *et al*. (2009), however these authors identified that this protective effect of exercise performed the day before a high fat meal was related to an exercise-induced increase in antioxidant capacity, which was not observed in this study. It is known that postprandial lipaemia impairs vascular function via oxidative stress (Bae et al., 2001), which may reduce NO bioavailability (Wallace et al., 2010). FMD is considered to be largely NO dependent (Green, 2005), but there was no effect of exercise on [TAS], or a relationship between FMD and [TAS] in the present study. However, Johnson *et al*. (2012) also reported no relationship between post exercise FMD and oxidative stress, and this may be related to the limitation of a single measurement of oxidative stress rather than rate of antioxidant depletion

(Dawson et al., 2013). Furthermore, the exercise bouts in this study were performed one hour, compared to 16-18 hours (Tyldum et al., 2009), before the ingestion of the high fat meal, and thus the process(es) underlying the response in pro/anti-oxidant state are likely to be mechanistically different. Indeed a recent investigation failed to observe any changes in postprandial antioxidant status after MIE and HIE when exercise was performed one hour after a high fat meal (Canale et al., 2014). Additionally, the potential influence of training status on the changes in pro/antioxidant status following the exercise bouts cannot be accounted for in this study (Bogdanis et al., 2013). However, based upon recommended $\dot{V}O_{2\ max}$ cut off values for cardiometabolic health (Adegboye et al., 2011), 5 of the boys and 2 of the girls included in this study could be identified as "at risk", and the $\dot{V}O_{2\ max}$ values observed in the present study were typically lower than those reported in trained groups (Armstrong and Barker, 2011).

Previous studies with healthy adults report that FMD either increases (Johnson et al., 2012, Tyldum et al., 2009), decreases (Dawson et al., 2013, Johnson et al., 2012) or remains unaltered (Dawson et al., 2008, Rognmo et al., 2008) after a single bout of exercise, however these data are difficult to interpret due to inconsistencies in the intensity, duration and modality of exercise, and the timing of the FMD measurement(s) (Dawson et al., 2013). The present study is the first to incorporate a work-matched exercise protocol in order to isolate the influence of exercise intensity on vascular function in adolescents, and demonstrate that FMD is increased one hour after HIIE but remains unaltered after MIE. In contrast, an exercise intensity dependent decrease in FMD has been shown immediately after cycling in adults (Birk et al., 2013), and exergaming in children (Mills et al., 2013). It is likely that this disparity is due to the timing of the FMD measure (one hour vs. immediately after exercise) as the FMD response post exercise is biphasic in nature (Dawson et al., 2008). Indeed, it is thought that the temporary blunting of FMD observed after high-intensity, but not MIE (Birk et al., 2013, Johnson et al., 2012, Mills et al., 2013), is the stimulus for subsequent improvements in FMD (Padilla et al., 2011), however no study has yet identified the time course of the FMD response following work-matched exercise in adolescents.

Changes in FMD after exercise have been attributed to differences in baseline arterial diameter and shear rate (Dawson et al., 2013). However, these remained unaltered between trials in the present study and there was no relationship between the magnitude of the FMD response and SR_{AUC}, which is consistent with existing data in children (Thijssen et al., 2009a) and following exercise in adults (Llewellyn et al., 2012). However, shear stress was not quantified during the exercise bouts. Given that the exercise trials were work-matched, it is likely that the disparate responses in FMD observed post exercise are related to the positive association between brachial artery shear and the intensity of cycling exercise (Green et al., 2002, Thijssen et al., 2009b). This has been shown to play a leading role in modulating the post exercise FMD response (Tinken et al., 2009, Tinken et al., 2010), probably due to an upregulation in eNOS and subsequent increase in the bioavailability of NO (Jenkins et al., 2012). It is not possible to partition out the influence of the high fat meal on the postprandial FMD response following MIE and HIIE. For example, it is possible that postprandial FMD could have been higher still following HIIE. However, considering that FMD has been demonstrated to return to baseline 2 hours post high-intensity exercise (Johnson et al., 2012), and the lack of change in [TAS] in the present study, it would appear that the inclusion of a high fat meal 1 hour after exercise did not modulate the post exercise NO bioavailability. Further study is needed to confirm this.

6.4.2 Microvascular function

A novel feature of this investigation was the simultaneous assessment of microvascular function during the FMD protocol. Whilst the endothelium only plays a part of the PRH response (Cracowski et al., 2006), impaired microvascular reactive hyperaemia is associated with elevated blood pressure (Serne et al., 2001), obesity (de Jongh et al., 2004), insulin resistance (Jaap et al., 1994), and has been identified in healthy children with clustered CVD risk factors (Khan et al., 2003). Therefore, it follows that the assessment of PRH as a surrogate of microvascular function in the current study may provide useful information regarding vascular health in asymptomatic individuals. A significant impairment in postprandial PRH was observed in CON, suggesting that a fatty

meal presents a global challenge to the vasculature. This dysfunction was prevented in both exercise trials, but not in an intensity-dependent manner. No other study has identified the effect of exercise intensity on subsequent postprandial microvascular function, however Gill *et al.* (2004) observed a similar protective effect of MIE performed the evening before a high fat meal in adults and this was endothelium-dependent.

Interestingly, the total reactive hyperaemic response was not lowered by the high fat meal in CON. Given that this microvascular outcome is not mediated by NO (Wong et al., 2003), it suggests that a reduction in NO bioavailability might be responsible for at least part of the attenuation in PRH in CON. Furthermore, these data indicate that prior HIIE may improve microvascular endothelial function beyond an increase in eNOS activity.

6.4.3 Postprandial lipaemia

Prior MIE (Tolfrey et al., 2008) and HIIE (Thackray et al., 2013) can attenuate postprandial lipaemia in adolescents boys. Additionally, Chapter 4 demonstrates that MIE and HIIE performed 1 hour prior to an identical high fat meal can lower postprandial lipaemia in adolescent girls. However these findings were not replicated in the present study, possibly due to the use of a one day protocol (Zhang et al., 1998) and a short (three hour) postprandial observation period. It has been hypothesised that exercise-induced changes in VLDL output may explain some of the reduction in postprandial lipaemia after a high fat meal (Magkos et al., 2006), particularly when the time between exercise cessation and consumption of the test meal is short due to the delay in the upregulation of lipoprotein lipase (Seip and Semenkovich, 1998). These data would appear to be consistent with this theory, as [3-OHB] was elevated three hours after the high fat meal in HIIE compared to CON, and significantly correlated with the reduction in TAUC-TAG, suggesting a shift towards hepatic fatty acid oxidation rather than re-esterification and VLDL synthesis during the HIIE condition (Gill et al., 2007). Thus, this data indicates that changes in VLDL-TAG metabolism may have occurred post HIIE in the girls in Chapter 4. However, it is acknowledged that plasma [3-OHB] is not a direct measure of hepatic VLDL-TAG output. Furthermore, recent evidence demonstrates that the

hydrolysis rate of VLDL is increased after exercise in adults (Ghafouri et al., 2015). Consequently, further study is necessary to elucidate the precise exercise-induced mechanisms underlying changes in postprandial lipaemia, and how they may be modulated by sex.

6.4.4 Translational perspective

This study demonstrates that exercise intensity is positively associated with postprandial macro- and micro-vascular function in adolescent boys and girls. Repeated sprint cycling the day before a high fat meal has previously been demonstrated to preserve postprandial macrovascular function in adolescents (Sedgwick et al., 2014). However, these authors reported that one third of the participants failed to complete the exercise protocol. In contrast, all participants in the present study completed the HIIE bout. Furthermore, the PACES data indicate that HIIE was perceived to be more enjoyable than MIE for both boys and girls, despite a greater physiological stress. This is encouraging considering that adolescents rarely sustain exercise for longer than 10 minutes (Riddoch et al., 2007), therefore low-volume, high-intensity exercise may be a suitable method of optimising this pattern of activity provided that the exercise is not an “all-out” effort. Further work is needed to identify the long term adherence to a HIIE training intervention in this group, however preliminary evidence is promising (Buchan et al., 2011). Indeed, these findings corroborate with Chapter 4 and add to a growing body of evidence which indicates that HIIE is a feasible and attractive alternative to MIE in adolescents (Crisp et al., 2012, Ratel et al., 2004).

6.4.5 Limitations and considerations

This is the first study to isolate the influence of exercise intensity on postprandial vascular function in adolescents. A further novelty of this study is the simultaneous assessment of microvascular function during the FMD protocol. However, these findings should be interpreted in light of a number of methodological considerations. Firstly, whilst post-occlusive reactive hyperaemia has been used as a marker of microvascular function in

adolescents (Roche et al., 2010), the mechanisms underlying the PRH response to 5 minutes of ischaemia following exercise and a high fat meal are yet to be fully determined, but likely involve other pathways in addition to changes in endothelial function (Cracowski et al., 2006). However, postprandial microvascular function has been shown to be improved following exercise elsewhere and this was endothelium-dependent (Gill et al., 2004). Therefore, it is likely that some of the improvements observed in macrovascular endothelial function via FMD in the present study are present at the microvascular level. Secondly, this study did not control for the menstrual cycle, which has been shown to influence FMD in women (Hashimoto et al., 1995). The median stage of maturity (Tanner 4) suggests that some girls would be pre or post menarche (Baxter-Jones et al., 2005), and whilst there was no significant interaction effect of sex on macro- or micro-vascular function in the present study, further work is necessary to explicitly establish whether sex influences this outcome in adolescents and in children. Thirdly, the high fat meal used in this study has limited ecological validity but provided a metabolic challenge in accordance with Chapters 4 and 5, and other postprandial investigations with adolescents (Sedgwick et al., 2012, Tolfrey et al., 2012, Tolfrey et al., 2014). This meal also provided an average of 35 g of sugar, which could plausibly have contributed to the postprandial responses (Ceriello et al., 2002), although this is equivocal (Padilla et al., 2006). Future work is needed to identify how prior exercise can alter macro- and micro-vascular function following more habitual fat loads and feeding regimes. Finally, this study did not determine endothelial-independent function via a sublingual spray of nitroglycerin (Corretti et al., 2002), and this remains an area of future research.

6.5 Conclusion

An impairment in macro- and micro-vascular function occur in concert after a high fat meal in adolescents. This study demonstrates that postprandial vascular function can be preserved after MIE, or improved after HIIE, and these changes were not related to plasma [TAG] or [TAS]. Whilst these findings cannot be extrapolated beyond healthy adolescents, they may have clinical importance as repeat impairment in endothelial function likely plays a key role in

the development of CVD, which is known to have its origins in childhood (Stary, 1989). Future work is needed to assess the efficacy of different exercise intensities on postprandial endothelial function in adolescents with risk factors for CVD (e.g. obesity, type I diabetes). Finally, HIIE was also perceived to be more enjoyable than MIE, despite the greater physiological stress. Taken together, low-volume HIIE may be a feasible and attractive strategy to reduce CVD risk from an early age.

Chapter 7

The acute effect of exercise intensity on vascular function in adolescents

7.1 Introduction

Whilst the clinical manifestations of CVD are not detectable until adulthood, it is well established that the atherosclerotic process originates in the first decade of life (Stary, 1989). Impaired vascular function is thought to precede structural adaptations to the vessel wall (Zeiher et al., 1991), and both macro- and micro-vascular function have been shown to be impaired in asymptomatic adolescents with CVD risk factors (Celermajer et al., 1992, Khan et al., 2003). Therefore, interventions which improve vascular function in young people are warranted.

Data are available demonstrating that time spent performing vigorous-, but not moderate-, intensity physical activity is related to improved macrovascular function (Hopkins et al., 2011) and attenuated cardiometabolic risk (Carson et al., 2014) in youth. Additionally, exercise interventions have been shown to improve macrovascular function in obese adolescents (Watts et al., 2004). It has been suggested that changes in vascular function after a single exercise bout provide the foundation for these chronic adaptations (Birk et al., 2013, Dawson et al., 2013). Consequently, there is value in identifying the acute vascular responses to a single bout of exercise.

Previous studies with adults report conflicting results on the effects of acute exercise on macrovascular function, with some reporting increases (Harris et al., 2008, Johnson et al., 2012), decreases (Birk et al., 2013, Johnson et al., 2012) and no change (Birk et al., 2013) in FMD. However, differences between exercise intensities, modalities, the timing of the post exercise FMD measurement(s) (Dawson et al., 2013) and the problems associated with reporting the ratio-scaled FMD statistic (Atkinson and Batterham, 2013), limit our understanding of the FMD response to an acute bout of exercise. Currently, only one published study has assessed FMD immediately post exercise in young people (Mills et al., 2013). These authors reported that FMD immediately decreased after high-intensity, but not low-intensity, exergaming in children, and concluded that repeating high-intensity exergaming may provide a stimulus for favourable macrovascular adaptations. However, the exercise bouts were not work-matched in this study and FMD was only assessed immediately post exercise. Given that changes in vascular function within ~ 2 hours of exercise are thought to be biphasic in nature (Dawson et al., 2013), it is important to

document the time course of the change in vascular function after a single bout of exercise in youth to establish the influence of exercise intensity on the FMD response.

An impairment in microvascular reactive hyperaemia has been identified in asymptomatic children with clustered CVD risk (Khan et al., 2003) and it is thought that microvascular dysfunction may play a primary role in the pathogenesis of insulin resistance (Pinkney et al., 1997). Microvascular function has been shown to be elevated in adolescent football players compared to their untrained peers (Roche et al., 2010), however no study has identified the time course of microvascular function following exercise at different intensities in young people or adults. Furthermore, post exercise changes in microvascular reactive hyperaemia have been shown to be unrelated to FMD (Shamim-Uzzaman et al., 2002). Therefore, it is inappropriate to adopt post exercise changes in FMD as a surrogate of microvascular function.

The purpose of this investigation was to test the hypothesis that macrovascular function is immediately impaired, and then subsequently improved, following HIIE, but remains stable following a work-matched bout of MIE in adolescents. A secondary aim was to identify the effect of exercise intensity on the time course of the microvascular response following exercise.

7.2 Methodology

7.2.1 Participants

Twenty 12 to 15 year old adolescents (10 males) volunteered to take part in this study. Written participant assent and parental consent were obtained before participation in the project, which was approved by the institutional ethics committee. Exclusion criteria included the use of any medication or substance known to influence fat metabolism or vascular function.

7.2.2 Experimental overview

This study required three visits to the laboratory and included a within-measures design. All exercise tests were completed using an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands).

7.2.3 Visit 1: Maximal oxygen uptake and gas exchange threshold

Participants were habituated to the cycle ergometer before completing a combined ramp and supramaximal test to exhaustion to establish the GET and $\dot{V}O_{2\ max}$ (Barker et al., 2011) as described in Section 3.4. All exercise was performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands).

7.2.4 Visits 2 and 3: Exercise interventions

Participants completed two experimental trials, separated by approximately one week. Following a ~ 12 hour overnight fast, participants were transported to the laboratory at 08:00 and then consumed 30 g of commercially available Corn Flakes with 130 mL of skimmed milk. The macronutrient contribution of this breakfast is unlikely to have influenced endothelial function (Vogel et al., 1997).

At 08:45, participants rested in a darkened, temperature-controlled (24°C) room for 15 minutes before the simultaneous assessment of macrovascular (FMD) and microvascular (PRH and total reactive hyperaemia) function.

At 09:15, one hour after breakfast, participants completed on separate days and in a randomised order: 1) ~ 30 minutes of continuous MIE at 90% of the GET; or 2) 23 minutes of HIIE. The HIIE bout consisted of a 3 minute warm up at 20 W, followed by 8 x 1 minute intervals at 90% of the peak power determined from the ramp test to exhaustion, interspersed with 75 seconds of recovery at 20 W, before a 2 minute cool down at 20 W. The duration of the MIE trial was calculated to match the total work performed during the HIIE bout. Thus, the exercise trials were identical in design to the MIE and HIIE bouts performed in Chapters 4 and 6. RPE was measured in the final 10 seconds of exercise

(Yelling et al., 2002), and participants completed the PACES questionnaire (Motl et al., 2001) immediately after exercise cessation. After their final exercise trial, each participant was asked to identify which exercise bout they preferred.

Macro- and micro-vascular function were reassessed immediately after exercise cessation, with further measures 1 and 2 hours post exercise to facilitate comparison between extant literature in adults (Dawson et al., 2013). Participants remained seated and were inactive at all times other than during the exercise bouts.

7.2.5 Measures of vascular function

Flow mediated dilation and SR_{AUC} were measured as described in Section 3.10.1, and in accordance with recent guidelines (Thijssen et al., 2011) and Chapter 6. The between-trial coefficient of variation for FMD was 9.7%.

During the FMD protocol, microvascular function was simultaneously assessed using a laser Doppler perfusion imager (Periscan PIM II, Perimed, Järfälla, Sweden) at a reproducible point on the distal third of the forearm (Cracowski et al., 2006). Methodological details are provided in Section 3.10.2. The between-trial coefficient of variation for PRH and the total hyperaemic response was 13.3 and 21.7% respectively.

7.2.6 Standardisation of diet and physical activity

With parental supervision, participants were asked to replicate their evening meal prior to each laboratory visit. Participants also completed a food diary during the 48 hour period immediately preceding each visit, which were subsequently assessed for total energy and macronutrient intake (CompEat Pro, Nutrition Systems, UK). Participants were instructed to avoid strenuous exercise and wear a tri-axial accelerometer on their wrist (GENEActiv, Activinsights Ltd, Cambridge, UK) during the 48 hour prior to each visit. Time spent performing moderate to vigorous activity was determined using established cut points for paediatric groups (Evenson et al., 2008).

7.2.7 Statistical analyses

The primary outcome for macro-vascular function was the difference between log-transformed peak and baseline arterial diameter, adjusted allometrically for baseline diameter (Atkinson and Batterham, 2013). Data were analysed using a linear mixed model with a random intercept (accounting for repeated measures within participants) plus fixed effects for condition (moderate/ high intensity), time (pre, post, 1-hour, 2-hour), and their interaction. As appropriate for a crossover trial, data were also adjusted for trial order. Differences on the log-scale were back-transformed to provide percent (ratio) effects. Point estimates are presented together with 95% CI. Additionally, the AUC for estimated shear rate was calculated from the last 30 s of occlusion until the time of peak dilation (SR_{AUC}) (Harris et al., 2010), however FMD was not related to SR_{AUC} at rest or at any point post exercise in either trial (*P* = 0.21 to 0.80, *r* = -0.1 to 0.4) which is consistent with Chapter 6 and other paediatric data (Thijssen et al., 2009a). Consequently, FMD was not normalised for SR_{AUC}.

Descriptive statistics were calculated using SPSS (version 19.0, Chicago, USA) and presented as mean ± SD. Mean differences in descriptive statistics between boys and girls were analysed using independent samples *t* tests. The mean differences in the physiological and perceptual responses of the boys and girls during HIIE and MIE were analysed using paired samples *t* tests. Parameters of macro- and microvascular function were analysed using a mixed model ANOVA with trial (MIE, HIIE) and sex (male, female) as the main effects. The inclusion of sex into the ANOVA model did not reveal a significant interaction effect for parameters of macro- and micro-vascular function. Data were subsequently pooled for these outcomes. Pairwise comparisons between means were interpreted using the *P* value, 95% CI and standardised *ES* to document the magnitude of the effect using the thresholds: small (0.2), moderate (0.5) and large (0.8) (Cohen, 1988). Relationships between changes in vascular outcomes and mechanistically important variables were explored using Pearson's correlations.

7.2.8 Power calculation

It is difficult to identify the sample size needed to appropriately power this investigation as no study has performed allometric scaling in order to partition out the confounding effect of the exercise bout on baseline diameter. However, conceptually similar studies in paediatric (Mills *et al*., 2014) and adult groups (Johnson *et al*., 2012, Birk *et al*., 2013) have identified an independent effect of exercise intensity on the acute ratio-scaled FMD response post exercise using samples sizes ranging from 10 to 15 participants. A power calculation was determined using the mean change in FMD (~5%) and standard deviation (3.5%) reported by Mills *et al*. (2014) immediately after high-intensity exergaming.

$$N = \frac{2\,(3.5)^2\,(1.96 + 0.84)^2}{5^2}$$

$$N = 8$$

7.3 Results

Baseline participant characteristics are presented in Table 7.1. The maturation status for boys and girls was as follows; Tanner stage 2, *n*=1 and *n*=0; stage 3, *n*=3 and *n*=0; stage 4, *n*=5 and *n*=7; stage 5, *n*=1 and *n*=3. No differences in energy intake, individual macronutrient contributions, or time spent performing moderate to vigorous physical activity were apparent for boys or girls during the 48 hour preceding each laboratory visit ($P>0.50$, $ES<0.20$; Table 7.2).

Table 7.1 Participant characteristics

	Boys (*n* = 10)	Girls (*n* = 10)	95% CI	*ES*
Age (y)	14.1 ± 0.3	14.1 ± 0.3	-0.3 to 0.4	0.00
Body mass (kg)	61.6 ± 15.9	54.9 ± 4.6	-4.9 to 18.3	0.57
Stature (m)	1.66 ± 0.10	1.65 ± 0.08	-0.08 to 0.09	0.11
$\dot{V}O_{2\,max}$ ($L \cdot min^{-1}$)	2.77 ± 0.80	2.04 ± 0.36	0.13 to 1.34	1.18
$\dot{V}O_{2\,max}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	44.8 ± 6.4	37.1 ± 5.3	2.2 to 13.2	1.26
GET ($L \cdot min^{-1}$)	1.36 ± 0.35	1.08 ± 0.17	0.02 to 0.54	1.02
GET (% $\dot{V}O_{2\,max}$)	49 ± 4	53 ± 6	-9 to 1	0.78

$\dot{V}O_2$, oxygen uptake; GET, gas exchange threshold; 95% CI, 95% confidence interval for the true difference; *ES*, effect size. Data presented as mean ± SD.

Table 7.2 Accelerometer and food diary data during the 48 hours preceding each trial

	MIE	HIIE	95% CI	*ES*
Moderate-vigorous activity ($min\ day^{-1}$)	38 ± 12	36 ± 15	-9 to 18	0.15
Total energy intake ($kcal\ day^{-1}$)	1945 ± 301	1887 ± 341	-163 to 279	0.18
Energy from carbohydrates (%)	47 ± 5	47 ± 5	-4 to 4	<0.01
Energy from fat (%)	38 ± 4	38 ± 6	-4 to 4	<0.01
Energy from protein (%)	15 ± 4	15 ± 3	-2 to 2	<0.01

MIE, moderate-intensity exercise trial; HIIE, high-intensity interval exercise trial; *ES*, effect size. 95% CI, 95% confidence limits for the true difference. Data have been pooled as ANOVA analysis revealed no main effect for sex

7.3.1 Exercise trials

The physiological and perceptual data from the exercise trials are presented in Table 7.3. All participants completed both exercise trials. The highest $\dot{V}O_2$ achieved during the HIIE condition equated to 96 ± 5%. Average length of the MIE trial was 25.8 ± 2.1 minutes. Nine boys and eight girls indicated that they preferred the HIIE exercise bout.

