Effect of acute exercise and diet manipulations on postprandial metabolism in boys and girls

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EFFECT OF ACUTE EXERCISE AND DIET MANIPULATIONS ON POSTPRANDIAL METABOLISM IN BOYS AND GIRLS

by

Alice Emily Thackray

A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of
Doctor of Philosophy of Loughborough University

October 2014

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ABSTRACT

Elevated postprandial triacylglycerol concentrations ([TAG]) are associated with the development and progression of atherosclerosis, and are established as an independent risk factor for future cardiovascular disease. Considering the majority of the daytime is spent in a postprandial state typically, and the paediatric origins of atherosclerosis are well established, lifestyle interventions including manipulations of exercise energy expenditure and dietary energy intake should be initiated early in life. Therefore, this thesis aimed to investigate the postprandial metabolic responses to different exercise and energy intake manipulations in boys and girls, with concentrations of circulating TAG representing the primary outcome of interest. To achieve this, a total of 60 healthy 11 to 13 year old boys and girls were recruited into five experimental studies.

The first experimental study (Chapter 4) demonstrated that a single session of high-intensity interval running (HIIR) involving 10 × 1 min intervals at 100% maximal aerobic speed (MAS) resulted in a moderate reduction in postprandial plasma [TAG] in 11 to 12 year old boys. In the second experimental study (Chapter 5), immediate replacement of the moderate-intensity exercise-induced energy deficit negated the reduction in postprandial plasma [TAG] in 11 to 13 year old boys. Furthermore, an exercise-induced energy deficit was required to promote an increase in whole-body fat oxidation. The importance of the associated energy deficit was explored further in Chapter 6, which demonstrated that a moderate-intensity exercise-induced energy deficit elicited a greater reduction in postprandial plasma [TAG] than an isoenergetic diet-induced energy deficit in 11 to 13 year old girls (21% vs. 10% respectively). Chapter 7 compared the effect of 10 × 1 min interval runs at 100% MAS (HIIR) and 5 × 1 min interval runs at 100% MAS combined with a mild reduction in habitual energy intake by 0.82 MJ (195 kcal; HIIR-ER) on postprandial metabolism in 11 to 13 year old girls. Acute manipulations of low volume HIIR and ER reduced postprandial plasma [TAG] and increased resting whole-body fat oxidation, with the magnitude of effect marginally, although not meaningfully, greater following HIIR than HIIR-ER. The final experimental chapter (Chapter 8) compared directly healthy 11 to 13 year old boys’ and girls’ postprandial TAG responses to acute HIIR. Although postprandial plasma [TAG] was substantially lower in boys compared with girls, the magnitude of reduction following HIIR was similar between the sexes (11% vs. 10% respectively).
Collectively, these studies demonstrate the efficacy of acute moderate- and high-intensity exercise, and to a lesser extent energy-intake restriction, to reduce postprandial plasma [TAG] and increase resting whole-body fat oxidation in boys and girls. Furthermore, the beneficial effect of exercise on postprandial metabolism appears dependent on the maintenance of the associated energy deficit. These lifestyle interventions have the potential to provide a practical, effective and engaging stimulus to promote a healthier cardiovascular risk profile in early adolescence.

**Keywords:** acute exercise, high-intensity, energy deficit, energy-intake restriction, postprandial, triacylglycerol, substrate oxidation, cardiovascular disease risk, boys, girls
ACKNOWLEDGEMENTS

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I am indebted to Lord Glendonbrook and the School of Sport, Exercise and Health Sciences for the resources and financial support provided throughout my PhD. I would also like to acknowledge the funding received from the NASPEM Marco Cabrera Student Research Award, which supported the work presented in Chapter 7. A special thank you goes to the participants and their families that were involved in my research studies. Without the sacrifice and commitment of these individuals, this work would not have been possible and I am extremely grateful for their help and enthusiasm. Furthermore, I would like to acknowledge the continued support of local schools in the Loughborough area, particularly Woodbrook Vale High School, Rawlins Academy and Charnwood College. I am also grateful to the undergraduate and postgraduate students that have assisted with the data collection for these studies: Josh Hill, Natalie Wheat, Jodie Allard, Kirsty Armstrong, Tetsuhiro Kidokoro, Sophie Wise, Emily Hellyer and Pardeep Pabla. Special thanks also go to Dr John Lenton, Dr Tom Paulson, Dr Matthew Sedgwick, Rachel Massie, Jack Garnham and James Smallcombe for assisting me in my hour of need.