Table 7.3 Physiological and perceptual responses to MIE and HIIE

	MIE	HIIE	95% CI	ES
Mean HR ($b \cdot min^{-1}$)*	129 ± 14	150 ± 14	17 to 25	1.50
Mean HR (% HR_{max})*	66 ± 6	77 ± 6	9 to 13	1.83
Mean $\dot{V}O_2$ ($L \cdot min^{-1}$)	1.19 ± 0.26	1.49 ± 0.37	0.23 to 0.39	0.94
Mean $\dot{V}O_2$ (% $\dot{V}O_{2\,max}$)	51 ± 8	63 ± 7	10 to 15	1.60
RER	0.91 ± 0.05	1.03 ± 0.06	0.10 to 0.15	2.17
RPE	4 ± 2	7 ± 1	2 to 4	1.90
PACES	57 ± 9	65 ± 7	5 to 13	0.99
Work performed (kJ)	117 ± 18	117 ± 18	-	-
Energy Expenditure (kJ)	770 ± 182	-	-	-

HR, heart rate; $\dot{V}O_2$, oxygen uptake; RER, respiratory exchange ratio; RPE, rate of perceived exertion; PACES, physical activity enjoyment scale; MIE, moderate-intensity exercise trial; HIIE, high-intensity exercise trial; 95% CI, 95% confidence interval for the true difference; *ES*, effect size. Data presented as mean ± SD and pooled for sex. n = 20 apart from * where n = 18 due to loss of telemetry

7.3.2 Macrovascular function

Baseline arterial diameter, SR_{AUC} and FMD are illustrated in Figure 7.1. There was a main effect of time (P<0.001), but not trial (P=0.68), or time by trial interaction (P=0.09) for baseline arterial diameter. Baseline arterial diameter was greater immediately after exercise compared to pre exercise values in MIE (P=0.03, 95% CI 0.01 to 0.22, *ES*=0.32) and HIIE (P=0.01, 95 CI 0.05 to 0.35, *ES*=0.51). Baseline diameter was not different from pre exercise values at any other point in either trial (P>0.21, *ES*<0.20 for all).

There was a main effect of time (P<0.001), but not trial (P=0.28), or time by trial interaction (P=0.75) for SR_{AUC}. Pairwise comparisons revealed that SR_{AUC} was elevated immediately after exercise compared to baseline in MIE (P<0.001,

95% CI 206 to 564, *ES*=1.20) and HIIE (*P*=0.001, 95% CI 205 to 704, *ES*=1.31). There was also a trend for SR_{AUC} to be greater 1 hour after MIE (*P*=0.06, 95% CI -10 to 358, *ES*=0.55) and HIIE (*P*=0.08, 95% CI -27 to 394, *ES*=0.64) compared to baseline. SR_{AUC} was not different from baseline 2 hours after exercise for either trial (*P*>0.14, *ES*<0.36 for both).

A time by trial interaction was present for FMD (*P*<0.001). No differences in mean FMD at baseline were apparent between trials (*P*=0.62, 95% CI -1.2 to 0.7, *ES*=0.12). Compared to baseline, FMD was attenuated immediately after HIIE (*P*<0.001, 95% CI -4.4 to -2.3, *ES*=1.20), but was unchanged immediately following MIE (*P*=0.28, 95% CI -1.5 to 0.4, *ES*=0.26). Consequently, FMD was lower in HIIE compared to MIE immediately post exercise (*P*<0.001, 95% CI -3.4 to -1.6, *ES*=1.57). FMD was not different from baseline 1 hour (*P*=0.67, 95% CI -0.8 to 1.2, *ES*=0.10) and 2 hours (*P*=0.72, 95% CI -0.8 to 1.1, *ES*=0.08) after MIE, however FMD was greater than baseline after HIIE at these time points (*P*<0.001, 95% CI 1.7 to 3.7, *ES*=1.33 and *P*<0.001, 95% CI 1.8 to 3.7, *ES*=1.36, respectively). Consequently, FMD was greater in HIIE compared to MIE 1 hour (*P*<0.001, 95% CI 1.8 to 3.8, *ES*=1.31) and 2 hours (*P*<0.001, 95% CI 1.8 to 3.8, *ES*=1.33) post exercise. Changes in FMD post exercise were not related to age, maturity (Tanner stage) or aerobic fitness in either MIE or HIIE (*r*<0.43 and *P*>0.10 for all).

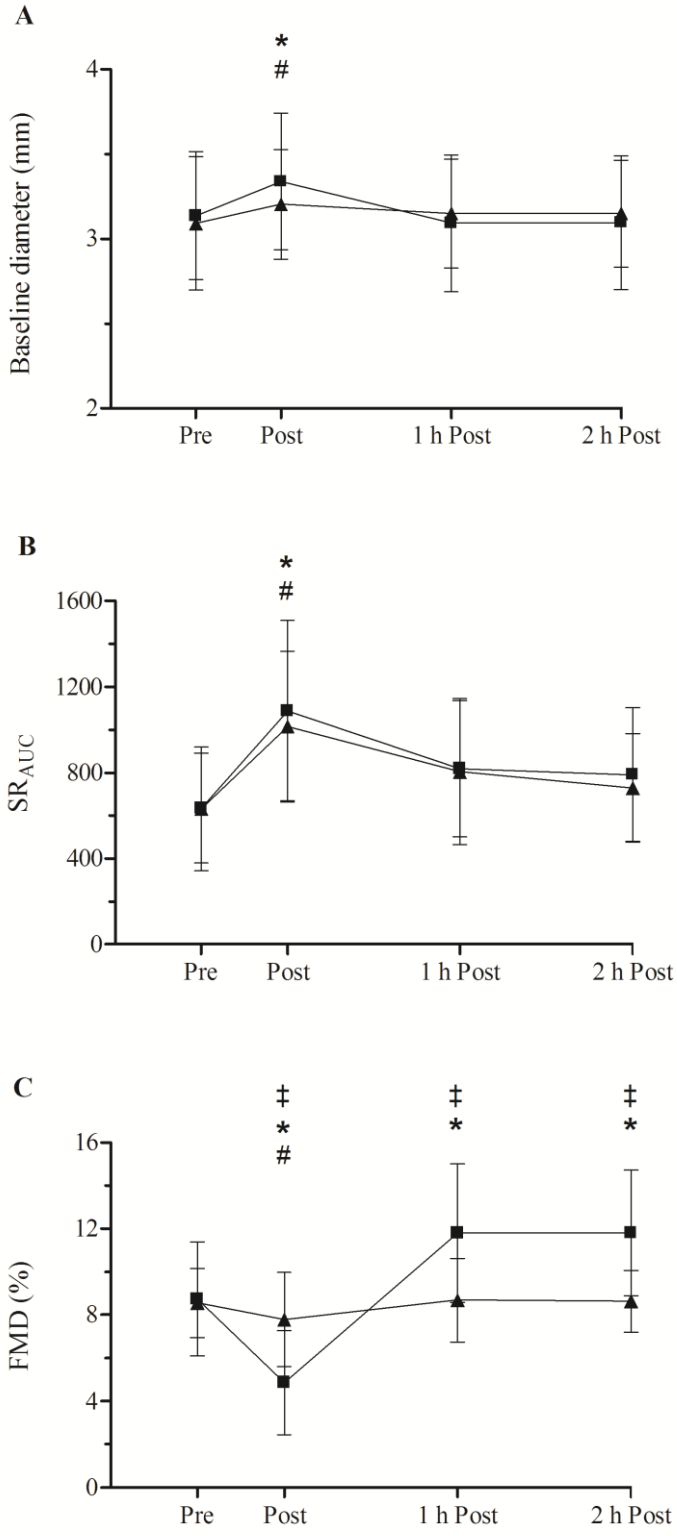

Figure 7.1 Mean baseline arterial diameter (A), area under the curve for shear versus time (SR_{AUC}; B) and flow mediated dilation (FMD; C) pre and post moderate-intensity exercise (▲) and high-intensity interval exercise (■). Error bars represent the standard deviation. Significant difference from pre exercise is denoted by $^{\#}$ for moderate-intensity exercise and * for high-intensity interval exercise. ‡ denotes significant difference between exercise trials. Refer to text for specific *P* values.

7.3.3 Microvascular function

Differences in parameters of microvascular function are presented in Figure 7.2. There was a main effect of trial (*P*=0.002) and time (*P*<0.001) for PRH, but no time by trial interaction (*P*=0.14). There were no differences between trials in mean PRH at baseline (*P*=0.51, 95% CI -0.18 to 0.09, *ES*=0.12). Compared to baseline, PRH increased immediately after MIE (*P*=0.048, 95% CI 0.02 to 0.46, *ES*=0.72) and HIIE (*P*<0.001, 95% CI 0.26 to 0.61, *ES*=1.16). PRH was greater in HIIE compared to MIE immediately after (*P*=0.02, 95% CI 0.05 to 0.44, *ES*=0.73) and 1 hour after exercise (*P*=0.002, 95% CI 0.13 to 0.48, *ES*=0.67). There was also a trend for PRH to be greater in HIIE 2 hours after exercise (*P*=0.08, 95% CI -0.03 to 0.42, *ES*=0.43).

There was a main effect of trial (*P*=0.01) and time (*P*<0.001) for the total hyperaemic response, but no time by trial interaction (*P*=0.17). There were no differences in total hyperaemic response between trials at baseline (*P*=0.65, 95% CI -28 to 18, *ES*=0.12). Compared to baseline, the total hyperaemic response was greater at all times after MIE (*P*<0.02 and *ES*>0.60 for all) and HIIE (*P*<0.001 and *ES*>1.18 for all). The total hyperaemic response was greater in HIIE compared to MIE immediately after (*P*=0.03, 95% CI 3 to 57, *ES*=0.67) and 1 hour after exercise (*P*=0.01, 95% CI 12 to 72, *ES*=0.62), with a strong trend for a statistical difference 2 hours after exercise (*P*=0.06, 95% CI -1 to 56, *ES*=0.45).

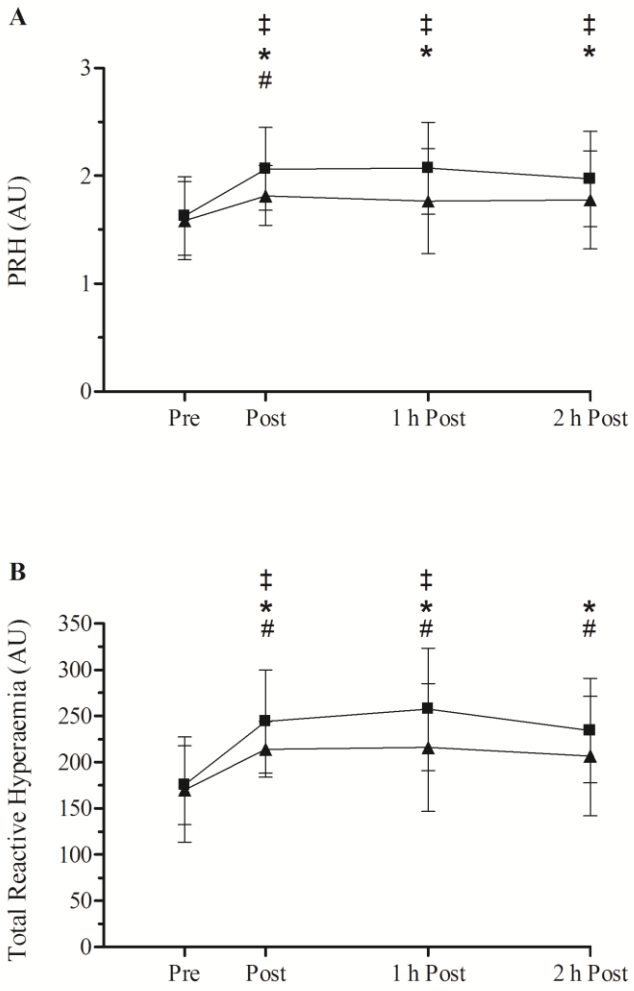

Figure 7.2 Mean peak reactive hyperaemia (PRH; A) and total reactive hyperaemia (B) pre and post moderate-intensity exercise (▲) and high-intensity interval exercise (■). Error bars represent the standard deviation. Significant difference from pre exercise is denoted by # for moderate-intensity exercise and * for high-intensity interval exercise. ‡ denotes significant difference between exercise trials. Refer to text for specific *P* values.

7.4 Discussion

The purpose of this investigation was to establish the effect of exercise intensity on macro- and micro-vascular function in adolescents, and to document the time course of the response. The novel findings from this study are: compared to baseline, 1) FMD is attenuated immediately following a single bout of HIIE but not MIE; 2) FMD is elevated 1 and 2 hours after HIIE, but unchanged in MIE; 3) PRH and total hyperaemic response are both increased during the 2

hours immediately following MIE and HIIE, and the magnitude of this increase is greater after HIIE than MIE. This is the first study to isolate the effect of exercise intensity and include serial measures of vascular function in adolescents after a single bout of exercise. The findings indicate that exercise intensity has an independent effect on macro- and micro-vascular function in young people, which likely have important implications for vascular health.

7.4.1 Macrovascular function

This study demonstrates that an immediate post exercise nadir in FMD is present following HIIE but not MIE, which is consistent with work-matched data in adults (Birk et al., 2013, Johnson et al., 2012) and the only available data in young people (Mills et al., 2013). Mills *et al*. (2013) hypothesised that this attenuation in FMD after high-intensity exercise might precede an increase in FMD, and might therefore be considered to be beneficial. However, these authors did not include serial measures of FMD in their investigation, and evidence of this response in endothelial function post exercise is scarce (Johnson et al., 2012). Furthermore, the "high-intensity" exergaming trial included by Mills *et al.* (2013) elicited a mean $\dot{V}O_{2\ peak}$ of 3.6 ± 2.5 metabolic equivalents, which the authors correctly classify as moderate-intensity (Norton et al., 2010). Therefore, the present study extends the work by Mills *et al.* and is the first to confirm that the initial impairment in FMD following high-intensity exercise precedes an increase in macrovascular function, and that this improvement is present at least two hours later. Thus, exercise which elicits a greater acute challenge on the vasculature may be associated with larger increases in FMD in adolescents, and the evidence of a biphasic response in FMD post high-intensity exercise is compelling.

The failure to observe any changes in FMD immediately after MIE is consistent with the data provided by Mills *et al*. (2013) following "low-intensity" exergaming, however this study extends their findings and report that endothelial function remained unchanged during the 2 hours that followed. Interestingly, the lack of change in FMD in the hours after MIE is consistent with some (Johnson et al., 2012, Birk et al., 2013), but not all (Harris et al., 2008, Tyldum et al., 2009) data in healthy adults. However, in addition to differences in exercise stimulus, timing

of the FMD measurement and interpretation of the ratio-scaled FMD statistic (Atkinson and Batterham, 2013, Dawson et al., 2013), an independent effect of training status (Harris et al., 2008) has been observed on the acute FMD response. Furthermore, evidence suggests that age might modulate vascular reactivity to the FMD protocol (Thijssen et al., 2009a). Although the data is unable to confirm a potential confounding effect of age, maturity (Tanner stage) or aerobic fitness on the change in FMD post MIE and HIIE, it appears that a direct comparison between the findings of the present study with apparently healthy adolescents and the available adult literature may be problematic.

Shear (when expressed as SR_{AUC}) is thought to be the main stimulus underlying the FMD response in healthy adults at rest (Pyke and Tschakovsky, 2007). However, the relationship between SR_{AUC} and FMD is not as robust following exercise (Llewellyn et al., 2012). Indeed, FMD remained elevated in the hours following HIIE despite a steady decline in SR_{AUC}. The relationship between SR_{AUC} and FMD has been shown to be weak in young people even at rest (Thijssen et al., 2009a), a finding also observed in this study. It is therefore not surprising that differences in the FMD response 1 and 2 hours post exercise were independent of changes in SR_{AUC}. Considering that baseline arterial diameter remained unchanged 1 and 2 hours following MIE and HIIE, and that data were analysed using recent statistical guidelines designed to partition out the influence of vessel calibre (Atkinson and Batterham, 2013), these findings are also not explained by this factor. It is therefore not possible to identify the mechanism(s) underlying the disparity in FMD response presented here.

It has been speculated elsewhere that the initial impairment in FMD immediately following exercise relates to an increase in oxidative stress (Dawson et al., 2013, Johnson et al., 2012), which would reduce the bioavailability of nitric oxide (Cai and Harrison, 2000). Whilst this outcome was not measured, an increase in oxidative stress following high-intensity exercise is not consistent with the augmented FMD response observed 1 and 2 hours after HIIE. Conversely, an exercise-intensity dependent increase in total antioxidant status has been reported during the hours following work-matched HIIE but not MIE (Tyldum et al., 2009), which would prevent the reduction in nitric oxide bioavailability associated with an increase in exercise-induced oxidative stress. However, this is not a consistent finding (Harris et al., 2008, Johnson et al.,

2012), and the changes in FMD 1 hour after identical HIIE in adolescents in Chapter 6 were not related to total antioxidant status. Alternatively, given that the exercise bouts were work-matched in the present study, these data may be explained by a positive association between the intensity of exercise and subsequent activity of endothelial nitric oxide synthase. Indeed, data in adults demonstrate that brachial artery shear increases with the intensity of cycling exercise (Thijssen et al., 2009b), and this has been demonstrated to play a leading role in the post exercise FMD response (Tinken et al., 2009). We did not quantify brachial artery shear during the exercise bouts as this is technically challenging during HIIE. However, Chapter 4 identified a reduction in postprandial systolic blood pressure in the 5 hours after HIIE, but not MIE, in adolescents, which would be consistent with an upregulation in endothelial nitric oxide synthase activity.

An interesting finding of the present study is that the magnitude of the increase in FMD observed 1 hour after HIIE was also present after 2 hours. Further study is needed to identify the precise decay in this favourable response after high-intensity exercise, although this benefit has been reported the following day in adults (Tyldum et al., 2009). Additionally, Chapter 6 demonstrates that a similar increase in FMD is present 4 hours after exercise despite the consumption of a meal which impaired FMD in a non-exercise control trial, whilst Sedgwick *et al.* (2014) reported an increase in postprandial FMD the day after repeated sprint cycling in adolescent boys. Therefore, a single bout of HIIE appears to provide a potent stimulus for macrovascular health, and may provide superior health benefits compared to MIE if repeated on a regular basis. Indeed, high-intensity interval training has been demonstrated to be more effectual in promoting macro-vascular function than moderate-intensity training in adults at risk of vascular dysfunction (Tjonna et al., 2008), and offer superior improvements in FMD than a multi-disciplinary approach in overweight adolescents (Tjonna et al., 2009). Furthermore, only time spent performing vigorous-, but not moderate-, intensity exercise is related to vascular function in children (Hopkins et al., 2011).

7.4.2 Microvascular function

A novel feature of this investigation was the simultaneous assessment of post-occlusive reactive hyperaemia in the cutaneous circulation (Cracowski et al., 2006) during the FMD protocol. Chapter 6 demonstrated that microvascular function is improved following both MIE and HIIE, and that the magnitude of this improvement is greater following HIIE. Furthermore, PRH and the total hyperaemic response to occlusion remained elevated 2 hours after exercise.

The data from this study show that transient improvements in microvascular function are possible following exercise without concomitant changes in FMD. No association has been demonstrated between FMD and reactive microvascular hyperaemia in adults post exercise (Shamim-Uzzaman et al., 2002), presumably because the post-occlusive cutaneous response is not mediated by nitric oxide (Wong et al., 2003). The finding that micro-, but not macro-, vascular function was improved in the hours after MIE is probably testament to the different mechanisms underlying the post-occlusive hyperaemic response in this investigation, i.e. only the latter is NO-mediated (Wong et al., 2003). Furthermore, the microvascular post-occlusive response may include both endothelial-independent and dependent pathways (Cracowski et al., 2006). It is therefore likely inappropriate to adopt measures of macrovascular health as an indication of global vascular function, especially as the earliest changes in vascular function due to the metabolic syndrome may be specifically linked to the capillary and arteriole beds, rather than the larger, conduit arteries (Pinkney et al., 1997). As a result, simultaneously assessing microvascular function alongside FMD may offer a novel insight regarding the effects of exercise intensity on vascular health.

This study is the first to show that a single bout of MIE or HIIE can improve microvascular function in the hours following exercise, and that HIIE may provide a superior benefit. Whilst this investigation is unable to identify the time course of the decay in these favourable responses post exercise, Gill *et al.* (2004) reported that endothelium-dependent microvascular function remained elevated 16-18 hours after 90 minutes of walking at 50% $\dot{V}O_{2\ max}$ in adults. Therefore, repeating a single bout of exercise may have some utility in promoting microvascular function the following day, although this needs to be

confirmed in adolescents. Conversely, there is evidence suggesting that the intensity of habitual physical activity may not influence microvascular endothelial function in adolescents (Radtke et al., 2013). However, this study determined microvascular function by means that are considered to be NO-dependent, which is mechanistically disparate from the method adopted in current investigation (Wong et al., 2003). Currently, no study has identified the efficacy of HIIE training on microvascular health in asymptomatic adolescents. Further study is therefore needed to identify whether the acute benefits in microvascular function observed in the present study translate into meaningful benefits in this group with time.

7.4.3 Translational perspective

Impairments in vascular function have been argued to be the earliest manifestation of CVD (Juonala et al., 2004, Ross, 1999) and the metabolic syndrome (Pinkney et al., 1997). Changes in vascular function after a single exercise bout may provide the foundation for chronic adaptations (Birk et al., 2013, Dawson et al., 2013). Therefore, the finding that a single bout of HIIE promotes superior improvements in macro- and micro-vascular function in the hours after exercise compared to MIE may have importance regarding the primary prevention of these diseases. Given that the HIIE was deemed to be more enjoyable than the MIE bout (which is consistent with Chapters 4 and 6), repeating HIIE may be more popular and effectual than MIE for the promotion of macro- and micro-vascular health in adolescents.

7.4.3 Limitations and considerations

This is the first study to isolate the effect of exercise intensity on vascular function in adolescents. The strengths of this investigation include a work-matched design, control of prior physical activity and dietary factors, serial measures of macro- and micro-vascular function and allometric scaling of the FMD statistic. However, apart from reporting SR_{AUC} and baseline arterial diameter, this study is not able to provide any mechanistic data which could potentially explain the changes in vascular function following MIE and HIIE. A

further limitation is that it was not possible to measure the time course of these changes beyond 2 hours post exercise. Thus, the rate of decay in microvascular function following MIE and HIIE, and macrovascular function following HIIE remains unknown. It also cannot be ruled out that an increase in skin temperature following exercise influenced the measurement of microvascular function. However, this unavoidable confounding effect is likely limited to the time point immediately post exercise as participants were acclimatised to the temperature-controlled (24°C) room for all other vascular measures. Furthermore, the analysis of the post-occlusive reactive hyperaemic response accommodates differences in baseline perfusion (Wong et al., 2003). Finally, this study cannot partition out the possible interaction between exercise intensity and diurnal variation in FMD. Data in adults suggests that FMD could decline by ~ 1% from baseline values over the course of the measurement period adopted in the present study (Ringqvist et al., 2000). However, the magnitude of this effect is far lower, and in the opposite direction, than the change observed following HIIE in the present study.

7.5 Conclusion

These data indicate that the intensity of exercise has an independent effect on macro- and micro-vascular function in adolescents. Specifically, macrovascular function was improved in the hours after HIIE but not MIE. Additionally, both exercise bouts enhanced microvascular function, although the magnitude of this increase was greater after HIIE. Therefore, it is likely that repeating high-intensity exercises may provide superior health benefits and lower cardiovascular disease risk than moderate-intensity activities. Given that HIIE was deemed to be more enjoyable than MIE, HIIE may provide an attractive, alternative to traditional MIE.

Chapter 8

Two weeks of high-intensity interval training on novel and traditional CVD risk factors in adolescents

8.1 Introduction

Whilst clinically overt CVD is not apparent until later life, the atherosclerotic process is known to originate in childhood (Stary, 1989) and the progression of this disease is related to CVD risk factor status in youth (Berenson et al., 1998, Mahoney et al., 1996). Furthermore, clustering of these risk factors track into adulthood (Bao et al., 1994, Katzmarzyk et al., 2001). Consequently, interventions which modify CVD risk factors in the first two decades of life are warranted for the primary prevention of CVD across the lifespan (McGill et al., 2000).