Finally, I am eternally thankful to my family and friends who have provided tremendous support over the years and kept me smiling even during the most challenging periods. I would especially like to thank my parents for providing me with the opportunity to fulfil my potential. Without your support, encouragement and unwavering belief in me, I would not be where I am today.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-OHB</td>
<td>3-hydroxybutyrate</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>EE</td>
<td>Energy expenditure</td>
</tr>
<tr>
<td>ES</td>
<td>Effect size</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
</tr>
<tr>
<td>iAUC</td>
<td>Incremental area under the concentration versus time curve</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
</tr>
<tr>
<td>MAS</td>
<td>Maximal aerobic speed</td>
</tr>
<tr>
<td>NEFA</td>
<td>Non-esterified fatty acids</td>
</tr>
<tr>
<td>RPE</td>
<td>Rating of perceived exertion</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>TAG</td>
<td>Triacylglycerol</td>
</tr>
<tr>
<td>TAUC</td>
<td>Total area under the variable versus time curve</td>
</tr>
<tr>
<td>TRL</td>
<td>Triacylglycerol-rich lipoproteins</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low-density lipoprotein</td>
</tr>
<tr>
<td>VO₂</td>
<td>Oxygen uptake</td>
</tr>
</tbody>
</table>
CHAPTER 1

Introduction

Cardiovascular disease (CVD) is a collective term describing the diseases affecting the heart and circulatory system (World Health Organisation, 2011a). Coronary heart disease represents the most common form of CVD and is characterised by a partial or complete blockage of one or more of the coronary arteries (World Health Organisation, 2011a). In 2008, CVD was responsible for 17.3 million deaths globally, representing 30% of all deaths worldwide (World Health Organisation, 2011a). Similarly, in the United Kingdom, CVD accounted for 32% of all-cause mortality in 2010 and, although mortality rates from CVD have been steadily declining since the 1980s, it remains the leading cause of morbidity and mortality in men and women (Townsend et al., 2012). Furthermore, the health consequences arising as a result of CVD were estimated to cost the health care system £8.6 billion in 2009 and result in a total economic burden of £19 billion per year (Leal et al., 2012). Therefore, continued efforts promoting the early prevention of CVD are imperative from a public health and economic standpoint.

While the clinical manifestations of CVD are delayed normally until mid-adulthood, atherosclerosis is a disease process central to the pathology of CVD that is initiated during childhood and progresses over the lifespan (Froberg and Andersen, 2005; McGill et al., 2000a). Several risk factors have been identified that augment the likelihood of developing CVD, which include non-modifiable factors such as age, sex, family history and ethnicity and others that are modifiable; for example, dyslipidaemia, hypertension, hyperglycaemia, obesity, poor diet and physical inactivity (World Health Organisation, 2011a). Risk factors for CVD have been identified in children and adolescents that are susceptible to track into adulthood (Bao et al., 1996; Eisenmann et al., 2004; Katzmarzyk et al., 2001; Nicklas et al., 2002). Considering obesity is known to aggravate other cardiovascular health complications, including insulin resistance and dyslipidaemia (Burke, 2006; Freedman et al., 1999), the prevalence of overweight and obesity in children and adolescents continues to provoke concern, and a recent public health report revealed that 28% of boys and girls aged 2 to 15 years are classified as overweight or obese in England (Health Survey for England, 2012c). Exposure to CVD risk factors during childhood and adolescence is associated with the development and progression of CVD in later life (Freedman et al., 2008; Juonala et al., 2010;
Raitakari et al., 2003), highlighting the importance of a lifelong approach for CVD prevention.

Dyslipidaemia is defined as abnormal circulating concentrations of plasma lipids and encompasses elevated concentrations of circulating total cholesterol, low-density lipoprotein (LDL) cholesterol and triacylglycerol (TAG) and suppressed high-density lipoprotein (HDL) cholesterol. Several adult studies support the independent association of adverse fasting concentrations of total cholesterol, LDL cholesterol, HDL cholesterol and TAG with CVD risk (Hokanson and Austin, 1996; Sharrett et al., 2001). However, most people spend the majority of the daytime in a postprandial state typically and the metabolic perturbations associated with a single meal are unlikely to subside before the consumption of subsequent meals. The transient increase in nonfasting TAG concentrations ([TAG]) is an independent risk factor for CVD incidence in men and women (Bansal et al., 2007; Nordestgaard et al., 2007), and childhood fasting [TAG] has emerged as an independent risk factor for young adult CVD (Morrison et al., 2009, 2012). Data from the National Health and Nutrition Examination Survey (NHANES) collected between 1999 and 2006 revealed that 10.2% of adolescents aged 12 to 19 years exhibited high concentrations of TAG and 20.3% displayed at least one abnormal lipid level, which becomes exacerbated in overweight and obese individuals (Centers for Disease Control and Prevention, 2010). Evidence of abnormal lipid concentrations in young people provides impetus to develop novel and engaging interventions that may be attractive to young people, whilst promoting a healthier cardiovascular profile under conditions of daily living.