Physical activity reduces the clustering of traditional CVD risk factors in adolescents independently of sedentary time (Ekelund et al., 2012). Despite this, few adolescents meet the current recommended minimum of 60 minutes of moderate-intensity physical activity per day (Riddoch et al., 2007), and interventions only have a small effect on increasing physical activity levels in paediatric groups (Metcalf et al., 2012). Encouragingly, recent observational studies indicate that accumulating small volumes (~ 7 minutes) of vigorous physical activity can reduce CVD risk in adolescents (Carson et al., 2014, Hay et al., 2012). Additionally, 2 weeks of low-volume, HIIT can improve aerobic fitness in adolescents (Barker et al., 2014), which is associated with a reduced risk of cardiovascular events in later life (Hogstrom et al., 2014). Furthermore, 7-12 weeks of HIIT can improve traditional CVD risk factors in adolescents (Logan et al., 2014). However, evidence indicates that favourable changes in traditional CVD risk factors with exercise may only account for ~ 60% of the reduction in CVD risk (Mora et al., 2007), and it is thought that improvements in endothelial function and cardiac autonomic modulation (HRV) may account for some of this “risk factor gap” (Green et al., 2003, Joyner and Green, 2009).

An impairment in endothelial function is present in asymptomatic children and adolescents with CVD risk factors (Celermajer et al., 1992), and is considered to be a sentinel event in the progression of atherosclerosis (Juonala et al., 2004). Poor autonomic function is also associated with clustered CVD risk in adolescence (Farah et al., 2014). It has been demonstrated that time spent performing high-intensity activities are most important for promoting vascular health (Hopkins et al., 2009) and autonomic function (Buchheit et al., 2007) in

children. Chapters 6 and 7 also demonstrate that a single bout of HIIE acutely promotes superior vascular health benefits than an equivalent MIE bout in adolescents. Therefore, performing small volumes of HIIE might provide a time efficient strategy for the modification of both traditional and novel CVD risk factors in adolescents.

Current understanding of how HIIT influences traditional and novel CVD risk factors in adolescents is limited to fasting measures, but adolescents may spend up to two thirds of the day in a postprandial state. As such, it is important to assess CVD risk factors after a meal. Indeed, elevated non-fasting [TAG] in youth predict future CVD (Morrison et al., 2009). Additionally, the consumption of a high fat meal promotes a transient impairment in endothelial function (Vogel et al., 1997), which is a pre-requisite for the development of fatty streaks (Juonala et al., 2004), and lowers HRV (Charlot et al., 2011). HIIT has been shown to attenuate postprandial lipaemia in at-risk adult groups (Freese et al., 2015). However, the reduction in postprandial lipaemia following 6 weeks of HIIT is comparable to that observed after a single bout of high-intensity exercise (Freese et al., 2015) indicating that this benefit is related to the last training session, rather than a chronic adaption *per se*. No data are available identifying whether this is also the case for endothelial function or HRV in apparently healthy adolescents.

Given the above, the primary purpose of this study was to test the hypothesis that 2 weeks of HIIT can improve traditional and novel CVD risk factors in both the fasted and postprandial state in adolescents. A secondary aim was to determine whether any benefits observed the day after training cessation were present three days after the last training bout.

8.2 Methodology

8.2.1 Participants

Sixteen 13 to 14-year-olds (7 girls) volunteered to take part in this study. Participant assent and parental consent were provided before participation in the project, which was approved by the institutional ethics committee. Exclusion criteria included any contraindications to exercise and the use of medications or

substances known to influence blood pressure, cholesterol or carbohydrate and fat metabolism. One boy and one girl failed to complete the HIIT protocol due to illness. A further boy did not complete the HIIT due to an unrelated injury. Thus 13 adolescents (6 girls) completed this investigation.

8.2.2 Experimental overview

Participants were transported to the laboratory on 4 separate occasions over a 3 week period. Visits 1 and 2 consisted of the pre-training outcome measures (PRE). Subsequently, participants completed six sessions of supervised HIIT, before completing the post-training measures the day after the last training session (visit 3, POST-1D) and three days after training cessation (POST-3D).

8.2.3 Visit 1: Anthropometric measures, maximal oxygen uptake and gas exchange threshold

Stature, body mass and somatic maturity were measured as described in Section 3.3. Participants completed a combined ramp and supramaximal test to exhaustion to establish $\dot{V}O_{2\,max}$ (Barker et al., 2011; Section 3.4).

8.2.4 Visits 2, 3 and 4: Pre and post training outcomes

A schematic of these visits is provided in Figure 8.1. Following a ~ 12 hour overnight fast, participants were transported to the laboratory at 07:45 and rested for 15 minutes before the assessment of SBP (Dinamap Carescape V100, GE Healthcare, USA), FMD and HRV at 08:00. Fasting capillary blood samples for plasma [TAG], [glucose], [total cholesterol (TC)], [HDL], [LDL] and [glutathione peroxidase (GTP)] were then collected. Participants then consumed a high fat and sugar meal consisting of a chocolate croissant with added chocolate spread, a chocolate muffin and a 300 mL commercially available fruit smoothie with 50 mL added double cream. This meal was consumed within 15 minutes and provided approximately 68 g of fat, 80 g of sugar and 7134 kJ. Plasma [TAG] and [glucose] were then assessed 30 minutes and 1, 2, 3 and 4 hours later. Plasma [GTP], FMD and HRV were re-assessed 3 hours after the test meal in order to coincide with the expected peak in plasma [TAG] based on

the findings of Chapters 4 and 6. During visit 4 only, participants repeated the combined ramp and supramaximal test to exhaustion (Barker et al., 2011) after the 4 hour postprandial observation period.

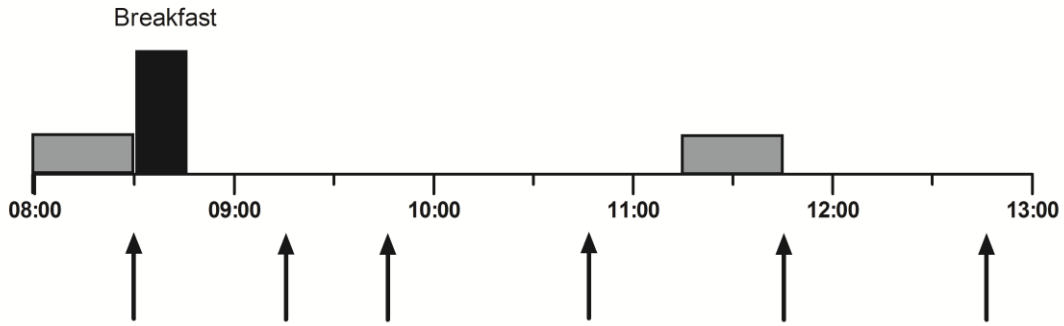

Figure 8.1 Schematic of fasted and postprandial assessments. Grey boxes indicate assessment of [glutathione peroxidase], flow mediated dilation and heart rate variability. Arrows represent capillary blood samples and assessment of systolic blood pressure. Please refer to text for precise timings of plasma [triacylglycerol], [glucose], [total cholesterol], [high-] and [low-density lipoproteins].

8.2.5 High-intensity interval training intervention

All HIIT sessions were performed using a mechanically-braked cycle ergometer (Monark 827e, Monark exercise AB, Sweden) located in a satellite laboratory at a local secondary school. Each participant completed six supervised HIIT sessions over a 2 week period. In accordance with Chapters 4, 6 and 7, the first training session included 8 repetitions of 1 minute at 90% of the peak power output determined from the prior ramp test to exhaustion, separated by 75 seconds of unloaded pedalling. The number of repetitions was increased to 9 during sessions three and four, and 10 for the remaining two sessions. A 3 minute unloaded warm up and cool down were provided. Participants cycled at a self-selected (70-95 revolutions·min^{-1}) but constant cadence and a RPE (Yelling et al., 2002) was noted after every other repetition.

8.2.6 Assessment of novel CVD risk factors

Flow mediated dilation and SR_{AUC} were measured as described in Section 3.10.1, in accordance with recent guidelines (Thijssen et al., 2011) and

Chapters 6 and 7. All analyses were performed by the primary investigator who was blinded to the measurement point.

HRV was simultaneously assessed during the FMD protocol using the time intervals between each ECG-gated image of the brachial artery (i.e. the R-R interval) during the 1.5 minutes prior to cuff occlusion. These data were screened for ectopic beats, and artefacts were removed and replaced by the mean of the adjacent beats. The root mean square of the squared differences between adjacent normal R-R intervals (RMSSD) was calculated using the Kubios HRV software (Biosignal Analysis and Medical Imaging Group, Joensuu, Finland), which has recently been used to establish reference values in adolescent boys (Farah et al., 2014).

8.2.7 Blood sampling and analyses

For each blood sample, ~ 900 µL of capillary blood was collected and centrifuged immediately at 13,000 *g* for 15 minutes at 4°C. Plasma was then removed and stored at -80°C for no more than one month, or analysed immediately for [glucose] (YS1 2300 Stat Plus Glucose and L-Lactate Analyzer, YSI Inc., Yellow Springs, USA). Plasma [TAG] and [GTP] (Cayman Chemical Company, MI, USA) were quantified in duplicate by enzymatic, colorimetric methods using an assay kit according to the manufacturer's guidelines. The within-batch coefficients of variation for plasma [TAG], [glucose], and [GTP] were 4.2, 1.4 and 3.8% respectively. [TC], [HDL] and [LDL] were assessed in capillary whole blood (CardioChek, Polymer Technology Systems, IN, USA) and the fasted TC:HDL ratio was calculated as a marker of CVD risk (Lemieux et al., 2001). Haematocrit and haemoglobin values were determined from the fasted capillary blood samples in order to calculate changes in plasma volume following HIIT (Dill and Costill, 1974).

8.2.8 Standardisation of diet and physical activity

With parental supervision, participants were asked to replicate their evening meal prior to each laboratory visit. Participants also completed a food diary during the 48 hour period immediately preceding each visit, which were

subsequently assessed for total energy and macronutrient intake (CompEat Pro, Nutrition Systems, UK). Participants were instructed to avoid strenuous exercise and wear a tri-axial accelerometer on their wrist (GENEActiv, Activinsights Ltd, Cambridge, UK) during the 48 hour prior to each visit. Time spent performing moderate to vigorous activity was determined using established cut points for paediatric groups (Phillips et al., 2013).

8.2.9 Statistical analyses

The TAUC analysis was performed using the trapezium rule (GraphPad Prism, GraphPad Software, San Diego, CA) to describe the postprandial differences in SBP and plasma [glucose], [TAG], [glucose] and [GTP] between visits. The IAUC was also calculated for plasma [TAG], [glucose] and [GTP]. All AUC analyses were calculated using the time point immediately before the test meal.

FMD was calculated as the difference between log-transformed peak and baseline arterial diameter, adjusted allometrically for baseline diameter (Atkinson and Batterham, 2013) and analysed using a linear mixed model with fixed effects for visit (PRE, POST-1D, POST-3D), time (fasted and postprandial), and their interaction. Differences on the log-scale were back-transformed to provide percent (ratio) effects and interpreted in the conventional manner.

Descriptive statistics were calculated using SPSS (version 19.0, Chicago, USA) and presented as mean ± SD. Differences between parameters of fitness and plasma volume before and after training were explored using paired samples *t* tests. Analysis of plasma [TAG], [glucose], [GTP], [TC], [HDL], [LDL], TC:HDL, blood pressure, SR_{AUC}, HRV, food diary data and time spent performing moderate to vigorous activity were initially performed using a mixed model ANOVA with visit (PRE, POST, POST-3D), time (fasted or postprandial) and sex as the main effects. However, the inclusion of sex into the ANOVA model did not reveal any significant interaction effects, so data were subsequently pooled. Pairwise comparisons between means were interpreted using the *P* value, 95% CI and standardised *ES* to document the magnitude of the effect

using the thresholds: small (0.2), moderate (0.5) and large (0.8) (Cohen, 1988). Statistical significance was accepted when $P<0.05$.

8.2.10 Power calculation

No study has identified the influence of 2 weeks of HIIE on FMD in either adult or paediatric populations. Therefore, a power calculation was based upon the pooled mean and standard deviation in FMD from chapters 6 and 7 (8.6 ± 1.6%), an alpha value of 0.05 and power set at 0.8. The equation below demonstrates that a sample size of 10 is required to detect a 2% change in FMD post training.

$$N = \frac{2\,(1.6)^2\,(1.96 + 0.84)^2}{2^2}$$

$$N = 10$$

8.3 Results

The mean stature and mass were 162.5 ± 8.0 cm and 58.0 ± 7.3 kg, respectively. Three boys and 1 girl were considered to be overweight according to age-appropriate body mass index cut points (Cole et al., 2000). The maturation status for boys and girls was as follows; Tanner stage 3, $n=2$ and $n=1$; stage, 4 $n=6$ and $n=1$; stage 5, $n=0$ and $n=5$. No differences in energy intake, individual macronutrient contributions, or time spent performing moderate to vigorous physical activity were apparent for boys or girls during the 48 hour preceding each laboratory visit ($P>0.18$, $ES<0.20$; Table 8.1). Mean RPE after the final cycling interval was 9±1 across all HIIT sessions. Plasma volume was not increased following HIIT ($P=0.11$, 95% CI 0.1 to 5.2, $ES=0.55$).

Table 8.1: Accelerometer and food diary data during the 48 hours preceding each visit

	PRE	POST-1D	POST-3D	PRE vs. POST-1D 95% CI	PRE vs. POST-3D 95% CI	POST-1D vs. POST-3D 95% CI
Moderate-vigorous activity (min day^{-1})	45 ± 30	63 ± 32	45 ± 20	-41 to 5	-23 to 22	-7 to 41
Total energy intake (kcal day^{-1})	1781 ± 449	1882 ± 324	2067 ± 270	-554 to 353	-801 to 233	-472 to 101
Energy from carbohydrates (%)	44 ± 8	48 ± 5	47 ± 9	-12 to 5	-11 to 5	-10 to 11
Energy from fat (%)	39 ± 9	36 ± 4	39 ± 5	-8 to 14	-8 to 8	-8 to 2
Energy from protein (%)	17 ± 4	17 ± 4	14 ± 4	-4 to 5	-2 to 9	-3 to 8

PRE, before intervention; POST-1D, 1 day after the intervention; POST-3D, 3 days after the intervention. 95% CI, 95% confidence limits for the true difference. Data have been pooled as ANOVA analysis revealed no main effect for sex. POST-1D includes the time spent performing the final HIIT bout (~ 27 min).

Table 8.2: Fasted and postprandial health outcomes before, 1 day after and 3 days after training

	PRE	POST-1D	POST-3D	PRE v POST-1D *ES*	PRE v POST-3D *ES*	POST-1D v POST-3D *ES*
Fasted						
SBP (mmHg)	116 ± 11	114 ± 9	116 ± 10	0.20	0.00	0.21
TAG (mmol·L^{-1})	0.37 ± 0.18	0.34 ± 0.07	0.40 ± 0.10	0.22	0.21	0.70
Glucose (mmol·L^{-1})	5.05 ± 0.21	5.05 ± 0.31	5.05 ± 0.21	0.00	0.00	0.00
GTP (mmol·L^{-1})	100.3 ± 19.2	95.6 ± 20.5	93.8 ± 17.6	0.24	0.35	0.09
TC (mmol·L^{-1})	3.88 ± 0.81	3.81 ± 0.66	3.87 ± 0.79	0.09	0.01	0.08
HDL (mmol·L^{-1})	1.32 ± 0.35	1.38 ± 0.36	1.42 ± 0.42	0.17	0.26	0.10
LDL (mmol·L^{-1})	3.09 ± 1.13	2.93 ± 0.98	2.86 ± 0.90	0.15	0.23	0.07
TC:HDL	3.00 ± 0.52	2.86 ± 0.60	2.84 ± 0.54	0.25	0.30	0.04
Postprandial						
TAUC-SBP (mmHg·4.25 h)	501 ± 41	497 ± 34	510 ± 32	0.11	0.24	0.39
TAUC-TAG (mmol·L^{-1}·4.25 h)	4.69 ± 3.32	4.04 ± 2.12	4.85 ± 2.30	0.23	0.06	0.37
IAUC-TAG (mmol·L^{-1}·4.25 h)	3.13 ± 2.84	2.67 ± 1.90	3.13 ± 2.00	0.19	0.00	0.24
TAUC-glucose (mmol·L^{-1}·4.25 h)	24.56 ± 1.49	24.74 ± 1.48	24.19 ± 0.94	0.12	0.30	0.44
IAUC-glucose (mmol·L^{-1}·4.25 h)	3.06 ± 1.46	3.32 ± 2.22	2.71 ± 1.40	0.14	0.24	0.33
GTP (mmol·L^{-1})	94.4 ± 25.4	91.3 ± 20.1	89.8 ± 16.3	0.14	0.22	0.08

PRE, before intervention; POST-1D, 1 day after the intervention; POST-3D, 3 days after the intervention; ES, effect size; SBP, systolic blood pressure; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TAUC, total area under the curve; IAUC, incremental area under the curve; [TAG], plasma triacylglycerol. ANOVA revealed no effect of trial for any outcome.

8.3.1 Traditional cardiovascular disease risk factors

The HIIT intervention increased peak power output by ~ 7% (217 ± 50 vs. 232 ± 51 W; *P*=0.002, *ES*=0.32) but did not improve $\dot{V}O_{2\ max}$ when expressed in absolute form (2.29 ± 0.63 vs 2.35 ± 0.63 L· min^{-1}; *P*=0.36, *ES*=0.10) or normalised for body mass (39.1 ± 9.2 vs 40.2 ± 9.3 mL· kg^{-1} · min^{-1}; *P*=0.31, *ES*=0.12).

Changes in fasted and postprandial outcomes are presented in Table 8.2. There was no effect of visit for fasted SBP (*P*=0.52), [glucose] (*P*=0.99), [TAG] (*P*=0.29), [GTP] (*P*=0.43) or TC:HDL (*P*=0.58). There was no effect of visit for TAUC-SBP (*P*=0.54), TAUC-TAG (*P*=0.22), IAUC-TAG (*P*=0.45), TAUC-glucose (*P*=0.18) or IAUC-glucose (*P*=0.65).

8.3.2 Novel cardiovascular disease risk factors

Mean differences in baseline arterial diameter, SR_{AUC} and FMD are presented in Figure 8.2. There was a visit by time interaction for baseline arterial diameter (*P*=0.05). Compared to PRE, postprandial baseline diameter was greater POST-1D (*P*=0.03, 95% CI 0.01 to 0.20, *ES*=0.39) and POST 3-D (*P*=0.01, 95% CI 0.04 to 0.21, *ES*=0.0.42), although not different between POST-1D and POST-3D (*P*=0.73, 95% CI -0.06 to 0.08, *ES*=0.04).

There was no effect of visit (*P*=0.34), time (*P*=0.15), or visit by time interaction (*P*=0.23) for SR_{AUC}. FMD was related to SR_{AUC} when fasted (*r*=0.39, *P*=0.02), but not following the test meal in any trial (*r* <0.31, *P*>0.30).

There was an effect of visit (*P*<0.001) and time (*P*<0.001), but not a visit by time interaction (*P*=0.41), for FMD. Fasted FMD was greater POST-1D compared to PRE (*P*=0.003, 95% CI 0.4 to 1.9, *ES*=0.70) and POST-3D (*P*=0.04, 95% CI 0.1 to 1.5, *ES*=0.48), with no difference between PRE and POST-3D (*P*=0.32, 95% CI -1.1 to 0.4, *ES*=0.22). The change between PRE and POST-1D fasted FMD was not related to PRE $\dot{V}O_{2\ max}$ (*r*=0.03, *P*=0.93).

Compared to the fasted measure, the test meal reduced FMD for all visits (*P*<0.001, 95% CI -2.0 to -1.1, *ES*=0.95). However, compared to PRE, postprandial FMD was greater POST-1D (*P*<0.001, 95% CI 0.9 to 2.4, *ES*=1.01)

and POST-3D (*P*=0.01, 95% CI 0.2 to 1.8, *ES*=0.60), with a trend for a difference between POST-1D and POST-3D (*P*=0.06, 95% CI 0.0 to 1.4, *ES*=0.42).

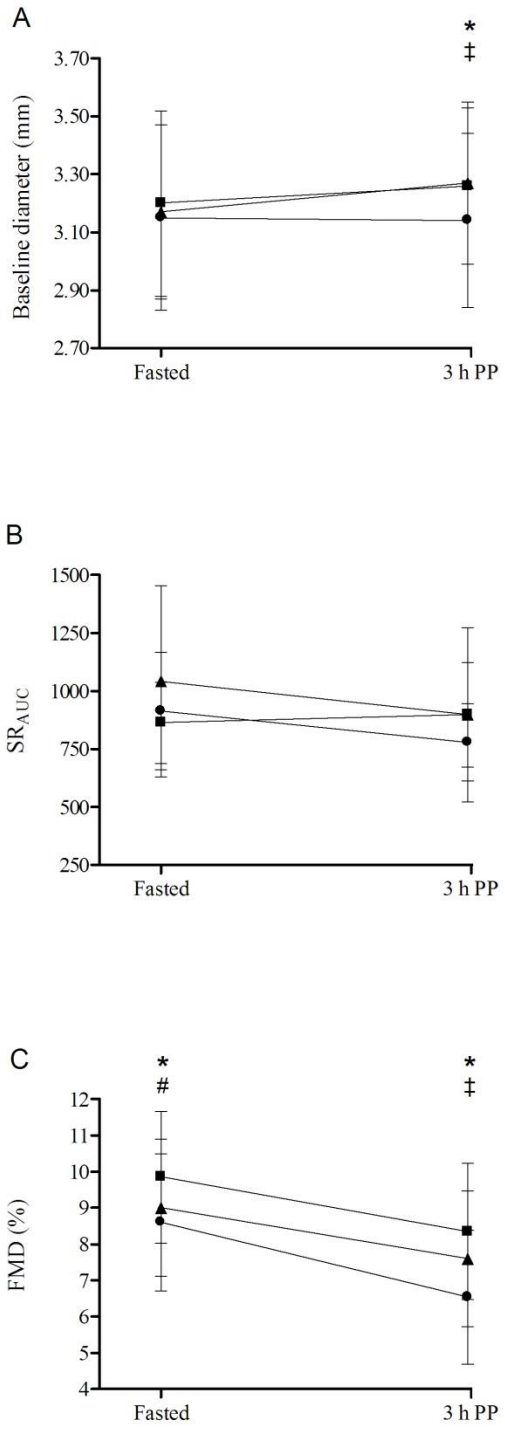

Figure 8.2 Mean baseline arterial diameter (A), area under the curve for shear (SR_{AUC}; B) and allometrically-scaled flow mediated dilation (FMD; C) before (●), 1 day after (■) and 3 days after (▲) the 2-week high-intensity interval training intervention. Error bars describe the standard deviation. 3 h PP, three hours postprandial. * *P*<0.05 for PRE vs POST-1D; ‡ *P*<0.05 for PRE vs POST-3D; # *P*<0.05 for POST-1D vs POST-3D.

Mean differences in HRV are presented in Figure 8.3. There was an effect of visit (*P*=0.02) and time (*P*<0.001), but not a visit by time interaction (*P*=0.13), for HRV. Compared to PRE, fasted HRV was greater POST-1D (*P*=0.001, 95% CI 9.1 to 27.2, *ES*=0.71) and POST-3D (*P*=0.02, 95% CI 2.5 to 20.5, *ES*=0.44), with a trend for a difference between POST-1D and POST-3D (*P*=0.08, 95% CI -0.9 to 14.2, *ES*=0.24). Compared to the fasted measure, postprandial HRV was lowered in all trials (*P*<0.001, 95% CI -20.0 to -7.9, *ES*=0.59), however there were no differences in postprandial HRV between trials (*P*>0.32 for all). Fasted HRV was not related to fasted FMD before the HIIT intervention (*r*=0.15, *P*=0.64). Changes in fasted HRV were not related to changes in fasted FMD (*r*=0.11, *P*=0.71) or plasma volume (*r*=0.01, *P*=0.98) following training.