Physical inactivity is associated independently with the clustering of CVD risk factors in childhood and adolescence (Andersen et al., 2006; Brage et al., 2004; Ekelund et al., 2007, 2012). In England, only 21% of boys and 16% of girls aged 5 to 15 years achieve the current physical activity recommendations of at least 60 min of daily moderate-intensity physical activity (Health Survey for England, 2012b). Given that physical activity participation has been shown to decline from childhood through adolescence (Health Survey for England, 2012b), and childhood and adolescent physical activity participation tracks into adulthood (Telama et al., 2005), it is particularly concerning that approximately 3.2 million deaths globally are a consequence of physical inactivity every year (World Health Organisation, 2009). There is compelling and irrefutable evidence in adults emphasising the health benefits of regular physical activity and exercise in the prevention and management of several chronic
diseases (e.g., CVD, obesity, Type 2 diabetes mellitus) (Warburton et al., 2006), and accumulating evidence in young people highlights the multitude of cardiovascular health benefits associated with a physically active lifestyle (Daniels et al., 2011; Janssen and LeBlanc, 2010). Furthermore, a single session of exercise has been shown to elicit transient improvements in blood lipids, blood pressure and glycaemic control (Thompson et al., 2001). The well-established health benefits of improving CVD risk factors advocate the promotion of lifestyle modifications targeting exercise and diet behaviours as a vehicle for health promotion early in life (Daniels et al., 2011; Froberg and Andersen, 2005; McGill et al., 2000a).

A wealth of evidence since the 1990s has demonstrated that acute exercise performed the day before a standardised meal reduces postprandial [TAG] in adults (Hardman, 1998; Maraki and Sidossis, 2013), although this effect appears short-lived (Herd et al., 1998) and may be dependent on the exercise energy expenditure (EE) (Gill et al., 2002a). However, the well-established differences in metabolic and hormonal responses to exercise between young people and adults highlights the demand for paediatric centred approaches (Boisseau and Delamarche, 2000; Riddell, 2008). Similar studies conducted to date in children and adolescents support the use of exercise as a tool to reduce postprandial [TAG] (Tolfrey et al., 2014b). Nevertheless, there remains a paucity of experimental studies in paediatric populations and further research is, therefore, required to identify novel and sustainable lifestyle interventions for health promotion in young people.

Consequently, the aim of this thesis was to examine the efficacy of acute exercise and diet interventions on postprandial metabolism in children and adolescents, with concentrations of circulating TAG representing the primary outcome of interest. Chapter 2 presents a critical review of the literature to date and provides a platform to address the primary research question in the subsequent experimental chapters. This is followed by an introduction to the general methods adopted consistently throughout the experimental chapters (Chapter 3). The first experimental chapter (Chapter 4) examined the effect of a single session of high-intensity interval running (HIIR) on postprandial plasma [TAG] in boys. Chapter 5 investigated the effect of acute moderate-intensity exercise with and without energy replacement on postprandial plasma [TAG] and whole-body fat oxidation in boys to ascertain the importance of the associated energy deficit. The contribution of the ensuing energy deficit is explored further in Chapter 6 by comparing the independent effect of acute moderate-
intensity exercise and energy-intake restriction on postprandial plasma [TAG] in girls. Subsequently, Chapter 7 extends the findings of the previous experimental chapters by determining the effect of HIIR alone and in combination with energy-intake restriction on postprandial plasma [TAG] and whole-body fat oxidation in girls. Finally, Chapter 8 combines and reanalyses data from Chapters 4 and 7 to compare the postprandial plasma TAG response following acute HIIR between boys and girls.
CHAPTER 2
Review of literature

This chapter first summarises the integrated pathways of lipid and lipoprotein metabolism under postabsorptive and postprandial conditions. Then it explores the disordered physiological processes associated with atherosclerosis and considers the implications of the postprandial state in relation to cardiovascular disease (CVD) risk. This is followed by a detailed review of the literature examining the role that exercise and diet interventions can play in modifying postprandial lipid metabolism in adults, with particular reference to concentrations of triacylglycerol (TAG), whole-body fat oxidation, acute exercise and the associated energy deficit. The review will finish by exploring the effect of lifestyle interventions on postprandial lipid metabolism in children and adolescents. Although the postprandial responses in children and adolescents are the focus of this thesis, the paucity of literature available in young people is supplemented with evidence in adults to provide a more comprehensive picture of postprandial lipid metabolism.

2.1 Lipid and lipoprotein metabolism

The major lipids are insoluble in plasma (e.g., cholesterol, cholesteryl esters, TAG and phospholipids) and, therefore, require specialised transport mechanisms. Cholesteryl ester and TAG particles are incorporated into the core of lipoproteins which are surrounded by a monolayer of phospholipids, free cholesterol and specific apolipoproteins. The major lipoproteins and their characteristics are presented in Table 2.1. Lipoprotein metabolism can be divided into two pathways: exogenous and endogenous. A summary of these pathways will be provided in the following section, but the reader is directed to the work of others for a more comprehensive insight (Frayn, 2010; Karpe, 1999). The exogenous pathway refers to the metabolism of intestinally derived lipoproteins called chylomicrons. The endogenous pathway relates to lipoproteins derived from hepatic tissue, which includes very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Chylomicron and VLDL particles, known as triacylglycerol-rich lipoproteins (TRL), are responsible for the delivery of TAG to peripheral tissues such as skeletal muscle. Conversely, LDL and HDL particles are principally involved in the transport of cholesterol.
Table 2.1  Characteristics of the major lipoproteins.

<table>
<thead>
<tr>
<th></th>
<th>Chylomicrons</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Site of synthesis</strong></td>
<td>Small intestine</td>
<td>Liver</td>
<td>Peripheral tissue capillaries</td>
<td>Small intestine, liver</td>
</tr>
<tr>
<td><strong>Density range</strong> (g·mL⁻¹)</td>
<td>&lt; 0.950</td>
<td>0.950 – 1.006</td>
<td>1.019 – 1.063</td>
<td>1.063 – 1.210</td>
</tr>
<tr>
<td><strong>Diameter (nm)</strong></td>
<td>80 – 1000</td>
<td>30 – 80</td>
<td>20 – 25</td>
<td>9 – 15</td>
</tr>
<tr>
<td><strong>Major lipids</strong></td>
<td>Dietary TAG</td>
<td>Endogenous TAG</td>
<td>Cholesterol, cholesteryl ester</td>
<td>Cholesteryl ester, phospholipids</td>
</tr>
<tr>
<td><strong>Protein</strong> (% by weight)</td>
<td>1</td>
<td>5 – 15</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td><strong>TAG</strong> (% by weight)</td>
<td>90</td>
<td>50 – 70</td>
<td>5 – 10</td>
<td>2</td>
</tr>
<tr>
<td><strong>Cholesterol</strong> (% by weight)</td>
<td>5</td>
<td>10 – 20</td>
<td>40 – 45</td>
<td>18</td>
</tr>
<tr>
<td><strong>Phospholipid</strong> (% by weight)</td>
<td>4</td>
<td>10 – 20</td>
<td>20 – 25</td>
<td>30</td>
</tr>
<tr>
<td><strong>Primary function</strong></td>
<td>Transport dietary fat</td>
<td>Transport endogenous fat</td>
<td>Transport cholesterol to peripheral tissues</td>
<td>Reverse transport of cholesterol</td>
</tr>
</tbody>
</table>

VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TAG, triacylglycerol.

Adapted from Frayn (2010) and McArdle et al. (2010).