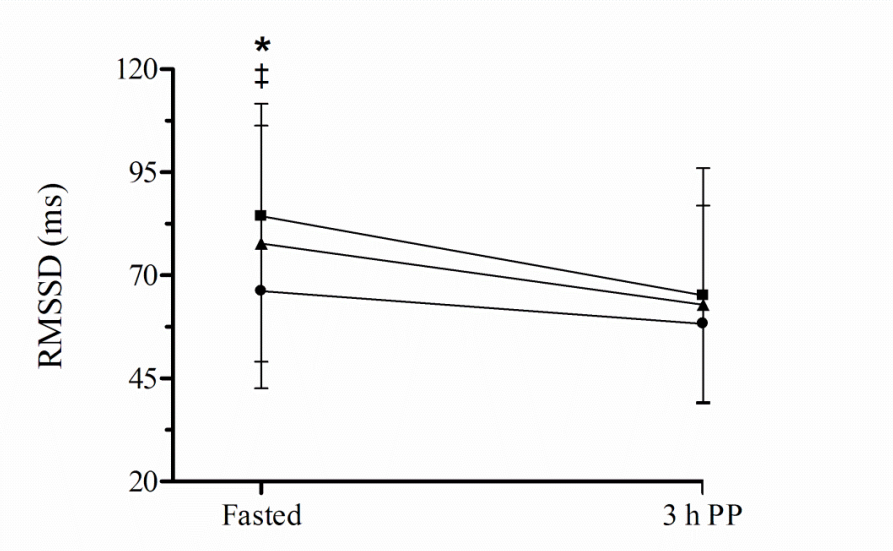

Figure 8.3 Mean heart rate variability before (●), 1 day after (■) and 3 days after (▲) the 2-week high-intensity interval training intervention. Error bars describe the standard deviation. RMSSD, root mean square of successive R-R intervals; 3 h PP, three hours postprandial. * *P*<0.05 for PRE vs POST-1D; ‡ *P*<0.05 for PRE vs POST-3D.

8.4 Discussion

This study is the first to identify the efficacy of a 2 week low-volume, HIIT intervention on traditional and novel CVD risk factors in adolescents. The key findings from this study are: 1) HIIT improved endothelial function and HRV in adolescent boys and girls; 2) improvements in novel CVD risk factors may occur in the absence of changes in traditional CVD risk factors; and 3) most of the

increase in endothelial function and HRV was lost 3 days after the HIIT intervention, indicating that the benefits of HIIT are likely related to the last training session.

8.4.1 Traditional cardiovascular disease risk factors

The present data indicate that 2 weeks of HIIT had no effect on traditional CVD risk factors or aerobic fitness. Previous studies have reported favourable changes in cholesterol (Racil et al., 2013), SBP (Corte de Araujo et al., 2012), body composition (Tjonna et al., 2008) and aerobic fitness (Corte de Araujo et al., 2012, Tjonna et al., 2009) in overweight and obese adolescents following 12 weeks of HIIT. It is possible that these effects of HIIT are reserved to adolescents with CVD risk factors (Buchan et al., 2013), however improvements in SBP and aerobic fitness have been observed following 7 weeks of HIIT in apparently healthy and normotensive adolescents (Buchan et al., 2011). Therefore, it is possible that the present intervention was of an insufficient duration to improve these health outcomes, although no study has yet identified the time course of these improvements with HIIT. However, it has previously been demonstrated that 2 weeks of repeated sprint interval training can elicit significant increases in $\dot{V}O_{2\ max}$ in adolescent boys (Barker et al., 2014). Thus it appears that improvements in fitness are achievable in 2 weeks provided that each training session requires a maximum (or “all-out”) effort.

Evidence in adults indicate that changes in traditional CVD risk factors may only account for ~ 60% of the reduction in CVD risk with exercise (Mora et al., 2007), i.e. ~ 40% of the benefit of exercise is currently unaccounted for. A strength of this study is the assessment of FMD and HRV as it has been proposed that exercise-induced improvements in endothelial function (DeSouza et al., 2000, Green et al., 2004) and autonomic function account for some of this “risk factor gap” (Green et al., 2003, Joyner and Green, 2009). Indeed, it has been demonstrated that improvements in endothelial function following exercise interventions in adults are not related to changes in traditional CVD risk factors (Green et al., 2003). Considering that CVD has its origins in youth (Stary, 1989), and that asymptomatic children and adolescents with CVD risk factors present with an impairment in endothelial function (Celermajer et al., 1992) and low

HRV (Farah et al., 2014), it is encouraging that improvements in both of these outcomes are apparent following just 2 weeks (6 sessions) of low-volume HIIT even in apparently healthy adolescents.

8.4.2 Endothelial function

The improvement in fasted endothelial function following HIIT in the present study is in agreement with cross-sectional data identifying that time spent performing higher-intensity activities is the most important predictor of vascular health in children (Hopkins et al., 2009). Given that data were allometrically scaled to partition out the influence of baseline diameter, and that no concomitant changes in SR_{AUC} or plasma [GTP] were observed, it is not possible to identify the mechanism(s) underlying the observed improvement in fasted FMD. Data from animal models demonstrate that eNOS gene expression is increased following 10 days of high-volume (2 hours per day) training (Sessa et al., 1994). It is known that the intensity of cycling exercise is positively associated with brachial artery shear (Thijssen et al., 2009), which is thought to play a leading role in the FMD response via an upregulation of eNOS activity (Tinken et al., 2009). Thus, it is plausible that just 2 weeks of HIIT is able to augment this pathway even in asymptomatic adolescents. However, ~ 65% of the improvement in fasted FMD was lost 3 days after training cessation, suggesting that most of the benefit in endothelial function may have been an acute response to the last training bout. Therefore, a single bout of high-intensity interval exercise may promote FMD for ~ 24 hours. Whilst Chapters 6 and 7 identify that endothelial function is acutely improved in the hours following a single bout of high-intensity interval exercise, the precise time course of the decay in FMD following HIIT is currently unknown, but may be rapid (Alomari et al., 2010). Consequently, it appears that regularly performing high-intensity interval exercise is important for the promotion of endothelial function in adolescents.

Atherosclerosis has long been purported to be a “postprandial phenomenon” (Zilversmit, 1979), and more recent evidence indicates that endothelial dysfunction is a requirement for the atherosclerotic progression (Juonala et al., 2004). A novelty of this study is the assessment of endothelial function following

a high fat and sugar meal, which impaired endothelial function, probably via an increase in oxidative stress (Bae et al., 2001, Ceriello et al., 2002). It has previously been demonstrated that postprandial endothelial function can be preserved the day after a single bout of sprint interval cycling in adolescent boys (Sedgwick et al., 2014), and improved following high-intensity exercise in adults (Tyldum et al., 2009). The latter study attributed this favourable response to an increase in [TAS], which would likely preserve the bioavailability of nitric oxide during the postprandial period (Cai and Harrison, 2000). In contrast, the test meal always attenuated FMD in the present study, which suggests that the bioavailability of nitric oxide was likely reduced via oxidative stress. In contrast, there were no differences in plasma [GTP] between trials. However, postprandial plasma [GTP] has recently been shown to remain unchanged after exercise (Canale et al., 2014), therefore a more comprehensive assessment of postprandial oxidative stress is warranted. Additionally, no relationship between [TAS] and postprandial FMD was apparent in Chapter 6, although the processes underlying the pro/anti-oxidant state are likely to be mechanistically different in the immediate hours post exercise. Nevertheless, postprandial FMD was greater 1 and 3 days post HIIT compared to baseline, which may suggest that the time course of the benefit of high-intensity interval exercise is different between fasted and postprandial FMD measures. Further work is needed to confirm this, however these findings are conceptually important considering the small volume of exercise performed by the adolescents, and that most of the day is spent in the postprandial state.

8.4.3 Autonomic function

The observed increase in fasted HRV is consisted with extant data following 9 weeks of HIIT in adolescents (Buchheit et al., 2008) and 2 weeks of HIIT in adults (Kiviniemi et al., 2014). Using recent HRV cut off points in adolescents (Farah et al., 2014), it is encouraging that participants improved from the 50th to the 75th percentile for the RMSSD outcome following just six sessions of HIIT. Furthermore, participants remained in the 75th percentile 3 days after training, although the magnitude of this improvement was diminished compared to the day following training cessation. Recent work in adults suggest that

improvements in autonomic function post HIIT are related to an increased vagal or baroreflex-mediated modulation of the sinoatrial node (Kiviniemi et al., 2014). Given that plasma volume did not change in the present study, these data suggest an increase in parasympathetic activity, although further work is necessary to identify the exact mechanisms underlying the improvement in HRV post HIIT. Interestingly, the RMSSD outcome has been shown to correlate with FMD in men (Pinter et al., 2012), possibly because both cardiac vagal activity (Chowdhary et al., 2000) and FMD (Green, 2005) are augmented by nitric oxide bioavailability. However, the changes in these parameters post HIIT were not related in the current study. Furthermore, there were no differences in postprandial HRV between trials, which is in contrast to the postprandial FMD data.

8.4.4 Postprandial cardiometabolic outcomes

Non fasting plasma [TAG] determined during youth may predict future CVD (Morrison et al., 2009), and a growing body of research in adolescents indicate that the lipaemic response to a high fat meal can be lowered by prior exercise (Tolfrey et al., 2014). A single bout of high-intensity interval running (Thackray et al., 2013) and sprint interval cycling (Sedgwick et al., 2014) performed the day before a test meal has been shown to attenuate postprandial lipaemia by 10% and 13% respectively in adolescent boys. Whilst a 14% reduction in TAUC-TAG was present the day after the last HIIT session, this did not reach statistical significance. However, a reduction in postprandial lipaemia the day after high-intensity interval exercise is not a universal finding (Tyldum et al., 2009). Additionally, the test meal in the present study included 80 g of sugar which may have blunted the lipaemic response (Cohen and Berger, 1990). In turn, it is plausible that this may have attenuated the magnitude of change in postprandial lipaemia following the last HIIT session.

Chapter 4 demonstrates that significant improvements in postprandial SBP are possible during the 4 hours following a single bout of HIIE. Additionally, an identical bout of HIIE can also improve glycaemic control in the hours following exercise cessation (Cockcroft et al., 2014). Given that no changes were apparent in these outcomes in the present study, it appears that these benefits

are transient and are likely to be lost the following day. Interestingly, 45 minutes of moderate-intensity exercise has been shown to improve insulin sensitivity in habitually low active adolescents 17 hours post exercise (Short et al., 2013). Further work is needed to establish the time course of the beneficial effect of a single bout of high-intensity interval exercise in this group. However, considering that even small volumes of high-intensity exercise are related to lower cardiometabolic risk (Carson et al., 2014), it is likely that repeating low-volumes of high-intensity exercise every day is important for health promotion in adolescents.

8.4.5 Translational perspective

This study demonstrates that improvements in novel, but not traditional, CVD risk factors are achievable following 2 weeks of low-volume HIIT in adolescents. These findings are pertinent given that few UK adolescents achieve the recommended minimum amount of daily exercise (Riddoch et al., 2007), and that improvements in traditional CVD risk factors may only account for ~ 60% of the benefit of exercise (Mora et al., 2007). Considering that this study is the first to comprehensively include traditional and novel CVD outcomes, other training studies which have not determined endothelial function or HRV may have "missed" some of the health benefits of exercise. Additionally, this study highlights that much of the benefit of the HIIT was lost after 3 days. This finding, in addition to the data presented in the preceding experimental chapters of this thesis, indicate that regularly performing HIIE may be an effectual, and feasible, strategy for the primary prevention of CVD.

8.4.6 Limitations and considerations

The strengths of this study include the control of diet and physical activity performed 48 hours prior to the mixed meal tolerance test, the comprehensive assessment of traditional CVD risk factors and the novel assessment of HRV using the ECG-gated images captured prior to cuff occlusion during the FMD protocol. However, this study did not control for the potential confounding influence of the menstrual cycle on FMD (Hashimoto et al., 1995), although this

is not a consistent finding regarding HRV (Leicht et al., 2003) and plasma [TAG] (Wendler et al., 1992). Additionally, this study did not include a non-exercise control group. However, this limitation is consistent with previous high-intensity training studies in adolescents (Barker et al., 2014) and adults (Whyte et al., 2010). Furthermore, the observed changes in FMD and HRV are unlikely to be influenced by maturation (Farah et al., 2014, Michels et al., 2013) or seasonal variation (Hopkins et al., 2011) during such a short time frame. It is also not possible to compare the present findings to an equivalent moderate-intensity training intervention. Given that the preceding experimental chapters of this thesis indicate an independent effect of the intensity of an acute bout of exercise on several of these outcomes, this remains a pertinent research question. Finally, HRV was not measured over a long enough period for the frequency domain analysis of autonomic function, which would provide greater mechanistic insight regarding changes in sympathovagal balance post HIIT.

8.5 Conclusion

The present study identifies that improvements in autonomic and vascular function are achievable in asymptomatic adolescents following 2 weeks of HIIT, and that these may occur in the absence of changes in traditional CVD risk factors. Additionally, most of these improvements were lost three days after the last HIIT session. These findings are in line with cross sectional data indicating that time spent performing high-intensity activities is important for vascular health (Hopkins et al., 2009) and autonomic function (Buchheit et al., 2007) in youth. Considering that few adolescents meet the current guideline of a minimum of 60 minutes of daily exercise (Riddoch et al., 2007), HIIT may offer an attractive, low volume alternative to moderate-intensity exercise interventions for the primary prevention of CVD.

Chapter 9

Synthesis

9.1 Summary of experimental chapters

The primary aim of this thesis was to identify the influence of exercise intensity on vascular health outcomes in adolescent boys and girls. The experimental chapters in this thesis provide a significant contribution to the literature in this field and may offer important information regarding the primary prevention of CVD in this age group. The purpose of this chapter is fourfold; 1) to briefly summarise the key experimental findings from these studies; 2) to speculate on potential mechanisms underlying the observed findings; 3) to identify methodological limitations underlying the experimental chapters in this thesis; and 4) to highlight the practical implications of this work and outline avenues for future research.

9.1.1 Chapter 4

The purpose of this study was to identify whether exercise intensity modulates postprandial lipaemia and blood pressure in adolescents, and to address whether these outcomes are influenced by sex. At the time of the investigation, no study with adolescents had isolated the influence of exercise intensity, and no data were available identifying whether sex might modulate the post-exercise lipaemic response despite evidence in adults (Freese et al., 2014), especially when the time between exercise cessation and the consumption of the test meal is short (Henderson et al., 2010).

The strengths of this investigation include; 1) the isolation of exercise intensity using a work-matched study design; 2) the adoption of the same test meal as previous work with adolescents (Tolfrey et al., 2012, Tolfrey et al., 2008) in order to facilitate between-study comparisons; 3) the measurement of postprandial substrate oxidation as a potential mechanism underlying the postprandial lipaemic response following exercise; and 4) the inclusion of girls to directly assess the influence of sex in this age group for the first time. Additionally, a novel feature of this study was the prescription of exercise 1 hour before the test meal, as this study design could be considered to isolate a different plasma [TAG]-lowering mechanism than lipoprotein lipase, the upregulation of which is delayed ~ 8-24 hours post exercise (Seip and

Semenkovich, 1998). However, it should be noted that lipoprotein lipase activity was not directly measured in this work.

The data from this study indicate that both MIE and HIIE performed 1 hour before a HFM can elicit moderate reductions in postprandial lipaemia in girls (*ES*=0.58 and 0.73), but not boys (*ES*=0.20 and 0.24), and that this was not influenced by exercise intensity. Therefore, this study demonstrated that the effect of sex on lowering postprandial lipaemia with exercise observed in adults (Freese et al., 2014, Henderson et al., 2010) is also apparent during adolescence. This sexual dimorphism highlights that it would be inappropriate to extrapolate the available exercise and postprandial lipaemia data in boys to both sexes.

This study also highlighted that exercise intensity modulates postprandial blood pressure and resting fat oxidation in adolescents. Specifically, HIIE, but not an equivalent bout of MIE, reduced postprandial SBP and increased fat oxidation, and these outcomes were not influenced by sex. Furthermore, the changes in postprandial [TAG] and fat oxidation were not related. Considering that HIIE was perceived to be more enjoyable than MIE, this work suggests that HIIE may be an attractive and feasible alternative to traditional MIE which is effectual at improving postprandial health outcomes in adolescents.

9.1.2 Chapter 5

Accumulating 1 hour of moderate-intensity exercise has been shown to induce modest reductions in postprandial lipaemia the following day in adolescent boys (Sedgwick et al., 2013). However, no data are available in adolescent girls, and it is not known whether the effect of accumulating exercise on postprandial health outcomes is modulated by exercise intensity. Having established that exercise on the same day as a high fat meal favourably influences several postprandial health outcomes, and that the intensity of exercise plays an important role in these responses (apart from postprandial lipaemia) in Chapter 4, the purpose of this study was to identify whether similar benefits are observed if the same total exercise stimulus is accumulated over the course of the day in adolescent boys and girls. This is a pertinent research question given

that adolescents rarely sustain exercise for > 10 minutes (Riddoch et al., 2007), and that current physical activity guidelines include the accumulation of exercise in their recommendations (Haskell et al., 2007). It is therefore important to identify how this pattern of exercise can be optimised (i.e. by manipulating exercise intensity) for health.

The strengths of this study include; 1) the same total exercise stimulus as performed in Chapter 4 to address the efficacy of accumulated exercise; 2) work-matching of the HIIE and MIE bouts; 3) limiting each exercise bout to < 7 minutes to reflect the exercise pattern of adolescents (Riddoch et al., 2007); 4) the inclusion of multiple meals, and 5) the comparison between boys and girls.

The data indicate that accumulating exercise (either MIE or HIIE) on the same day as two test meals does not attenuate postprandial lipaemia in adolescent boys and girls. However, favourable changes in postprandial SBP and fat oxidation were observed in the HIIE, but not MIE, trial, which is in agreement with data presented in Chapter 4. Interestingly, the magnitude of the change in postprandial SBP was greater in Chapter 4 compared to when HIIE was accumulated in this study (*ES*=0.68 and 0.31, respectively), indicating that the pattern of exercise might be important for this outcome. In contrast, the *ES* for the increase in resting fat oxidation in the HIIE trial was comparable between Chapters 4 and 5 (*ES*=0.88 and 0.74), suggesting that the pattern of exercise does not influence this outcome provided that the total stimulus is the same. Finally, a novel finding of this study was the improved glycaemic control observed in the HIIE, but not MIE trial. This was not observed following HIIE in Chapter 4, and potential mechanisms underlying this favourable change are discussed in Section 9.2.1.

Taken together, it would appear that exercise intensity is important to consider if the brief pattern of exercise typically performed by adolescents (Riddoch et al., 2007) is to be optimised for health. Specifically, HIIE is consistent with superior postprandial health benefits than an equivalent MIE stimulus when accumulated across the day. Whilst HIIE was not perceived to be more enjoyable than MIE (as reported in Chapter 4), the PACES data indicate that these exercise protocols were comparable. Therefore, HIIE continues to be a feasible, attractive and effectual alternative to MIE for health promotion in this population.

9.1.3 Chapter 6

The purpose of this study was to extend the work performed in Chapter 4 and provide insight into how exercise intensity may influence the attenuation in postprandial vascular function which has been observed in adults (Gaenzer et al., 2001, Vogel et al., 1997, Gill et al., 2004, Tyldum et al., 2009) and adolescents (Sedgwick et al., 2013, Sedgwick et al., 2014, Sedgwick et al., 2012). In addition to replicating the robust methodological design adopted in Chapter 4, the strengths of this study include; 1) the simultaneous assessment of macro- and micro-vascular function; 2) allometric scaling of the FMD statistic to partition out the confounding impact of baseline artery diameter; 3) the determination of plasma [3-OHB] as a candidate mechanism for putative exercise-induced reductions in postprandial lipaemia (based on the findings of Chapter 4 in the girls); and 4) assessment of plasma [TAS] as a potential mediator of changes in the postprandial vascular response.

This study demonstrated that macro- and micro-vascular function is augmented 1 hour after HIE but not MIE. This investigation also demonstrated that macro- and micro-vascular function (PRH, but not the total hyperaemic response) were compromised following a high fat meal, and that prior exercise can protect against these unfavourable responses. Specifically, compared to CON, postprandial macro- and micro-vascular function (PRH) were greater in MIE and in HIIE, and postprandial FMD was greater in HIIE than MIE. Interestingly, these differences in vascular function were independent to changes in plasma [TAG] and [TAS]. Therefore, this study demonstrates that the intensity of an acute bout of exercise independently influences vascular function both before and after a high fat meal, even in the absence a concomitant reduction in postprandial plasma [TAG] or [TAS]. Furthermore, there was no interaction effect of sex on these outcomes.

Finally, this study provided novel information regarding postprandial lipaemia and changes in [3-OHB] in adolescents, and how this marker of hepatic fatty acid metabolism, mainly VLDL-TAG output, is influenced by exercise intensity. Specifically, [3-OHB] increased following HIIE, but not MIE. Furthermore, this outcome demonstrated a strong relationship with the change in postprandial lipaemia in HIIE (P=0.01, r=0.61). Therefore, this data indicates that changes in

VLDL-TAG metabolism may have played an important role in the postprandial response observed in Chapter 4, and in other investigations which adopted a 1-day protocol.

9.1.4 Chapter 7

This investigation was the first to assess the time course of the macro- and micro-vascular response post exercise in adolescents, which is important as it has been suggested that the acute changes post exercise provide the basis for chronic adaptation and subsequent vascular remodelling (Birk et al., 2013, Dawson et al., 2013). This study provides novel data as only one study has examined the acute macrovascular response to different exercise intensities in a paediatric cohort (Mills et al., 2013). Unfortunately, this study only measured FMD immediately after exercise, which is problematic as a recent review suggests that the post-exercise FMD response is biphasic in nature (Dawson et al., 2013), and did not include an assessment of microvascular function. Furthermore, the exercise bouts were not work-matched. Chapter 7 also extends the findings presented in Chapter 6, as the inclusion of serial vascular measures post exercise detail the time course of the macro- and micro-vascular responses post HIIE and MIE, and the confounding effect of a high fat meal was removed. The strengths of this study include; 1) work-matching of the exercise bouts; 2) the simultaneous measures of macro- and micro-vascular function; 3) serial measures of vascular function post exercise; and 4) allometric scaling of the FMD statistic.

This study demonstrated that the intensity of an acute bout of exercise independently influences macro- and micro-vascular function in the 2 hours following exercise cessation. It confirms that the post exercise nadir in FMD previously observed after high-intensity exercise in children (Mills et al., 2013) precedes an augmented response which is evident 1 and 2 hours later, as predicted by a recent review (Dawson et al., 2013). In contrast, no changes in macrovascular function were observed following MIE at these time points. Additionally, this study identified that both MIE and HIIE increased NO-independent microvascular during the 2 hours post exercise cessation, and that the magnitude of this improvement is greater following HIIE than MIE.

The changes in macrovascular function observed following HIIE were not related to baseline arterial diameter or SR_{AUC}. Thus, this data suggest an upregulation of eNOS activity in the hours after HIIE due to a positive association between exercise intensity and the brachial artery shear stimulus during the exercise bout. Furthermore, the findings from this study identify that the superior effect of HIIE on microvascular function is independent of changes in NO bioavailability. Thus, a single bout of HIIE is a powerful stimulus for acute improvements in macro- and micro-vascular function, and that these favourable changes are mediated by different underlying mechanisms.

9.1.5 Chapter 8

The purpose of this study was to address the efficacy of performing the same HIIE protocol adopted in Chapters 4, 6 and 7, three times a week for two weeks, on traditional and novel CVD risk factors in apparently healthy adolescent boys and girls. Given that changes in traditional CVD risk factors following exercise only explain 59% of the reduction in CVD risk (Mora et al., 2007), it is pertinent to look “beyond” the health outcomes which are typically measured in training interventions in children and adolescents (Buchan et al., 2011a, Buchan et al., 2011b, Baquet et al., 2001). It has been proposed that some of the 41% “risk factor gap” can be explained by improvements in endothelial and autonomic function following exercise interventions (Joyner and Green, 2009). Considering that asymptomatic adolescents with clustered CVD risk present with an impairment in FMD (Celermajer et al., 1992) and low HRV (Farah et al., 2014), it is pertinent to include these outcomes to provide a more comprehensive understanding of how regular HIIE may influence CVD risk in youth.

The strengths of this study include; 1) the comprehensive measurement of traditional and novel CVD risk factors in both the fasted state and after a mixed meal tolerance test; 2) the simultaneous assessment of macrovascular and autonomic function; 3) the determination of whether changes in these outcomes remain three days post intervention; and 4) the identification of whether our HIIE protocol (also adopted in Chapters 4, 6 and 7) may attenuate postprandial lipaemia when performed the day before the test meal.

This intervention failed to improve any traditional CVD risk factors, whether in the fasted or postprandial state. In contrast, both FMD and HRV were improved the day after the last training session. However, the magnitude of these improvements deteriorated 3 days after training cessation (the improvement in FMD was no longer significant at this time point). Thus improvements in vascular and autonomic function may occur in the absence of improvements in traditional risk CVD risk factors. However, these benefits may be lost ~ 72 hours after the last training session, indicating the importance of performing regular HIIE for health.

9.2. Synthesis of findings and discussion of candidate mechanisms

This section collates the outcomes from the experimental chapters and speculates on the potential mechanisms underlying the observed findings.

9.2.1 Postprandial lipaemia

The assessment of postprandial lipaemia following exercise is a central theme in this thesis. The most pertinent questions raised by the initial findings from Chapter 4 are; what is(are) the mechanism(s) responsible for lowering postprandial lipaemia in the girls but not boys? And why does exercise intensity fail to influence it(them)?

Given that 30 minutes of jogging at 55% $\dot{V}O_{2\ peak}$ (Tolfrey et al., 2012) and performing 10 x 1 minutes of running at the maximal aerobic speed (Thackray et al., 2013) has been shown to attenuate postprandial lipaemia in adolescent boys the following day, the failure of both HIIE and MIE to lower plasma [TAG] in the boys in Chapter 4 is likely related to the adoption of a 1-day protocol. Completing exercise one hour prior to the test meal, rather than the day beforehand, is consistent with a blunted reduction in lipaemia in adults (Zhang et al., 1998), potentially due to the delayed upregulation of lipoprotein lipase (Seip and Semenkovich, 1998). Indeed, in the only other study to adopt a 1-day protocol with adolescents, postprandial lipaemia was not attenuated despite the completion of 135 minutes of walking (Sisson et al., 2013). Therefore, if the

study design diminishes the importance of the exercise-induced upregulation of lipoprotein lipase, and is unlikely to have affected gastric emptying rates (Feldman and Nixon, 1982), it is possible that the lower lipaemic response post exercise in the girls is related to an increased clearance, or decreased secretion of VLDL-TAG, or both (Bellou et al., 2013, Magkos, 2009, Katsanos, 2006).

It was beyond the scope of this thesis to quantify changes in specific lipoprotein fractions and their subclasses, however this potential explanation gains credibility considering that sex is known to influence VLDL kinetics post exercise (Bellou et al., 2013, Magkos et al., 2006). Furthermore, the lower post exercise lipaemic response observed in the girls in this thesis is consistent with the work by Henderson *et al*. (2010) who observed that postprandial plasma [TAG] is reduced in women, but not men, when a high fat meal is consumed 1 hour after exercise. These authors speculated that this may be due to the lower observed increase in post exercise plasma free FA in women than men (Henderson et al., 2007, Henderson et al., 2010), which could plausibly lead to a lower VLDL-TAG synthesis rate (Lewis et al., 1995). Support for this mechanism is provided by Davitt *et al*. (2013) who demonstrated using a stable isotope tracer technique that the reduction in postprandial lipaemia in women, when the test meal is consumed 30 minutes post exercise, is due to a reduced abundance of endogenous FA in plasma [TAG], rather than an increased clearance rate. Together, these findings highlight that sex does influence the postprandial [TAG] response when the duration between exercise cessation and consumption of the test meal is short. However, it is an assumption that these sex differences in plasma [FA] reflect changes in hepatic VLDL-TAG output, as this outcome was not measured in these studies. Indeed, these findings may be consistent with an increase in VLDL clearance, rather than hepatic output, which has recently been demonstrated to play an important role in the postprandial lipaemic response post exercise (Ghafouri et al., 2015). These authors demonstrated an increase affinity of $VLDL_1$, a key determinant of postprandial lipaemia (Tan et al., 1995), for lipoprotein lipase the day after exercise in men *ex vivo*. This methodological approach has yet to be performed in women, or in the immediate hours post exercise, however it provides evidence that changes in VLDL clearance rate, rather than just hepatic VLDL secretion, occur following exercise.

The lack of an interaction effect of exercise intensity on postprandial lipaemia in the girls was surprising given that HIIE has recently been shown to be more effectual than MIE in adult males (Trombold et al., 2013, Gabriel et al., 2012). However, these investigations were performed the afternoon before the test meal, and therefore the processes underlying the attenuation in lipaemia are likely to be mechanistically different. Our data confirm that the attenuation in postprandial lipaemia after exercise is not related to substrate oxidation during the exercise bout (Malkova et al., 1999), and identified that changes in this outcome were not related to differences in fat oxidation during the postprandial period, which is also not in line with data reported by Trombold *et al*. (2013). Additionally, correlation analyses failed to identify any meaningful relationship between participant characteristics (i.e. body fatness (Lee et al., 2013, MacEneaney et al., 2009)) and the postprandial response. However, these analyses are limited due to the small sample size and homogenous nature of the sample population in this thesis. Finally, the IAUC-TAG statistic partitions out the potentially confounding effect of a reduction in "baseline" plasma [TAG], thus the lower lipaemic response observed is not an artefact of a lower plasma [TAG] post exercise in the girls.

The data presented in this thesis are unable to explain why exercise intensity failed to affect the postprandial response in the adolescent girls using the data from Chapter 4. However, in the aforementioned investigation, Davitt *et al*. (2013) reported that the blunted appearance of endogenous TAG was similar following 1 hour of resistance exercise or 1 hour of exercise at 60-65% $\dot{V}O_{2\ peak}$ in sedentary women. Additionally, exercise intensity has not been shown to influence plasma [3-OHB] immediately post exercise (Bassami et al., 2007), which is plausibly the candidate mechanism underlying our findings in the girls in Chapter 4. Thus, the modulation of VLDL-TAG kinetics in the immediate hours following exercise cessation in women and probably in adolescent girls may not be influenced by exercise intensity.

In order to address this further, plasma [3-OHB] was measured during the 4 hour post exercise period in Chapter 6, and these data demonstrate that an upregulation in plasma [3-OHB] does occur after HIIE, but not MIE. Whilst it is acknowledged that plasma [3-OHB] is only indicative of hepatic VLDL output, which was not measured in any experimental chapter, it appears that changes

in VLDL-TAG metabolism one hour post exercise may play a role in attenuating postprandial lipaemia as the difference in TAUC-3-OHB between HIIE and CON explained 44% of the change in TAUC-TAG between these trials (r=-0.66, P=0.003). This finding adds credibility to the speculation made in Chapter 4, that the adoption of a 1-day protocol likely isolates different mechanisms than traditional 2-day study designs; the former may favour post exercise changes in VLDL-TAG metabolism rather than an upregulation of lipoprotein lipase activity. However, plasma [3-OHB] was not increased post MIE in the girls. This effect of exercise intensity is consistent with an increase in fasted plasma [3-OHB] the day after HIIE, but not an isoenergetic bout of MIE in men (Trombold et al., 2013), but not with the lack of effect observed immediately post exercise (Bassami et al., 2007). However, no data are currently available which identify the influence of the intensity of exercise performed 1 hour before a HFM on [3-OHB] or VLDL-TAG metabolism. Therefore the plasma [3-OHB] data presented in Chapter 6 is unable to explain why postprandial lipaemia was lower in both HIIE and MIE trials in the girls in Chapter 4. However, such insights are speculative as there were no main effects of sex or trial on the TAUC-TAG or IAUC-TAG in Chapter 6.

Given that the exercise bouts and both the timing and composition of the HFM in Chapters 4 and 6 were identical, the failure to attenuate postprandial lipaemia in the girls in either the MIE or HIIE trial in Chapter 6 may be related to the use of a 3 (rather than 4) hour postprandial period in this study. Closer scrutiny of the data indicates that plasma [TAG] was returning to baseline in the girls, but not the boys, 3 hours after the meal (Figure 9.1), which is consistent with effect of sex observed by Henderson *et al*. (2010). Thus we may have “missed” the benefit of exercise on the TAUC-TAG or IAUC-TAG outcomes in this investigation due to the adoption of a 3 hour postprandial measurement period.

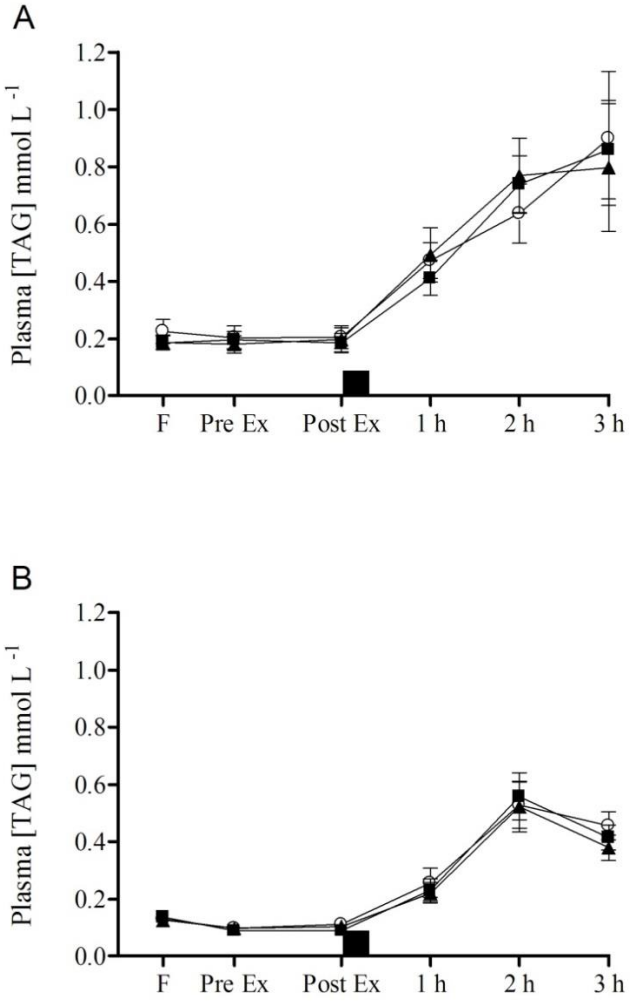

Figure 9.1 Mean plasma triacylglycerol (TAG) concentrations for the control (○), moderate-(▲) and high-(■) intensity exercise trials in Chapter 6. Data are split for sex; boys (A) and girls (B). Error bars describe the standard error of measurement. The high fat meal (HFM) is represented by the black rectangle.

In order to address this further, the 3 h TAUC-TAG and IAUC-TAG from Chapter 4 have been calculated and analysed using a mixed model ANOVA with trial and sex as the main effects (i.e. in a manner which is consistent with both Chapter 4 and 6). This analysis revealed no differences in the 3 h TAUC-TAG (main effect of trial P=0.40; main effect of sex P=0.84; trial by sex interaction P=0.47) or 3 h IAUC-TAG outcome in Chapter 4 (main effect of trial P=0.21; main effect of sex P=0.71; trial by sex interaction P=0.06). Thus it is likely that this disparity between Chapters 4 and 6 is explained, at leas in part, by the truncated postprandial observation time in the latter.

To further shed light on this discrepancy, the 3 h IAUC-TAG data was pooled between these two studies and analysed using a mixed model ANOVA with trial (CON, MIE, HIIE), sex (male, female) and study (Chapter 4, Chapter 6) as the main effects. This is appropriate as the exercise bouts and the composition and timing of the high fat meal were identical between studies. Accordingly, there was a main effect of study (*P*=0.03), but not trial (*P*=0.33) or sex (*P*=0.16) for the pooled 3 h IAUC-TAG. Specifically, the 3 h IAUC-TAG was always lower in Chapter 6 than in Chapter 4 (*P*=0.03, 95% CI -0.67 to -0.04, *ES*=0.73), and it is possible that this may have limited the absolute change in postprandial lipaemia observed in Chapter 6.

Data from Chapter 8 provides some insight into whether the HIIE bout in Chapter 4 may have lowered postprandial lipaemia the following day, as plasma [TAG] was measured every hour for 4 hours after a mixed meal tolerance test (which provided 68 g of fat), the day after performing 10 x 1 min intervals cycling at ~ 90% peak power output. However, because this meal contained a high glucose load (80 g of sugar) which may have lowered postprandial lipaemia (Cohen and Berger, 1990), such comparisons are made with caution.

Chapter 8 demonstrates a 14% reduction in the IAUC-TAG the day after the last training session, although this was not significantly different to the postprandial response before training (*P*=0.24, 95% CI -1.25 to 0.34, *ES*=0.20). However, the magnitude of this change is similar to the 10% (*ES*=0.39) reduction observed the day after 10 x 1 minute of high-intensity running in adolescent boys by Thackray *et al*. (2013). Additionally, Trombold *et al.* (2013) reported a 14% reduction in postprandial lipaemia the day after HIIE in adults (calculation of an *ES* not possible from the manuscript). Thus, when expressed as a percentage change, the date presented in Chapter 8 are in line with the current evidence base regarding the efficacy of HIIE to attenuate postprandial lipaemia.

Surprisingly, there was no main effect of sex for plasma [TAG] in Chapter 8 (*P*=0.16), so this attenuation in postprandial lipaemia was pooled for boys and girls. It is not immediately apparent why this study was unable to replicate the effect of sex observed in Chapters 4 and 5, and demonstrated in the immediate hours post exercise in adults (Henderson et al., 2010). Interestingly, Tolfrey *et al*. (2014a) suggests that an energy expenditure of ~ 1500 kJ may be necessary

to attenuate postprandial lipaemia the following day in girls, but not boys (Tolfrey et al., 2012). When the data in Chapter 8 are split for sex, there does appear to be a disparity in the IAUC-TAG outcome in boys (*P*=0.08, *ES*=0.37) compared to girls (*P*=0.43, *ES*=0.26). However, this analysis is limited due to the small sample size of boys (*n*=7) and girls (*n*=6), and thus requires further work. Indeed, Tolfrey *et al*. (2014a) attribute their observations to a greater individual variation in the postprandial lipaemic response in the girls, which might be due to the confounding influence of the menstrual cycle (Gill et al., 2005). Thus, it is likely that a larger sample size is needed, or menstrual cycle phase controlled for, or both, in order to form robust conclusions regarding the efficacy of HIIE on postprandial lipaemia the following day in adolescents, and whether this is modulated by sex.

Data in adults demonstrate that accumulating MIE the day before the test meal is as effectual at lowering postprandial lipaemia as performing the same exercise stimulus in a single bout (Miyashita et al., 2006). In contrast, postprandial lipaemia was not attenuated in the girls in Chapter 5, even though the total exercise accrued in this study was equivalent to the MIE and HIIE bouts performed in Chapter 4. A plausible explanation for this finding is that 75% of the exercise bouts were accumulated during the postprandial period. It has previously been demonstrated in adults that 1 hour of treadmill running at 60% $\dot{V}O_{2\ max}$ attenuates postprandial lipaemia (38%) when performed 1 hour before, but not 1 hour after a high fat meal (Zhang et al., 1998). Furthermore, Sisson *et al*. (2013) demonstrated that 135 minutes of walking during the postprandial period failed to lower postprandial lipaemia in adolescent boys and girls.

Currently, no data are available comparing the efficacy of accumulated and continuous exercise of any intensity on postprandial lipaemia using a 1-day protocol. Given that Chapter 6 indicates that acute increases in plasma [3-OHB] (which likely reflect changes in VLDL-TAG output) may play a leading role in the favourable postprandial response in the immediate hours post exercise, it is possible that accumulating brief (< 7 min) bouts of exercise is an insufficient stimulus for the upregulation of this mechanism, although may have been sufficient to increase lipoprotein lipase activity the following day (Miyashita et al., 2006). However, no dose-response data are available concerning the

increase in plasma [3-OHB] in the 4 – 6 hours post exercise, and no study in adolescents has compared accumulated versus continuous exercise on the subsequent lipaemic response. Considering that accumulating brief exercise bouts better reflects the pattern of physical activity performed by this age group (Riddoch et al., 2007), this remains an important area for future research.

9.2.2 Postprandial fat oxidation

The assessment of postprandial fat oxidation in Chapters 4 and 5 was primarily to determine whether differences in postprandial lipaemia between trials are explained by changes in resting fat oxidation following MIE or HIIE, which has been observed the day after MIE and HIIE in men (Trombold et al., 2013). However, this outcome is also important as exercise training-induced increases in resting fat oxidation in adults are a significant determinant of fat loss (Barwell et al., 2009).

The positive association observed between exercise intensity and post-exercise fat oxidation in Chapters 4 and 5 is consisted with some (Broeder et al., 1991, Phelain et al., 1997), but not all (Melanson et al., 2002, Henderson et al., 2007) data reported in adults, and this discrepancy is probably due to the differences in the duration of the post-exercise observation time. Whilst the precise mechanisms underlying this phenomenon are yet to be fully established (Henderson et al., 2007), the lack of change in RMR indicate that the increase in fat oxidation reflects a shift in substrate use, rather than a function of an exaggerated excess post-exercise oxygen consumption, as mean RER was lowered post HIIE but RMR remained unchanged. Additionally, the lower RER observed 2 hours post HIIE probably does not reflect a conservation of 'non-metabolic' carbon dioxide for the replenishment of bicarbonate as Stringer *et al*. (1992) demonstrated that disturbances to the bicarbonate pool return to baseline 30 minutes after 6 minutes of high-intensity exercise. Furthermore, it is known that young people are characterized by a better acid/base balance than adults during repeated bout of high-intensity exercise (Ratel et al., 2002). Thus, it is likely that perturbations to the bicarbonate pool would have returned to baseline when RMR and substrate oxidation were reassessed post HIIE in Chapter 4, although this was not directly assessed.

Data in adults have linked the elevation in fat oxidation post high-intensity exercise with greater circulating noradrenaline concentrations (Quisth et al., 2005) and growth hormone (Henderson et al., 2007), both of which are positively associated with exercise intensity during the recovery period (Pritzlaff-Roy et al., 2002, Romijn et al., 1993). It is also plausible that the increase in fat oxidation following HIIE reflects a post-exercise sparing and re-synthesis of glycogen which would have been utilised to a greater degree during HIIE than MIE. However, data from Chapter 4 revealed no relationship between mean RER during exercise and TAUC-fat oxidation for HIIE (r=0.22, P=0.36) or MIE (r=-0.16, P=0.51).

It is important to note that no other investigation has included a high fat meal prior to their assessment of changes in post-exercise substrate utilisation, and evidence is available which suggest that this might have obscured our findings (Richard and Rivest, 1989, Poehlman and Horton, 1989). Indeed, our data indicates a significant increase in fat oxidation following consumption of this meal even during the non-exercise control day in Chapters 4 and 5 (main effect of time, P<0.001 for both Chapter 4 and 5; Figure 9.2). Nevertheless, considering that the test meal was also included in the MIE and HIIE trials, the between-trial differences presumably reflect differences in substrate use which are caused by the exercise bouts.

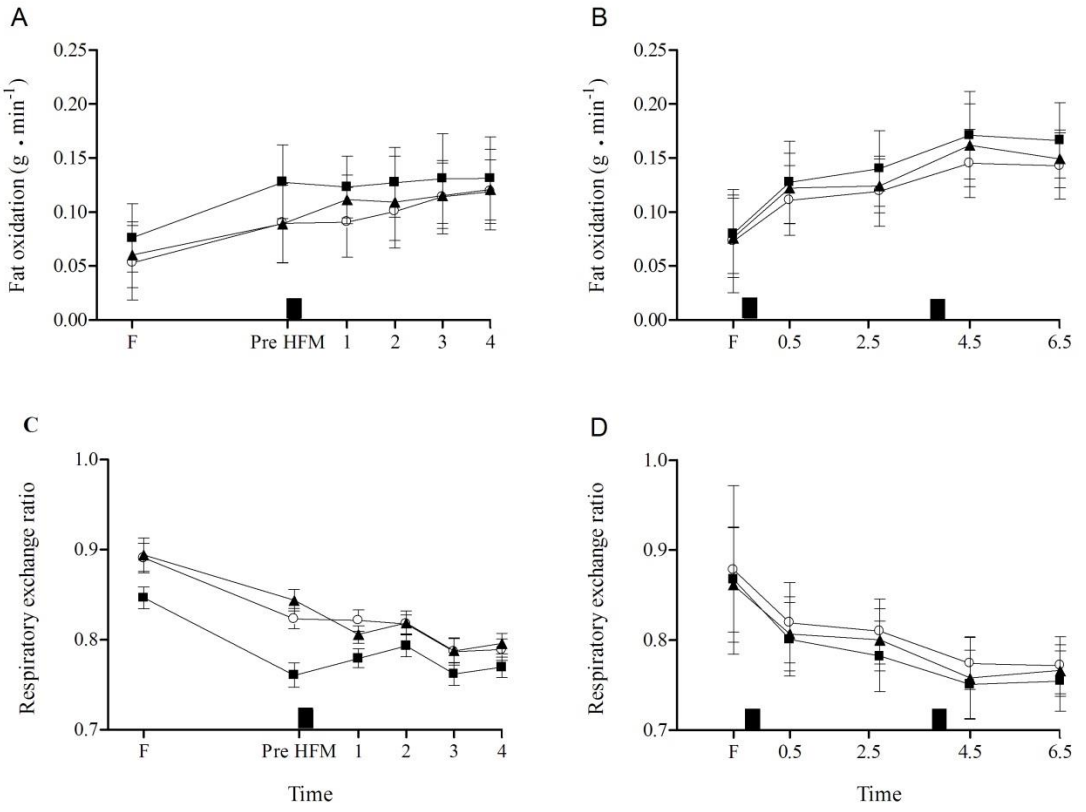

Figure 9.2 Mean fat oxidation and respiratory exchange ratio for the control (○), moderate-(▲) and high-(■) intensity exercise trials in Chapters 4 (A and C) and 5 (B and D). Error bars describe the standard error of measurement. The black rectangle represents a high fat meal (HFM).

9.2.3 Postprandial blood pressure

Uetani *et al*. (2012) demonstrated that an increase in SBP in the hours following a meal is associated with insulin resistance, carotid thickness and arterial stiffness in the elderly. Acute exercise is known to transiently lower SBP, and Forjaz *et al*. (2004) demonstrated that the fall in SBP during the 90 minutes after exercise is positively associated with exercise intensity. The data reported in Chapters 4 and 5 are consistent with this finding, as we observed a reduction (*ES*=0.68) in postprandial TAUC-SBP following HIIE, but not MIE (*ES*=0.14), compared to CON.

The absence of a postprandial hypotensive response following MIE in Chapters 4 and 5 is in agreement with some (Jones et al., 2007, MacDonald et al., 1999a, MacDonald et al., 1999b), but not all (Floras and Senn, 1991) investigations with adults. It has been argued that the magnitude of the hypotensive response

post exercise is greater in individuals with higher initial blood pressure (Kenney and Seals, 1993), which may offer some explanation regarding the failure of MIE to lower SBP. However, this argument does not explain the favourable response post HIIE.

Unfortunately, the comparison between TAUC-SBP in these experimental chapters and the available literature is clouded by our inclusion of a high fat meal. Indeed, a mean increase in SBP of 4 mmHg was observed in Chapter 4 following the consumption of the test meal, and we speculated at the time that this reflected a probable impairment in endothelial function and transient increase in arterial stiffness, which had been observed elsewhere (Vogel et al., 1997). The findings from Chapter 6 and 8 confirm that an impairment in endothelial function is likely to have taken place in CON.

Considering that postprandial resting heart rate was elevated following HIIE compared to MIE and CON in Chapter 4, it is logical that the accompanying lower SBP reflects a reduced total vascular resistance, perhaps caused by an increase in the bioavailability of NO due to an upregulation in eNOS activity, or a reduction in sympathetic drive, or both. The case for the former is consistent with the findings from Chapter 6 and 7, and suggests that the greater shear stress during the HIIE bout compared to MIE increased eNOS activity. Whilst adult data are available indicating that the post-exercise fall in total peripheral resistance increases with exercise intensity (Jones et al., 2007), these authors, and others (Forjaz et al., 2004) do not support an intensity-dependent fall in systemic vascular resistance as the primary mediator of the post-exercise hypotensive response. Unfortunately, SBP was not measured in Chapters 6 and 7 so an assessment of the relationship between the change in vascular function and the change in TAUC-SBP is not possible.

The hypotensive response immediately post exercise has also been attributed to a reduction in baroreflex sensitivity (Heffernan et al., 2007). The delay in the “resetting” of baroreflex sensitivity post exercise has been shown to increase with exercise intensity (Niemela et al., 2008), and these authors speculated that this phenomenon was attributed to a greater withdrawal of vagal modulation and an augmented sympathetic vasomotor tone as the intensity of exercise increases. HRV analysis (RMSSD) of the ECG gated FMD images pre-

occlusion in Chapters 6 and 7 (Figure 9.3) demonstrate that vagal outflow is decreased immediately post MIE (*P*=0.01, 95% CI -23.2 to -4.1, *ES*=0.86) and HIIE (*P*<0.001, 95% CI -42.9 to -24.5, *ES*=2.50), but remained blunted 1 hour after HIIE only (Chapter 6; *P*=0.01, 95% CI -12.6 to -5.1, *ES*=0.83, Chapter 7; *P*=0.02, 95% CI -18.8 to -2.1, *ES*=0.71). Thus our data suggest a greater delay in the recovery of baroreflex sensitivity post HIIE. However, the interplay between the putative mechanisms underlying the post exercise "hypotensive" response (i.e. changes in systemic vascular resistance, stroke volume, cardiac output, sympathovagal balance, plasma volume) following exercises at different intensities, and how these may be modulated by resting blood pressure, fitness and sex (Senitko et al., 2002), is a complex phenomenon and currently not well defined in either adult or paediatric groups.

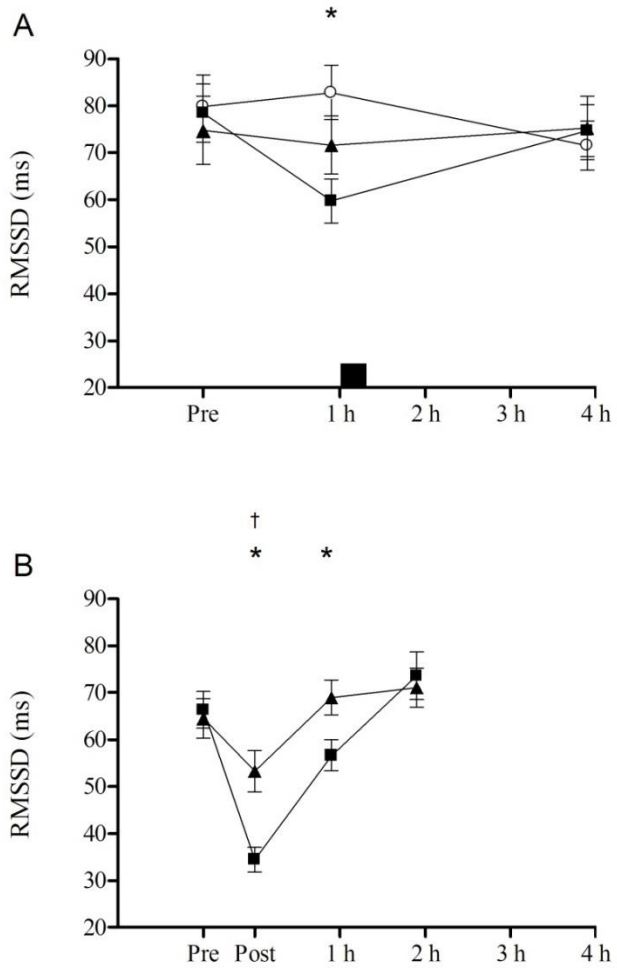

Figure 9.3 Mean heart rate variability for the control (○), moderate-(▲) and high-(■) intensity exercise trials in Chapters 6 (A) and 7 (B). Error bars describe the standard error of measurement. The black rectangle represents a high fat meal. * *P*<0.05 compared to Pre in HIIE; † *P*<0.05 compared to Pre in MIE.

9.2.4 Macrovascular function

Chapters 6 and 7 demonstrate that endothelial function is augmented 1 hour after HIIE, but is not influenced by MIE. Additionally, Chapter 7 highlights that this increase in FMD post HIIE remains 2 hours after exercise cessation. Furthermore, Chapter 6 demonstrates that macrovascular function remains elevated 4 hours post HIIE, despite the consumption of a HFM which attenuated FMD in CON. These findings cannot be explained by the observed changes in SR_{AUC} (which were not related to FMD) or baseline artery diameter as the FMD statistic was allometrically scaled to partition out the influence of this potential confounder (Atkinson et al., 2009, Atkinson et al., 2013). Furthermore, no differences in physical activity performed the day before each laboratory visit were apparent between trials, the importance of which is demonstrated by the increase in endothelial function observed the day after the HIIT protocol (POST-1D) in Chapter 8.

Whilst no study has documented the change in brachial artery shear stimulus during exercise at different intensities in adolescents, the FMD responses observed in this study are consistent with data observed in adults 1 hour after exercise (Johnson et al., 2012). It is likely that the favourable macrovascular response 1 and 2 hours post HIIE is due to an upregulation in the activity of eNOS and subsequent increase in NO bioavailability. Furthermore, this pathway is probably exercise intensity dependent, as cycling intensity is positively associate with brachial artery shear in adults (Green et al., 2002, Thijssen et al., 2009a), and it is understood that the exercise-induced change in shear provides the principle physiological stimulus for the adaptation of endothelial function (Tinken et al., 2010). Consequently, it is plausible that the MIE bout in Chapters 6 and 7 did not provide enough of a shear-stimulus for the post exercise increase in eNOS activity.

Chapter 7 reported an attenuation in FMD immediately post HIIE but not MIE, which is consistent with the only available data in youth (Mills et al., 2013), and adults (Birk et al., 2013, Johnson et al., 2012). Considering that the FMD data were allometrically scaled, this observation is not a statistical artefact caused by an increase in baseline arterial diameter. Therefore, this post HIIE nadir in endothelial function reflects a physiological event, and is probably related to an

increase in oxidative stress which is known to be positively associated with exercise intensity (Goto et al., 2003). Indeed, this post exercise impairment in endothelial function can be prevented via antioxidant supplementation (Silvestro et al., 2002). An increase in oxidative stress lowers the bioavailability of NO, and thus attenuates FMD (Green, 2005), as NO preferentially scavenges free radicals, forming peroxynitrite (Beckman and Koppenol, 1996). Furthermore, peroxynitrite itself is a potent oxidant that uncouples eNOS (Wallace et al., 2010), and encourages the production of O_2- (Forstermann and Munzel, 2006), thereby reducing NO production and further compromising the bioavailability of NO.

Presumably, the MIE bout did not increase circulating reactive oxygen species to a level which exceeded the body's antioxidant defences, although the same cannot be said immediately following the HIIE bout. Whilst this thesis is unable to provide any specific insight regarding the time course of changes in redox state following MIE and HIIE, such a conclusion is in line with available evidence from adult studies (Parker et al., 2014, Wang and Huang, 2005). However, the elevated FMD observed 1 and 2 hours post HIIE might indicate that this pro-oxidative state is short-lived, or that the magnitude of the likely increase in NO production 1 and 2 hours post HIIE may have exceeded the amount required to buffer this postulated increase in circulating free radicals, or both. However, markers of oxidative stress were not determined in Chapter 7, and the specific time course of the change in oxidative balance post MIE and HIIE is not well established.

Total antioxidant status was determined in Chapter 6, as data in adults demonstrate that postprandial lipaemia impairs macrovascular function via oxidative stress (Bae et al., 2001) and Tyldum *et al.* (2009) previously identified that the favourable influence of exercise intensity on FMD was related to an increase in plasma [TAS]. However, although plasma [TAS] was significantly lowered in the postprandial period, we were unable to replicate this interaction between exercise intensity and this outcome 1 hour post exercise in Chapter 6. This discrepancy is plausibly related to the 2-day study design adopted by Tyldum *et al*. (2009). Indeed, no differences in postprandial circulating antioxidants were observed when moderate- and high-intensity exercise were performed 1 hour following the test meal in adults (Canale et al., 2014). The

data in Chapter 6 therefore indicate that oxidative balance might not have been compromised 1 hour post MIE and HIIE in Chapter 7. In other words, the oxidative stress presumed to be responsible for the impairment in endothelial function immediately post HIIE is short-lived (< 1 hour). However, it is a limitation that a more comprehensive assessment of redox state could not be performed in this study. Therefore, such conclusions are speculative and deserve further study.

Fasted and postprandial plasma [GTP] was determined the day after the last training session in Chapter 8 as this antioxidant enzyme is lowered after a high fat meal and associated with a concomitant fall in endothelial function in adults (Tsai et al., 2004). Importantly, these measurements coincide with the timing of the [TAS] measures by Tyldum *et al*. (2009). However, [GTP] was not altered either by the HIIT intervention or the test meal in Chapter 8, which does not corroborate with the findings by Tyldum *et al.* (2009). However, during data collection for this investigation, Canale *et al*. (2014) published data demonstrating that [GTP] remains unchanged following a high fat meal despite concomitant reductions in other antioxidant enzymes, such as superoxide dismutase and catalase. Given that the fat loads and postprandial measures were comparable between Tsai *et al*. (2004) and Canale *et al.* (2014), the cause of this inconsistency is not immediately apparent. Thus, a more comprehensive assessment of oxidative stress would have been beneficial in Chapter 8.

The inclusion of a high fat meal in Chapter 6 means that it is not possible to identify whether the magnitude of the postprandial increase in FMD above baseline (*ES*=1.56) 4 hours post exercise was compromised in the HIIE trial. However, the magnitude of this increase in FMD 4 hours post HIIE (i.e. 3 hours post HFM) in Chapter 6 is similar to that observed 1 hour (*ES*=1.33) and 2 hours (*ES*=1.36) after HIIE in Chapter 7. Interestingly, Chapter 8 demonstrates that fasted FMD was greater ~ 18 hours after the final HIIT bout compared to before training (*ES*=0.70). Given that this finding may have been a response to the last HIIT session, rather than a training effect *per se*, these data indicate that a single HIIE bout of < 30 minutes in duration provides a profound stimulus for improvements in endothelial function in adolescents that may last for just short of 2 days. However, it is not possible to confirm that the observed increase in fasted FMD following 2 weeks of HIIT in Chapter 8 is solely an

artefact of the last training session as this study did not assess changes in FMD the day after the initial training bout. Thus the magnitude of the change in FMD following the first and last training sessions cannot be compared. It has been demonstrated that eNOS gene expression is augmented in dogs following 10 days of high volume (2 x 1 hour per day) training, and it is thought that this reflects a systematic vascular adaption, rather than a "last bout effect" (Jenkins et al., 2012). Given the aforementioned positive association between exercise intensity and shear stress (Green et al., 2002, Thijssen et al., 2009a), and that shear plays a leading role is augmenting eNOS activity following both acute (Tinken et al., 2010) and chronic (Hambrecht et al., 2003) exercise in humans, it is plausible that this favourable adaptation may have occurred following 2 weeks of HIIT in Chapter 8. Furthermore, Kingwell *et al*. (1997) demonstrated that the increase in basal NO production following 4 weeks of cycle training (30 minutes at 65% maximum power output) returned to baseline following 48 hours detraining. Currently, no data exist in either humans or animals which identify the time course of the return of either eNOS gene expression or activity following a single bout of HIIE, and this remains an area for future research.

9.2.5 Microvascular function

The simultaneous assessment of microvascular function during the FMD protocol is also a unique feature of Chapters 6 and 7. Unfortunately, microvascular function was not determined in Chapter 8 due to equipment failure.

The initial work in Chapter 6 demonstrated that PRH was impaired 3 hours after the test meal in CON, but not in the MIE and HIIE trials. The mechanisms underlying the post-occlusive hyperaemic response in the cutaneous circulation are not well established, but thought to include both endothelium dependent and independent pathways (Cracowski et al., 2006). The protective effect of exercise on postprandial microvascular function observed in Chapter 6 is consistent with the work by Gill *et al*. (2004), who demonstrated that this favourable postprandial response was endothelium-dependent using the iontophoresis of acetylcholine and sodium nitroprusside. Whilst the PRH outcome is different, data in adults show that measure of microvascular function

significantly correlates with the maximum peak perfusion following the iontophoresis of acetylcholine (r=0.62, P<0.001) (Hansell et al., 2004). Thus, the findings from Gill *et al*. (2004) suggest that the improvement in postprandial PRH in the exercise trials in this thesis may, in part, be due to an augmented endothelial function in the cutaneous circulation.

Data in adults indicate that post-occlusive reactive hyperaemia may be favourably modulated by antioxidant status (Franzoni et al., 2004). Interestingly, Gill *et al*. (2004) found no significant correlations between the exercise-induced change in microvascular function and the changes in markers of inflammation, and speculated that the improvement in postprandial microvascular function might reflect the putative increase in eNOS activity due to elevations in post exercise blood flow and shear. Whilst plasma [TAS] was attenuated after the test meal in all trials (P=0.04) in Chapter 6, the lack of a time by trial interaction for this outcome (P=0.53) and the lack of a significant relationship between changes in plasma [TAS] and PRH or the total hyperaemic response (r<0.2 and P>0.05 for all) in Chapter 6 indicate that differences in oxidative stress might not underlie the protective effect of exercise on postprandial microvascular function. However, it is acknowledged that Chapter 6 is unable to provide a more comprehensive measurement of changes in redox state. Given that FMD was simultaneously determined in Chapter 6, and this is thought to be NO-mediated (Green, 2005), it is attractive to use the FMD data as a surrogate for microvascular NO bioavailability in order to identify whether changes in NO might underlie the PRH response. However, existing data in adults demonstrate that the changes in the PRH outcome do not significantly correlate with changes in FMD adults (Hansell et al., 2004, Shamim-Uzzaman et al., 2002). Indeed, no significant relationship was apparent between PRH and FMD in either exercise trial (r<0.24, P>0.33 for both), or in CON (r=0.39, P=0.12). Therefore, the reduction in PRH is likely to be mechanistically disparate to the concomitant attenuation in FMD.

In order to gain more insight regarding the potential mechanisms influencing the change in microvascular function, the total hyperaemic response following occlusion was calculated as described in Section 3.9.2. This outcome has been shown to be uninfluenced by NO-blockade in adults (Wong et al., 2003), and is therefore considered to be independent of changes in NO bioavailability.

Consequently, the data in Chapter 6 demonstrates that the high fat meal does not impair NO-independent microvascular function as the total hyperaemic response was not attenuated in CON. Furthermore, the total reactive hyperaemia in Chapter 6 suggest that HIIE (and probably MIE; *P*=0.08, *ES*=0.42) may improve microvascular function beyond the putative increase in eNOS activity post exercise. Given the aforementioned findings by Gill *et al*. (2004), it appears that other endothelial-dependent mechanisms are responsible for the microvascular response.

One such mechanism could be an upregulation in prostaglandin release post-occlusion, as Engelke *et al*. (1996) demonstrated a 22% reduction in the PRH response following inhibition of prostaglandins using ibuprofen. Interestingly, the authors reported that this inhibition did not influence the total reactive hyperaemic response. Therefore, this endothelium-dependent vasodilatory response may account for some of the improvement observed in the PRH outcome in Chapter 6, but may not have influenced the findings by Gill *et al.* (2004) as analysis of the iontophoresis technique involves the calculation of the area under the flux versus time curve.

Data from Chapter 7 enables further speculation regarding the potential mechanisms underlying the augmented microvascular response post MIE and HIIE, as the confounding effect of the test meal is removed. Therefore, it is possible that the post exercise microvascular responses are not influenced by greater oxidative stress, as indicated by the lower plasma [TAS] after the high fat meal in all trials in Chapter 6 (main effect of time, *P*=0.04). In this experimental chapter, the total hyperaemic response was greater immediately after, and 1 and 2 hours after both MIE and HIIE which is in line with data presented in Chapter 6. However, the total hyperaemic response, and PRH, were greater immediately after, and 1 hour after HIIE compared to MIE. This effect of exercise intensity on both the total hyperaemic response and PRH outcomes was not observed 1 hour after exercise in Chapter 6, and the discrepancy between these two investigations is not immediately apparent. Using a stepwise regression analysis, Radtke *et al*. (2012) demonstrated that only maturity (and not sex, stature, SBP, BMI or habitual physical activity) was an independent predictor of microvascular reactivity in adolescence. However, the Tanner staging data indicate that the pubertal status of the participants in

Chapters 6 and 7 were broadly comparable. Accordingly, when the microvascular data are pooled for all participants in Chapters 6 and 7 (*n*=37), PRH is greater 1 hour post HIIE compared to 1 hour post MIE (*P*=0.002, 95% CI 0.09 to 0.36, *ES*=0.45). Similarly, the total reactive hyperaemic response is greater 1 hour post HIIE compared to 1 hour post MIE (*P*=0.02, 95% CI 6 to 64, *ES*=0.38). Thus, it appears that these parameters of microvascular function are positively associated with exercise intensity.

Given that Gill *et al*. (2004) reported that the favourable effect of prior exercise on postprandial microvascular function is endothelium-dependent, and that NO-blockade (L-NMMA) (Wong et al., 2003) and prostaglandin inhibition (ibuprofen) (Engelke et al., 1996) do not influence that total hyperaemic response post occlusion, it is likely that other endothelial vasoactive substances (such as endothelium-derived hyperpolarising factor) play a substantive role in the observed post-occlusive response. Currently, no data are available identifying the contribution of endothelium-derived hyperpolarising factor to the reactive hyperaemic response. Interestingly, although L-NMMA (Wong et al., 2003) and ibuprofen (Engelke et al., 1996) fail to modify the post-occlusive microvascular response, Engelke *et al*. (1996) demonstrated a 33% reduction in the total hyperaemic response when the NO-blockade is administered after ibuprofen. Therefore, it has been argued that prostaglandin inhibition might “unmask” a role for NO in the reactive hyperaemic response.

Finally, it is pertinent that Gill *et al*. (2004) reported that the magnitude of the decrease in postprandial endothelium-independent reactivity (i.e. following the iontophoresis of sodium nitroprusside) was blunted following exercise compared to a non-exercise control group. Therefore, prior exercise might preserve endothelial-independent vasoactive pathways, including the involvement of sensory nerves in the axon reflex response (Cracowski et al., 2006). Indeed, Larkin and Williams (1993) demonstrated a 72% reduction in the post-occlusive hyperaemia response following combined sensory nerve and prostaglandin inhibition. Consequently, the microvascular outcomes in this thesis probably represent a complex interaction of a number of endothelium dependent and independent mechanisms. Currently, the specific contributions of NO, endothelium-derived hyperpolarising factor, prostaglandins and sensory nervous input are not known. However, given that changes in these

microvascular outcomes failed to correlate with FMD, which is in accordance with data in adults (Hansell et al., 2004, Shamim-Uzzaman et al., 2002), and that the earliest detectable manifestation of the metabolic syndrome may reside in the micro- and not macro-vasculature (Pinkney et al., 1997), the inclusion of microvascular function Chapters 6 and 7 is both novel and pertinent, even in the absence of a comprehensive mechanistic understanding.

9.2.6 Autonomic function

A novelty of Chapter 8 is the analysis of the ECG-gated FMD images (during the 90 seconds prior to occlusion) to determine HRV as a measure of autonomic function. Low HRV is associated with clustered CVD risk in adolescents (Farah et al., 2014, Zhou et al., 2012), and improvements in autonomic function following exercise have been proposed to explain some of the ~ 40% "gap" in the benefit of exercise on CVD risk (Green et al., 2008, Joyner and Green, 2009). Given that normative values for the frequency and time domain analyses of HRV are now available for adolescent boys (Farah et al., 2014), this measure is an attractive outcome to determine how exercise might modulate CVD risk.

The improvement in HRV observed in Chapter 8 is consistent with favourable changes in autonomic function following two weeks of HIIT in adults (Kiviniemi et al., 2014). These authors included frequency domain analyses, which indicate that the improvements in HRV are likely related to an increase in vagal or baroreflex-modulated modulation of the sino-atrial node. Accordingly, nine weeks of HIIT has also been demonstrated to improve post exercise parasympathetic activity in adolescent boys (Buchheit et al., 2008).

Improvements in cardiac vagal modulation 2 days after a single bout of HIIE have been attributed to an increase in plasma volume (Buchheit et al., 2009), and these authors speculated that the subsequent baroreflex activation would inhibit sympathetic activation and promote parasympathetic outflow. However, this is not a consistent finding (Convertino, 2003), and no increase in plasma volume following HIIT was apparent in Chapter 8.

A potential mechanism underlying the improved HRV post training in Chapter 8 is an increase in basal NO bioavailability, as NO has been shown to directly influence cardiac vagal function (Chowdhary et al., 2000). Indeed, the RMSSD statistic has been shown to significantly correlate with FMD in apparently healthy adult males (r=0.39, P<0.01), whilst genetic variations in the eNOS gene have been demonstrated to explain part of the inter-individual variability in the observed parasympathetic adaptations post training (Silva et al., 2011). However, fasted HRV was not related to FMD before the HIIT intervention (r=0.15, P=0.64) in Chapter 8. Furthermore, the change in fasted HRV was not related to the change in FMD following HIIT (r=0.11, P=0.71), and the improvement in postprandial FMD the day after training was not mirrored by a concomitant improvement in postprandial HRV. Thus, the data presented in Chapter 8 cannot provide any understanding regarding the mechanism(s) responsible for the favourable change in fasted HRV. Greater insight would likely be provided by in the inclusion of time and frequency domain analyses of HRV. Additionally, it is not clear why this benefit of was lost in the postprandial period. Data in adults demonstrate that postprandial vagal withdrawal is more pronounced when the test meal (~ 40 g fat) is consumed 3 hours after 60 minutes of cycling at 70% $\dot{V}O_{2\ max}$ (Charlot et al., 2011), however no study has identified if changes in postprandial HRV are observed the day after exercise. Greater insight would likely be provided by in the inclusion of time and frequency domain analyses of HRV.

9.2.7 Exercise enjoyment

It could be argued that the “best” exercise for the primary prevention of CVD is that which is most enjoyable, and thus most likely to be repeated daily and incorporated into a lifestyle. Indeed, the perceived enjoyment of exercise is known to mediate the success of school-based physical activity interventions in adolescents (Dishman et al., 2005). Therefore, the meaningfully higher PACES score for HIIE compared to MIE for both boys and girls in Chapters 4, 6 and 7 is a pertinent finding. This is also encouraging given the comparable (e.g. TAG) and mostly superior health benefits observed post HIIE compared to MIE in all experimental chapters, and the improvements in novel CVD risk factors

following HIIT in Chapter 8. Furthermore, Chapter 8 demonstrates that a school-based, low-volume, HIIT programme is feasible and effective with this age group. Taken together, these findings add to a growing body of literature indicating that HIIE may be an attractive alternative to continuous moderate-intensity exercise (Buchan et al., 2011a, Crisp et al., 2012, Ratel et al., 2004).

Given that the exercise bouts were consistent between Chapters 4, 6 and 7, pooling the modal score for each PACES question might provide some insight regarding why HIIE was perceived to be more enjoyable than MIE in these investigations (Figure 9.4, *n*=60). This analysis highlights that the greatest disparity between the exercise trials was that participants frequently reported the MIE bout to be more "boring" than HIIE. Considering that the duration of the HIIE and MIE bouts were broadly comparable across all studies (23 ± 0 minutes compared to 26 ± 3 minutes, respectively), the HIIE may have been perceived to be less "boring" due to its intermittent nature. This conclusion is consistent with findings by Crisp *et al*. (2012), who demonstrated that normal weight and overweight 8 – 12 year old boys preferred cycling at an intensity equivalent to the maximum rate of fat oxidation for 30 minutes when 4 seconds of sprint intervals were included every 2 minutes. It could also be argued that the intermittent nature of exercise at a high-intensity better reflect the nature of physical activity performed by adolescents and children than continuous MIE (Riddoch et al., 2007, Trost et al., 2002). Indeed, time spent performing invasion-game sports, which are typically characterised by repetitive high-intensity running (Stroyer et al., 2004), are a major contributor to total physical activity levels in adolescents (Gordon-Larsen et al., 2000, Sallis et al., 2000), and accelerometer data identify that very few adolescents sustain exercise for longer than 10 minutes (Riddoch et al., 2007). Thus, it is perhaps not surprising that the continuous MIE bout was considered to be more "boring" than HIIE given that adolescents do not typically choose to perform this type of exercise. Indeed, it has been reported that intermittent MIE is perceived to be more enjoyable and "achievable" than continuous MIE in obese adolescents (Daley et al., 2008). Consequently, this pattern of activity is probably important in promoting adherence to exercise interventions in adolescent populations. It would therefore be interesting to identify the perceived enjoyment of HIIE compared to a work-matched bout of intermittent MIE, however the data in this

thesis demonstrate that the health benefits of the former would likely be superior. Considering that the intermittent nature of HIIT appears to be important for maximising physiological adaptations in adults (Cochran et al., 2014), it is encouraging that this type of exercise may be more enjoyable for adolescents.

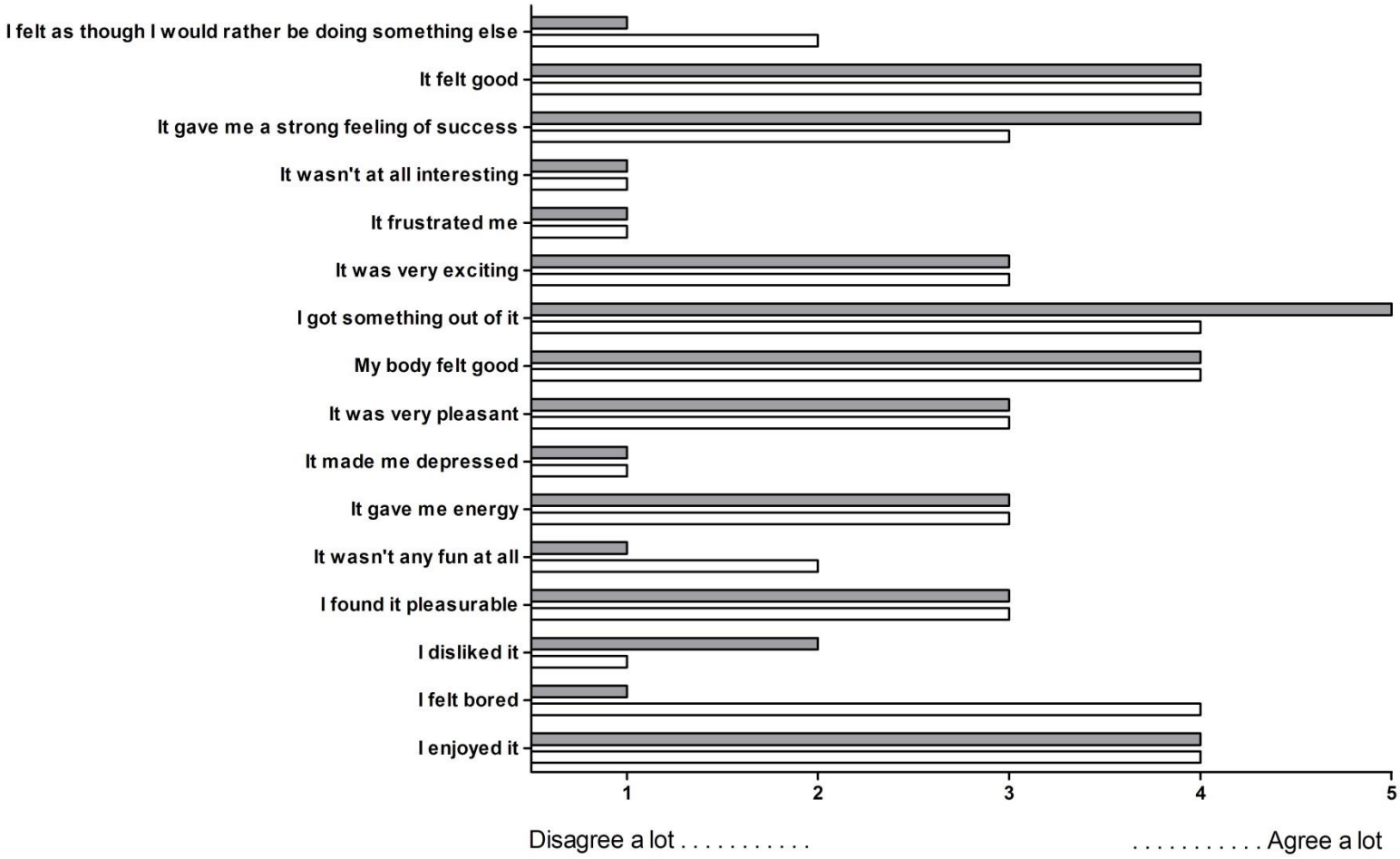

Figure 9.4 Modal answers to the physical activity enjoyment questionnaire following high-intensity interval (grey) and moderate intensity (clear) exercise. Data are pooled from Chapters 4, 6 and 7 (boys *n*=30, girls *n*=30). Of these participants, 25 boys and 24 girls preferred the high-intensity interval exercise.

A further interesting finding highlighted in Figure 9.4 is that the modal answers for “It gave me a strong feeling of success” and “I got something out of it” were greater in HIIE (agree and strongly agree, respectively). It therefore appears that participants perceived a greater sense of achievement following the HIIE compared to the MIE bout. This is important as motivation to exercise may be positively associated with intrinsic reward and feelings of personal achievement in adolescents (Craggs et al., 2011), which is a correlate of habitual physical activity levels in 4-18 year olds (Van Der Horst et al., 2007).

In contrast to Chapters 4, 6 and 7, the PACES data from the accumulated HIIE and MIE trials in Chapter 5 were comparable (62 ± 12 and 62 ± 9 respectively, *P*=0.93, *ES*=0.03; data are pooled for sex). MIE was perceived to be more “boring” than HIIE in Chapters 4, 6 and 7, however data from Chapter 5 indicates that this effect was lost, probably due to the very brief nature (< 7 minutes) of the accumulated exercise bouts (Figure 9.5). Additionally, the greater sense of achievement previously observed in the HIIE trials in Chapters 4, 6 and 7 was not apparent in Chapter 5, and this is also probably a function of the reduction in uninterrupted exercise time.

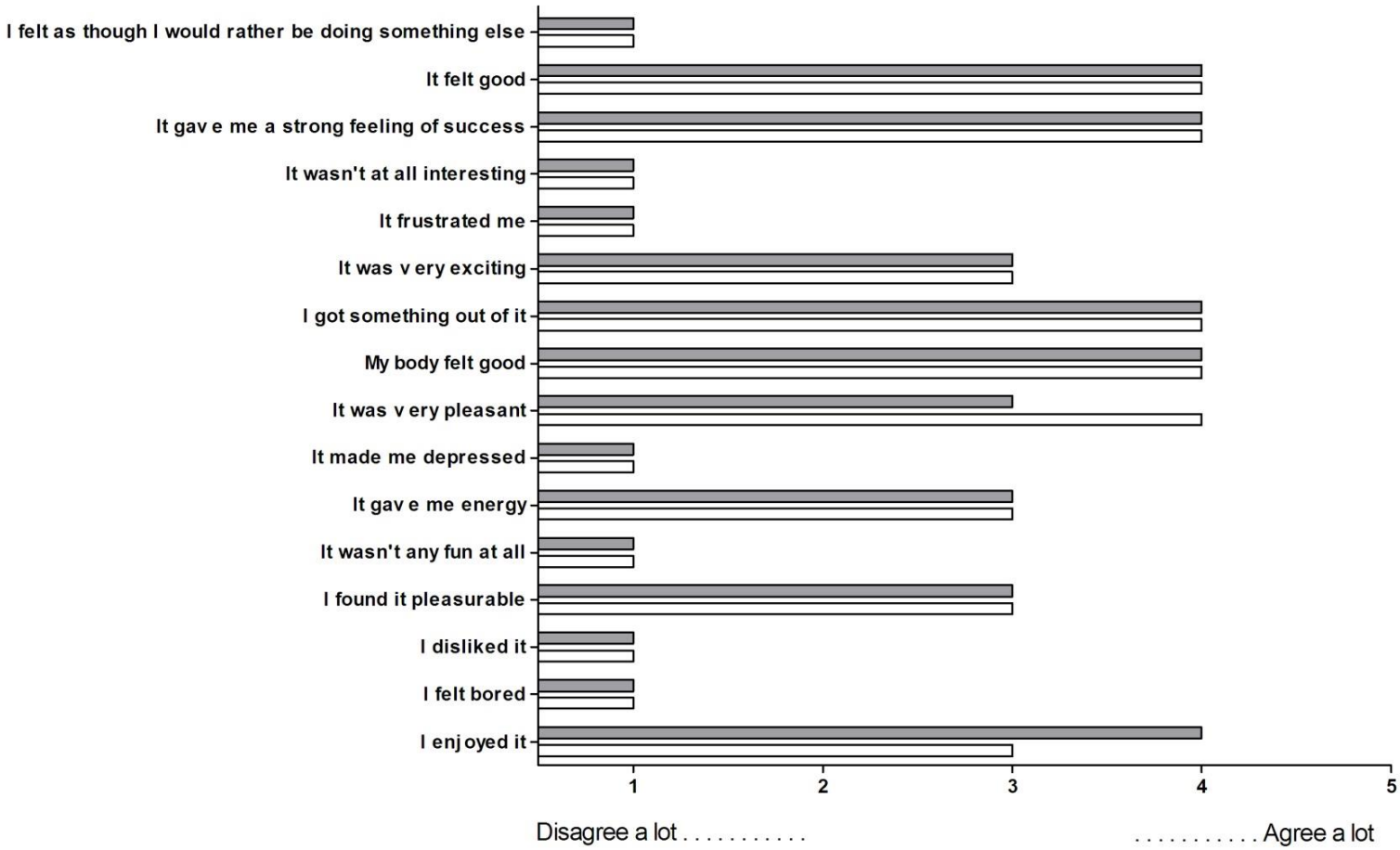

Figure 9.5 Modal answers to the physical activity enjoyment questionnaire following high-intensity interval (grey) and moderate intensity (clear) exercise in Chapter 5 (boys *n*=9, girls *n*=10). Of these participants, 8 boys and 3 girls preferred the high-intensity interval exercise.

9.3 Methodological considerations and areas for future investigation

The trade-off between the validity and precision of a study design, and the feasibility of an investigation is a well-established challenge for research scientists. This compromise is exaggerated when working with paediatric populations due to ethical restraints and limitations in non-invasive techniques. Consequently, whilst the experimental chapters of this thesis are consistent with a number of methodological strengths (e.g. Tanner staging, control of prior physical activity and diet, work-matching of HIIE and MIE, allometric scaling of the FMD technique), these data should be considered in light of a number of limitations.

Firstly, the inclusion of adolescent girls was an important goal of this thesis as this population is typically under-represented in the postprandial literature. This is a pertinent issue because CVD is the leading cause of death in women (WHO, 2011) and adolescent girls are consistently reported to be less physically active than their male counterparts (Riddoch et al., 2007, Trost et al., 2002). Additionally, sex is known to influence postprandial lipaemia (Cohn et al., 1988, Couillard et al., 1999) and FMD (Benjamin et al., 2004). However, the menstrual cycle has been shown to have a confounding influence on these outcomes (Gill et al., 2005, Hashimoto et al., 1995), and this was not accounted for in any of the experimental chapters. Furthermore, in the girls who had achieved menarche, the potential influence of an irregular menstrual cycle cannot be ruled out. Therefore it could be argued that further studies which control for menstrual cycle phase are needed in order to explicitly identify the influence of sex on the outcomes included in this thesis. The girls were also at an advanced maturity compared to boys, meaning advanced biological age may account for some of the sex differences (e.g. postprandial lipaemia in Chapter 4), and this remains to be examined.

The test meal utilised in Chapters 4, 5 and 6 replicated the high fat meal provided in other postprandial investigations in adolescents (Tolfrey et al., 2012, Tolfrey et al., 2008, Tolfrey et al., 2014a), facilitating comparison between studies. Furthermore, the amount of fat this meal provided (1.5 $g \cdot kg^{-1}$, or approximately 75 g) is broadly consistent with the extant adolescent (Tolfrey et al., 2014b) and adult (Freese et al., 2014) evidence base. This meal provides a

metabolic “challenge”, designed to exaggerate the possible changes in postprandial lipaemia following an exercise bout so that a chronic process can be observed in an acute setting. However, the ecological validity of this fat load is questionable. Whilst alarming evidence indicates that 30% of adolescents in the United States of America consume high fat “fast food” at least 3 times per week, and that this number might be rising (Bauer et al., 2009), the inclusion of more representative meals are warranted. Furthermore, given that most of the waking day may be spent in the postprandial state, it is pertinent to address the influence of multiple meals on markers of postprandial health. Whilst this was a key feature of Chapter 5, this study was not able to include a direct assessment of macro- or micro-vascular function, or autonomic function.

The mechanisms underlying the attenuation in postprandial lipaemia following exercise remain to be fully elucidated, and the data presented in this thesis are unable to precisely identify the mechanism(s) underlying the favourable influence of exercise observed in adolescents (Tolfrey et al., 2014b). Postprandial lipaemia was only significantly lowered in Chapter 4, and this reduction was not related to substrate oxidation during the exercise or postprandial period. It was not possible to determine changes in lipoprotein lipase activity post exercise, although evidence in adults indicates that this is not a pre-requisite for an exercise-induced attenuation in postprandial lipaemia (Ferguson et al., 1998, Peddie et al., 2012) and is unlikely to have influenced the postprandial response in the immediate hours post exercise (Seip and Semenkovich, 1998, Zhang et al., 2002). Additionally, it has been argued that the major determinant of postprandial lipaemia is $VLDL_1$, rather than the total VLDL pool (Tan et al., 1995), and this lipoprotein fraction was not quantified in any of the experimental chapters. However, Gill *et al*. (2007) demonstrated a strong and inverse correlation between the change in plasma [3-OHB] and the exercise-induced fall in [$VLDL_1$] (r=-0.72, P=0.02), and this outcome was therefore included in Chapter 6. Barring venous blood sampling for the serial measures of specific lipoprotein fractions, the inclusion of plasma [3-OHB] in future work would likely provide useful insight into hepatic-mediated changes in plasma [TAG]. Alternatively, plasma FA kinetics and plasma lipoprotein fractions can be determined non-invasively and *in vivo* using stable isotope tracers and nuclear magnetic resonance spectroscopy, as adopted elsewhere in

adults (Magkos et al., 2006). Thus, the inclusion of these techniques may provide greater insight regarding the appearance and removal of plasma [TAG] following exercise bouts of different intensities.

A key mechanistic link between elevated plasma [TAG] and atherosclerosis is thought to be the vascular insult caused by a oxidative stress (Bae et al., 2001, Sies et al., 2005) and the oxidative modification of LDL particles (Steinberg, 2009). Oxidative stress refers to a "balance" of free radicals and antioxidants, and thus the global redox state is difficult to infer from the measure of a single antioxidant and/or free radical. However, it was not feasible to measure several individual free radicals or antioxidants in this thesis. Consequently, a commercially available analysis kit was used Chapter 6 to identify changes in postprandial plasma [TAS] following exercise, and this is in line with a key adult study in this field (Tyldum et al., 2009). However, this outcome only provides a broad measure of the numerous complex interactions underlying the pro/anti-oxidative state, and therefore cannot provide insight regarding the roles of specific free radicals and their counterpart buffers on endothelial function. Accordingly, we quantified plasma [GTP] in Chapter 8, as data in adults demonstrates an inverse relationship between this antioxidant, postprandial lipaemia and endothelial function (Tsai et al., 2004), and evidence suggests that plasma [GTP] may play a role in the prevention of atherosclerotic plaque accrual (Lapenna et al., 1998). However, no differences in plasma [GTP] were apparent between visits, which is consistent with recent postprandial data in adults (Canale et al., 2014). Therefore, the data presented in this thesis are not able to identify how postprandial lipaemia might influence parameters of vascular health via oxidative stress. Further research using a more sensitive assay to lipid peroxidation, such as thiobartituric acid reactive substances and 8-epi-prostaglandin-$F_{2\alpha}$ (Wallace et al., 2010) is warranted.

The positive association between exercise intensity and FMD (at least 1 hour post exercise) in Chapters 6 and 7 was attributed to an upregulation in eNOS activity due to a greater shear stress during HIIE compared to MIE. However, whilst this is consistent with data in adults (Green et al., 2002, Thijssen et al., 2009a), it is a limitation that neither the magnitude, nor pattern of brachial shear was quantified in either of these experimental chapters. Furthermore, given that exercise mode influences shear pattern (Thijssen et al., 2009a), and that the

balance between antegrade and retrograde shear is an important determinant of the acute (and probably chronic) endothelial response (Thijssen et al., 2009b), the findings that HIIE was superior to MIE in the hours after exercise cannot be extrapolated beyond cycling exercise. For example, leg kicking exercise is consistent with a greater retrograde shear profile than walking and cycling (Thijssen et al., 2009a). Therefore, the influence of swimming intensity on vascular function in adolescents is an appealing area of future research, especially given that 64% of girls and 57% of boys aged 11-15 would like to swim more (Health, 2007).

In accordance with current guidelines (Harris et al., 2010, Thijssen et al., 2011), the FMD statistic was allometrically scaled and the shear stimulus was presented in Chapters 6, 7 and 8. However, these studies did not assess changes in endothelium-independent vasodilation via the sublingual administration of nitroglycerine, which would provide information regarding whether improvements in smooth muscle function and arterial compliance were playing any role in the observed changes in FMD (Corretti et al., 2002). Furthermore, this thesis cannot provide information regarding the influence of an acute bout of MIE and HIIE, or HIIT, on several other important determinants of vascular health, including endothelial progenitor cells, vascular endothelial growth factor and endothelial adhesion markers. Given that the number of endothelial progenitor cells independently predicts endothelial function in children (Bruyndonckx et al., 2014), and that these numbers can be increased following a single 20 minute bout of HIIE (Zaldivar et al., 2007) and after 12 weeks of combined aerobic and resistance training (Park et al., 2012) in paediatric groups, studies identifying how these vascular health outcomes are influenced by exercise intensity would be mechanistically insightful.

As discussed in Section 9.2.5, the mechanisms underlying the post-occlusive hyperaemic response in the cutaneous circulation are not well defined. In comparison, laser Doppler imaging combined with the iontophoresis of acetylcholine or sodium nitroprusside is an established technique to determine endothelium dependent and independent microvascular function respectively (Ferrell et al., 2002). Therefore, whilst the assessment of microvascular function during the FMD protocol is a strength of Chapters 6 and 7, further work is needed to elucidate the mechanisms responsible for the observed changes.

Similarly, the analysis of HRV from the ECG-gated images captured during the 1.5 minutes prior to cuff occlusion in Chapter 8 provides an important outcome regarding HIIT and CVD risk. Yet this length of observation is insufficient for the analysis of frequency domains (Task force of the European Society of Cardiology, 1996), which provide more information regarding sympathovagal balance. Despite this limitation, it is understood that the time domain analysis of HRV is strongly correlated (r=0.85) to each frequency domain variable (Kleiger et al., 1991). Therefore, the time domain assessment of HRV might provide a surrogate for frequency domain measures (Stein et al., 1994).

Given that Chapters 4, 5, 6 and 7 all demonstrate an independent effect of exercise intensity on the majority of vascular health outcomes studied, a limitation of Chapter 8 is the lack of a group randomised to two weeks of work-matched MIE. However, existing data in 12 year olds demonstrate that time spent performing vigorous, but not moderate, intensity physical activity is favourably related to HRV indices (Buchheit et al., 2007). Furthermore, 2 weeks (6 sessions) of HIIT has been shown to provide superior improvements in HRV than aerobic endurance training in adults (Kiviniemi et al., 2014). The inclusion of a non-exercise control group in Chapter 8 would also provide information regarding the coefficient of variation for each outcome, enable any changes post HIIT to be attributable to the intervention, and identify the influence of growth and maturation. However, the influence of the latter is unlikely in to be meaningful within two weeks, and the lack of a control group is consistent with other adolescent (Barker et al., 2014) and adult (Burgomaster et al., 2008, Whyte et al., 2010) 2 week HIIT interventions.

Collectively, the experimental chapters in this thesis demonstrate that performing HIIE is important for vascular health outcomes, however the optimal HIIE or HIIT protocol remains unclear. For example, Chapter 8 demonstrates that 2 weeks of HIIT does not improve traditional risk factors or aerobic fitness in adolescents. In contrast, 2 weeks of repeat sprint cycling can improve fitness in this group (Barker et al., 2014). Similarly, this study cannot comment on the feasibility or adherence of a longer intervention involving high-intensity interval cycling, or whether this intervention would have been more effectual during the winter months when FMD is lower (Hopkins et al., 2011). Preliminary data in adolescents indicate that longer HIIT interventions are feasible, and provide

similar benefits as MIE training despite an 85% lower training volume (Buchan et al., 2011a). However, no study has included a work-matched exercise training design to isolate the influence of exercise intensity on cardiovascular risk factors in this group, and this remains a pertinent area for future research. Furthermore, data in adults indicate that 6 weeks of HIIT is more effectual than a comparable continuous high-intensity training intervention (Cochran et al., 2014). These authors suggest that the intermittent nature of the HIIE bouts plays an important role in the subsequent training response, thus it would be interesting to compare HIIT against an equivalent moderate-intensity interval exercise training intervention.

Further work is also needed to identify the minimal amount of exercise for cardiometabolic health in adolescents, which might be as little as 3 minutes of sprint cycling per week according to data in adults (Metcalfe et al., 2012). However recent work with adolescent boys indicates that repeat sprint cycling may be consistent with unpleasant side effects (vomiting) and low adherence rates (Sedgwick et al., 2014). Therefore, studies are needed to identify a HIIE protocol which provides optimal health benefits with minimal risk and dropout rates in adolescents.

Finally, apart from age (<11 years and >16 years), the presence of relevant allergies, any contraindications to exercise or the use of any relevant medications and supplements, there were no specific exclusion criteria for these studies. It is not unlikely that the recruitment process for the studies in this thesis resulted in a biased sample, i.e. one where BMI, aerobic fitness and habitual physical activity are not representative of the wider adolescent population. Table 9.1 describes the participant characteristics for each study, and uses recent age-appropriate cut points to determine how many boys and girls were overweight or obese (Cole et al., 2000) and how many failed to achieve the minimum recommended level of aerobic fitness for cardiometabolic health (Adegboye et al., 2011). Recent data from the UK Department of Health indicate that ~ 30% of adolescent boys and girls are overweight based upon normative British growth curves (Health, 2013), whilst current evidence suggests that ~ 40% of adolescents are “unfit” (Bailey et al., 2012). Finally, the most recent objectively determined physical activity data by the Department of Health report that 7% of boys and < 1% of girls aged 11 – 15 years achieve at

least 60 minutes of moderate to vigorous physical activity per day (Department of Health, 2008). Consequently, the majority of participants can be characterised as “asymptomatic”, if not “healthy”, and are probably broadly representative of the wider UK adolescent population. However, HIIT has been demonstrated to improve cardiometabolic risk factors in overweight adolescents (Tjonna et al., 2009) and restore vascular function in obese adolescents (Watts et al., 2004). Furthermore, the improvement in glycaemic control observed in the HIIE, but not MIE, trial in Chapter 5 suggests that the intensity of accumulated exercise may play an important role in controlling blood glucose concentrations in adolescents with type I or II diabetes, and this remains an interesting avenue for future research.

Table 9.1. Participant characteristics across all experimental chapters

	Overweight/Obese	Low fit	< 60 min MVPA day^{-1}
Chapter 4			
Boys (*n* = 10)	1	2	-
Girls (*n* = 10)	1	4	-
Chapter 5			
Boys (*n* = 9)	2	5	7
Girls (*n* = 10)	2	2	8
Chapter 6			
Boys (*n* = 10)	1 (Obese)	5	3
Girls (*n* = 10)	2	2	1
Chapter 7			
Boys (*n* = 10)	3	6	8
Girls (*n* = 10)	0	2	10
Chapter 8			
Boys (*n* = 7)	3	4	3
Girls (*n* = 6)	1	2	5
Total			
Boys (*n* = 46)	10 (22%)	22 (48%)	21 (46%)
Girls (*n* = 46)	6 (13%)	12 (35%)	24 (52%)

Age appropriate cut off points for overweight and low fit determined from (Cole et al., 2000) and (Adegboye et al., 2011), respectively. MVPA, moderate to vigorous physical activity. An ActiGraph GT1M Accelerometer (ActiGraph, LLC, Pensacola, USA) was used in Chapter 5 and interpreted using cut points provided by (Evenson et al., 2008). A GENEActiv wrist-worn accelerometer (GENEActiv, Activinsights Ltd, Cambridge, UK) was used for Chapters 6, 7 and 8, and interpreted using cut points provided by (Phillips et al., 2013). Accelerometer data are not available for Chapter 4 due to data loss and poor compliance. **N.B.** Accelerometer data should be interpreted with caution as participants were instructed to refrain from organised physical activity during accelerometer wear time.

9.4 Practical implications

Given that < 10% of UK adolescents achieve the recommended *minimum* of 60 minutes of moderate to vigorous physical activity per day (Department of Health, 2008, Riddoch et al., 2007), and that interventions are largely unsuccessful in increasing physical activity levels in paediatric groups (Metcalf et al., 2012), it is essential to identify how smaller volumes of exercise can be optimised for the primary prevention of CVD.

This thesis demonstrates, for the first time, that a single bout of HIIE is consistent with either similar (i.e. postprandial lipaemia in girls) or superior cardiometabolic health benefits compared to an equivalent bout of MIE in adolescents. Furthermore, postprandial health benefits are observed when HIIE, but not MIE, is accumulated in brief (< 7 minutes) bouts over the course of the day, which is important as adolescents rarely sustain exercise for longer than 10 minutes (Riddoch et al., 2007). Thus, repeating HIIE may be more effectual than MIE for the primary prevention of CVD across the lifespan.

It is pertinent that HIIE was considered to be more enjoyable than MIE, which is a key determinant of the success of physical activity interventions in this age group (Dishman et al., 2005). Indeed, Chapter 8 adds to a growing body of literature which demonstrate that HIIT interventions are feasible and favourably modulate CVD risk factors in adolescents (Buchan et al., 2011a, Buchan et al., 2013). Therefore, this thesis strongly supports the promotion of time spent performing HIIE in adolescents.

The experimental chapters in this thesis were not designed to elucidate the optimal or smallest volume of HIIE for cardiovascular health. However, the HIIE bouts were 8-10 minutes in length, which is consistent with observational data demonstrating that just 7 minutes of vigorous physical activity per day is necessary for cardiometabolic health (Hay et al., 2012). Given that this exercise stimulus may be performed either in a single bout or over the course of the day, these findings are encouraging and may provide a realistic goal for most adolescents.

It has been suggested that schools should play an increasing role in physical activity promotion (Pate et al., 2006), and it is understood that school-based

HIIE interventions are successful provided that staff members are engaged with the process (Buchan et al., 2012). Furthermore, HIIE interventions may require very little equipment (Ratel et al., 2004) and can successfully be incorporated into physical education classes to increase time spent performing vigorous physical activity in adolescent boys and girls (Fairclough and Stratton, 2005). Therefore, it initially appears that schools could facilitate a meaningful increase in the total time spent performing vigorous physical activity in adolescents, even if interventions to promote moderate to vigorous physical activity in children only have a small effect (~ 4 minutes) on increasing physical activity (Metcalf et al., 2012).

Importantly, evidence indicates that UK adolescents are most active in the hour immediately after school, before becoming more sedentary in the late afternoon and evening (Riddoch et al., 2007). Therefore, optimising the physical activity performed in the after school period might provide the best opportunity to increase the time spent performing vigorous physical activities. Additionally, longitudinal data suggest that the amount of activity performed after school declines from childhood into adolescence (Arundell et al., 2013). Therefore efforts could be made to "protect" this period of the day from sedentary behaviours for the effectual promotion of vigorous physical activities in adolescence. Finally, adolescents are less active at the weekend than during the week (Riddoch et al., 2007). Given that low-volume HIIE is consistent with superior cardiometabolic health benefits than MIE in adults (Nybo et al., 2010), and that parental activity is a correlate of adolescent activity (Van Der Horst et al., 2007), interventions aiming to promote HIIE may wish to include parents and families.

More work needs to be done in order to identify that HIIE may be a safe, as well as effectual, alternative to MIE. There is need for larger studies which assess the feasibility and efficacy of longer HIIE interventions (> 7 weeks) with adolescents. Additionally, given that Chapter 8 is the first to assess endothelial function and HRV following HIIT in asymptomatic adolescents, the benefits of HIIT on these novel CVD risk factors may have been "missed" in other studies. Furthermore, it needs to be established that HIIE interventions are successful in improving cardiometabolic health outcomes in adolescents who are low active and in diseased groups. However, the findings of the experimental chapters in

this thesis are consistent with data identifying that time spent performing vigorous physical activity is more important than moderate-intensity activities for endothelial function (Hopkins et al., 2009), autonomic function (Buchheit et al., 2007) and cardiometabolic risk (Carson et al., 2014, Hay et al., 2012) in adolescents. It is also likely that the findings of this thesis apply to other groups. For example, HIIT has been demonstrated to improve fitness, endothelial function and insulin sensitivity in overweight and obese adolescents (Tjonna et al., 2009), whilst the pilot work by Hulzbos *et al.* (2011) demonstrates that HIIT markedly improved fitness in a patient with cystic fibrosis. Indeed, the superior cardiometabolic improvements following HIIE compared to MIE observed in this thesis are entirely consistent with findings from adult clinical groups (Gibala et al., 2012, Whyte et al., 2012, Wisloff et al., 2007). Thus, it is likely that the data presented in this thesis have significant implications for health promotion beyond asymptomatic adolescents.

In summary, this thesis demonstrates that repeating HIIE might provide superior health benefits than more traditional MIE in adolescents. Furthermore, excluding the warm up and active recovery intervals, the HIIE bouts totalled just 8 minutes. Therefore, if school-based interventions only increase physical activity by ~ 4 minutes (Metcalf et al., 2012), it is important that these are performed at a high-intensity.

9.5 Conclusion

Cardiovascular diseases have their origins in youth. Physical activity plays an important role in lowering future CVD risk, however few adolescents achieve the recommended minimum amount of daily physical activity and interventions fail to meaningfully increase activity levels. Therefore, it is important to understand how lower volumes of exercise might be optimised for cardiovascular health. Chapters 4, 5, 6, 7 are the first to isolate the effect of exercise intensity on several vascular health outcomes in adolescent boys and girls. The data presented in these experimental chapters identify that HIIE attenuates postprandial lipaemia to the same extent as an equivalent bout of MIE in adolescent girls, whilst providing superior improvements in glycaemic control, blood pressure, resting fat oxidation, macro- and micro-vascular health in both

boys and girls. Therefore, the intensity of exercise is positively associated with vascular health outcomes in adolescents. Additionally, HIIE may be more enjoyable than MIE, suggesting that low-volume HIIT might provide a feasible and more effectual alternative to traditional MIE interventions. Indeed, Chapter 8 demonstrates that improvements in novel CVD risk factors (FMD and HRV) are possible following just 2 weeks of HIIT. Therefore, this thesis indicates that repeating HIIE may provide superior vascular health benefits than MIE in adolescents and has laid the groundwork for future investigations in this area.

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Appendix

Appendix 1: Certificates of ethical approval

Chapter 4

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: 2012/314

Title: The influence of a single bout of high intensity interval training (HIT) and moderate exercise on postprandial lipaemia in adolescents

Applicants: Mr Bert Bond with Ms Carly Isic

The proposal was reviewed by the Ethics Committee and was approved from June 2012 until December 2012.

Signature: 

Name/Title of Ethics Committee Administrator: Alison Hume, Student Services Manager

Chapter 5

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: 2012/391

Title: The efficacy of accumulating bouts of high intensity interval exercise throughout the day on postprandial lipaemia and blood pressure in adolescents

Applicants: Mr Bert Bond with Prof Craig Williams and Dr Alan Barker

The proposal was reviewed by the Ethics Committee and was approved from October 2012 until April 2013.

Signature: 

Name/Title of Ethics Committee Administrator: Alison Hume, Student Services Manager

Chapter 6

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: 2013/704

Title: The effect of exercise intensity and sex on postprandial macro- and micro- vascular endothelial function in adolescents

Applicants: Mr Bert Bond with Dr. Alan Barker, Prof. Craig Williams, Ms Lucy Corless

The proposal was reviewed by the Ethics Committee and was approved from 16 May 2013 until 31 May 2014.

Signature:

Name/Title of Ethics Committee Administrator: Alison Hume, Student Services Manager

Chapter 7

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

Title: The acute effect of exercise intensity on vascular function in adolescent boys and girls

Applicants: Bert Bond (PhD student), Dr Alan Barker (primary supervisor), Professor Craig Williams (secondary supervisor)

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

Decision: This proposal has been approved until September 2014.

Signature: Mark Wilson Date: 11/3/14

Name/Title of Ethics Committee Reviewer: Dr Mark Wilson

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

Title: The influence of 2 weeks of low-volume, high-intensity interval training on cardiovascular health outcomes in adolescents

Applicants: Bert Bond

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

Decision: This proposal has been approved until October 2014.

Signature: Mark Wilson Date: 18/3/14

Name/Title of Ethics Committee Reviewer: Dr Mark Wilson

Appendix 2: Standard health screening form

HEALTH SCREEN FOR CHILD VOLUNTEERS (PARENTAL FORM)

Name: ……..

It is important that volunteers participating in research studies are currently in good health amd 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.

Thank you for your cooperation

Appendix 3: Example information pack to participants (Chapter 6)

Parent/Guardian Information Sheet

Project Title: The effect of exercise intensity on blood vessel function after a fatty meal in adolescents

Thank you for expressing an interest towards the ongoing research at the Children's Health and Exercise Research Centre (CHERC), Sport and Health Sciences, University of Exeter. Over the past 25 years, scientists at CHERC have made crucial advances in the fields of paediatric exercise physiology and health promotion, and the department has developed into a world-leading research centre. However, these advances have only been made possible by the enthusiasm of the children who volunteer to take part in the exciting studies at the centre, and the support of their parents/guardians. This letter is an invitation for your child to be included in a study which aims to look at the health benefits of moderate and high intensity exercise.

We will be running a study in the coming weeks, and we are looking for Year 10 girls and boys to volunteer to take part. This may be a great opportunity for those interested in health, exercise and nutrition, and we aim for each study to be as fun and as informative as possible for those who volunteer. It also provides an excellent opportunity for students to experience a University atmosphere and the process of scientific research first hand. Please take time to read the following information carefully before deciding whether or not to let your child be included in this study. We would welcome any questions or concerns you have about this study, so please do not hesitate to contact a member of the research team on the details provided at the end of this information sheet.

1. What is the purpose of the study?

Heart attacks and strokes are the leading causes of death in the UK, and they are attributable to the progression of atherosclerosis (a slow build up of fatty plaques in the blood vessels), a process which begins in childhood. We know that exercise can promote the health of blood vessels in both adults and children, but we don't know which type of exercise (small volumes of high-intensity, intermittent exercise or longer durations of continuous moderate intensity exercise) is best for young people. Current physical activity guidelines of 60 minutes per day are only met by 32% of boys and 24% of girls in the UK. Furthermore, the 2008 Health Survey for England identified that only 7% of boys and <1% of girls aged 11-15 years achieve this amount of physical activity. A lack of time is often cited as a possible reason why so few adolescents meet these guidelines. It is therefore important to identify whether brief, high-intensity exercise can benefit the health of blood vessels in youth. Ultimately, this study will from part of a larger field of research which may help shape future health promotion guidelines for children and adolescents.

2. What does this study involve?

The project will require a total of 4 visits to an exercise laboratory at CHERC, which is situated at St. Luke's Campus on Heavitree Road in central Exeter. Please note, we will pick up your child from school and drop them off either back at school or at home. For clarity, the protocol has been divided into 2 sections; baseline and experimental measures. The details of each visit are provided in this information pack.

Section 1 – Baseline Measures

The first visit will familiarise your child to the laboratory setting and the equipment we will use. Upon arrival, we will measure your child's height, sitting height, body weight and estimate body fatness by measuring their skinfolds at the arm, back and hip. This is performed by very gently pinching the skin and measuring the width of the skinfolds. Your child will also be familiarised with cycling at different intensities on a stationary bike machine until he or she feels confident.

It is important that we control for the influence of pubertal development on the results. Because children start puberty at different times, and mature at different rates, we will ask your child to self assess their pubertal development at home and in private. This will require your child to look at scientific drawings showing 5 stages of pubertal development and to identify which stage best describes their own development before sealing this information into an envelope which we will provide. This is a routine procedure which is frequently performed within this population for scientific research.

Ensuring that your child is comfortable with the equipment and has had the opportunity to ask any questions they may have, your child will then be required to complete an exercise test and a number of measurements will be taken. This will involve pedalling on an exercise bike for as long as possible. This exercise test starts against very light resistance, but gradually the pedals become harder to turn making the test progressively more difficult. This will feel like cycling up a hill which becomes steeper and steeper until your child cannot cycle any further. At this point, they will be allowed to rest and their recovery will be closely monitored. This is a routine exercise test which is well tolerated. However, it is important to stress that it demands a maximal effort and that the final moments of the test will therefore be strenuous for your child.

Section 2 – Experimental Measures

We will collect your child from school at 07:15. It is important that your child has not eaten since 8:00pm the previous evening. **This includes not having a breakfast as this will be provided**. Blood pressure will be monitored, and the amount of fat and antioxidant enzymes in your child's circulation will be measured using a very small amount (less than 1 mL) of blood from the fingertip. This is a routine process which we have frequently performed with this age group with no adverse effects; it will feel like a small pin prick.

We will then provide your child with a breakfast consisting of a bowl of Kellogg's® Cornflakes with semi-skimmed milk. Half an hour after breakfast we will assess the ability of your child's brachial (arm) artery to dilate. This is an indication of blood vessel health – the greater the dilation, the healthier the artery. We will inflate a blood pressure cuff around the forearm, just below the elbow, for 5 minutes. The cuff is then rapidly deflated, and we measure the amount of dilation of the artery using ultrasound. At the same time, we will measure the changes in blood flow in the smaller blood vessels of the forearm. This process involves shining a laser onto the forearm skin and recording the light reflected back. This process is considered to be safe, and is non-invasive and pain-free. We will provide your child with their scores at the end of the study.

One hour after they have consumed breakfast, you child will either (1) perform some high intensity cycling, (2) cycle at a moderate intensity or (3) rest for half an hour. Each of these conditions will be separated by at least one week and performed in a random order.

1) High intensity cycling

This will involve cycling for 60 seconds at 90% of the resistance achieved at the end of the initial fitness test performed during Visit 1, before cycling at a very light resistance for 75 seconds. This pattern of intense cycling, followed by active recovery will be repeated for a total of 8 repetitions, before a cool down period. This exercise attempts to replicate the vigorous nature of game activities and will last a total of 23 minutes (including the rest intervals). This type of exercise is well tolerated by children, and your child will be closely monitored throughout the session.

2) Moderate intensity cycling

This exercise bout will last for approximately 20 minutes and will be set at a resistance equating to roughly 50% of the maximum resistance achieved during the initial test performed on Visit 1. As such, this should feel quite easy.

3) Rest

Your child may read a book, watch a film or complete school work during this time provided that they remain inactive.

One hour after your child has completed the exercise/rest condition, we will re-assess the ability of the brachial artery to dilate using ultrasound, before providing them with a milkshake comprised of Cornish ice cream and double cream. Once this has been consumed we will ask your child to rest for a total of 3 hours in the lab. When at rest, your child may complete homework or coursework, revise, watch a film or play computer games provided that they are inactive. No other food may be consumed during this period, however water will be freely available. Less than 1 mL of blood will be collected from your child's

finger tip every hour until the end of the 3 hour duration, whereupon we will perform the final ultrasound assessment. Blood pressure will also be measured at these times. Your child will finish the day by 2:30pm and can be collected from our lab, or we can drop them home or back to school by 3:00pm.

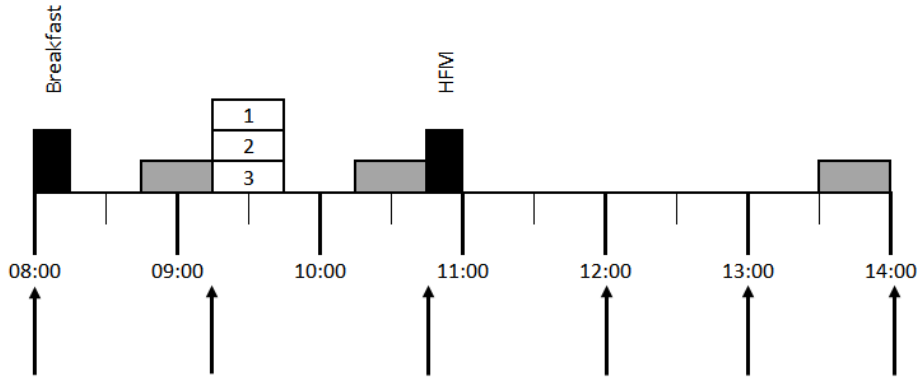

Figure 1. Outline of the day. The arrows represent the small amount of blood (<1mL) taken from the fingertip to measure fat and antioxidant enzyme content. The grey boxes indicate when we will be scanning the brachial (arm) artery using ultrasound. 1 = rest; 2 = moderate-intensity exercise; 3 = high-intensity intermittent exercise.

We will require your child to wear an accelerometer (which is a small device worn at the hip and measures amount and type of physical activity) and complete a food diary for the 48 hour period prior to visiting the lab. The food diary should include the amounts of all items which have been eaten or drunk during this time. It is also essential that the evening meal your child consumes is replicated as closely as possible for the evenings of the other 2 conditions as this may affect the results.

3. What else will my child have to do?

If your child wishes to take part, we would ask that they refrain from strenuous exercise during the day before arriving at the laboratory. Your child will also need to bring suitable kit for the exercise sessions, and some schoolwork or a DVD to watch during the 3 hour rest and observational period. We also have a Sony Playstation 3 and desktop computers with age-appropriate games and educational software which your child may play on during the rest period. We would also encourage your child to ask as many questions as they please; we hope that their visit to the laboratory inspires them to think about their health and the potential of higher education.

4. What are the possible risks of my child taking part?

The testing procedures and exercise sessions are both safe and routine amongst children and adolescents. An assessment of your child's health will be made before any involvement to confirm it is safe for them to take part. Your child will be carefully monitored and observed to ensure their safety and well-being throughout all testing. Please note that by signing the statement of informed consent and by allowing your child to take part, you are confirming the absence of any allergies which are relevant to the Cornflake breakfast and

milkshake (e.g. nut allergies, lactose intolerance). The present study has been thoroughly reviewed and granted ethical approval by the departmental ethics committee within Sport & Health Sciences, University of Exeter. All measures are considered to be non-invasive and pain-free.

5. What are the potential benefits of my child taking part?

The main aim of this study is to develop our understanding of the benefits of different types of exercise in children and adolescents. Ultimately this project forms part of a broader research theme which hopes to identify how we can best promote and protect the health of our children as they develop into adults. Whilst this may not immediately benefit your child, we hope that your child will enjoy their time in the University laboratories and the chance to be part of a scientific edge study. At CHERC, we pride ourselves on ensuring that each volunteer has an enjoyable and informative experience throughout every research project. We hope that we can inspire your child to take an interest in their health and in the science of exercise, and that this project proves to be both interesting and fun.

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

Your child's 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 child's 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 child's individual data with a full explanation of what it represents should you so wish.

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

If your child would like to take part in the study you must give your permission by completing the following forms which are included in this information pack:

- The parental consent form
- The child assent form (signed by your child)
- The personal information form
- The health screen questionnaire

You should then return these forms to Miss V Gill in the P.E. Department. We will then make contact with you about arranging your child's first visit. Any questions should be forwarded to Bert Bond (the principle investigator) without hesitation.

Taking part is entirely voluntary and it is up to you to decide whether or not your child is involved. If you do allow your child to take part you are still free to withdraw your child at any time, without giving a reason.

Parent/Guardian Consent Form

I have read the information sheet 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.

I understand that:

- My child will perform an incremental cycle test to exhaustion.
- My child will participate in 3 different trials: a rest trial, a moderate exercise trial, and a high intensity interval trial at the Children's Health and Exercise Research Centre.
- My child will have their height, weight and body fat measured.
- My child will assess their pubertal status according to 5 drawings of secondary sexual characteristics. The purpose of this has been made clear to me.
- Fingertip blood samples will be taken.
- My child will have to consume a breakfast of Kellogg's® Cornflakes with semi-skimmed milk, and a specially prepared milkshake (consisting of ice cream and double cream). My child will not have eaten breakfast.
- I can confirm the absence of any food allergies related to this study.
- My child will be asked to record dietary information and consume the same meal no later than 8:00pm before each trial.
- I am free to request further information at any stage.

I know that:

- My child's participation in the project is entirely voluntary and my child is free to withdraw from the project at any time without giving reason or affecting his relationship with either the research team or the school.
- The results will be stored on computer in coded form and individual results will be confidential to the Children's Health and Exercise Research team.
- The results of the project may be published but my child's anonymity will be preserved.

Signed …….. Date ……..……

On behalf of my child ……..

Assent form for Participant

Name……

I agree to take part in the study as described in the enclosed information sheet. The study has been clearly explained to me.

I understand that:

- I will complete a maximal exercise test to exhaustion on my first visit.
- I will participate in 3 different trials: a rest trial, a moderate exercise trial, and a high intensity interval exercise trial at the Children's Health and Exercise Research Centre.
- I will have my height, weight and body fat measured.
- I will need to assess my pubertal status according to 5 drawings of secondary sexual characteristics. The purpose of this has been made clear to me.
- Fingertip blood samples will be taken.
- I will have to eat a bowl of Kellogg's® Cornflakes with semi-skimmed milk, and a specially prepared milkshake. I am not allergic to any of these foods.
- I will be asked to record dietary information. I must also eat the same meal before each visit, and this must be eaten by 8:00pm.
- I am free to ask any questions at any time.

I know that:

- I can withdraw from the study at any time without giving a reason or affecting my relationship with either the research team or the school.

Signed ……

Date ……

Appendix 4: Food diary (used in all experimental chapters)

Food Diary

Name:

Day 2

Date:

Was today a normal eating day? YES NO

Time	Food and Drink	Measured amount (e.g. 1 tsp, 1 apple etc)	Brand/Description
Breakfast			
Mid-morning Snack			
Lunch			
Afternoon Snack			
Dinner			
Evening Snack			

Day 2

Date:

Was today a normal eating day? YES NO

Time	Food and Drink	Measured amount (e.g. 1 tsp, 1 apple etc)	Brand/Description
Breakfast			
Mid-morning Snack			
Lunch			
Afternoon Snack			
Dinner			
Evening Snack			