2.1.1  Chylomicron metabolism

Within the enterocytes of the small intestine, fatty acids are re-esterified into TAG, and are packaged with phospholipids, un-esterified cholesterol and the apolipoproteins B-48 and A-I into chylomicrons, the largest of the circulating lipoprotein particles. Some short-chain fatty acids enter the capillary plasma directly as non-esterified fatty acids (NEFA), but the majority of dietary fatty acids are long chain and undergo re-esterification to form chylomicrons. Each chylomicron particle is characterised by a single apolipoprotein B-48 molecule which remains with the particle throughout its lifetime, and is frequently used as a surrogate marker to quantify the circulating chylomicron lipoprotein concentration. Chylomicrons enter the circulation via the lymphatic system and, through interaction with other lipoproteins, acquire
Apolipoproteins C-II and E. Apolipoprotein C-II is synthesised by the liver and enables the activation of the enzyme lipoprotein lipase (LPL) located on the cell surface of capillary endothelial cells in peripheral tissues, particularly adipose tissue, skeletal muscle and heart muscle. The hydrolysis of TAG by LPL liberates NEFA which surrounding cells can take up for oxidation or storage. Simultaneously, redundant cell surface material (i.e., free cholesterol, phospholipids and some apolipoproteins) is transferred to other particles such as HDL, resulting in smaller lipoprotein remnants enriched in cholesteryl ester. The loss of apolipoprotein C-II prevents further hydrolysis by LPL, but further de-lipidation may occur in the presence of hepatic lipase in the liver. Removal of chylomicron remnants, facilitated by apolipoproteins B-48 and E, primarily occurs at the liver via the LDL receptor and LDL-receptor related pathway.

2.1.2 VLDL and LDL metabolism

VLDL particles are responsible for TAG transport in the postabsorptive and postprandial state. They are synthesised and secreted into the circulation from hepatic cells in the liver and are characterised by a hydrophobic core enriched in TAG and cholesteryl ester, with apolipoproteins B-100, C and E on the lipoprotein surface. The rate of VLDL synthesis and secretion is largely determined by substrate availability (i.e., NEFA), with the main source of NEFA derived from adipose tissue lipolysis mediated by hormone-sensitive lipase (Barrows and Parks, 2006). Furthermore, partially hydrolysed TRL remnants and circulating NEFA released from TRL lipolysis in skeletal muscle and adipose tissue can also contribute to the hepatic NEFA flux, in addition to the liver’s capacity for de novo TAG synthesis (Barrows and Parks, 2006; Karpe, 1999). Each VLDL particle encompasses a single apolipoprotein B-100 molecule which remains throughout its lifetime and additional apolipoproteins C and E are acquired once in the circulation. Similar to chylomicrons, repeated cycles of LPL-mediated hydrolysis of TAG in the presence of apolipoprotein C-II provides NEFA for peripheral tissues. Redundant cell surface material is mainly transferred to HDL particles, with the remaining cholesteryl ester-enriched remnants either taken up directly by the liver via the LDL receptor facilitated by apolipoproteins B-100 and E, or having undergone several cycles of lipolysis by LPL, remain in the circulation as smaller intermediate-density lipoproteins (IDL). Circulating IDL particles are degraded further by the action of LPL and hepatic lipase to become LDL particles consisting of a cholesteryl ester core surrounded by a shell of phospholipid, free cholesterol and the integral apolipoprotein B-100 molecule. LDL
particles remain in the circulation for approximately 3 days and are predominantly cleared via the LDL receptor to tissues that require cholesterol. Hydrolysis of the cholesteryl ester liberates free cholesterol which contributes to the cellular cholesterol pool and is used to synthesise cell membranes, steroid hormones, bile salts and VLDL particles. Other LDL particles that have been chemically modified (e.g., through oxidation) may be taken up by macrophage scavenger receptors and contribute to the process of atherosclerosis described in Section 2.2.

2.1.3 HDL metabolism

HDL particles are involved primarily in the removal of cholesterol from peripheral tissues to the liver in a process known as the ‘reverse cholesterol transport’ pathway, which ultimately leads to cholesterol excretion in bile. Nascent HDL particles originate from apolipoprotein A-1 molecules associated with phospholipids and are secreted from the liver and small intestine. They acquire excess free cholesterol through the interaction with peripheral cells (e.g., macrophages) and uptake of excess surface material from TRL hydrolysis. Mature, cholesterol-rich HDL particles are formed when the enzyme lecithin-cholesterol acyl transferase (LCAT), activated by apolipoprotein A-1, catalyses the esterification of free cholesterol to cholesteryl esters. Removal of cholesteryl esters occurs directly through receptor-mediated uptake in the liver allowing cholesterol-depleted HDL particles to be recycled, or indirectly through the action of cholesterol ester transfer protein (CETP) which catalyses the movement of cholesteryl esters from HDL to TRL in exchange for TAG. The TAG-enriched HDL particles become substrates for hepatic lipase to form smaller cholesteryl-ester depleted HDL particles that re-enter the HDL pathway.

2.1.4 Regulation of TRL metabolism

In the postabsorptive state (e.g., after an overnight fast), upregulation of hormone sensitive lipase mediated by decreased insulin action promotes adipose tissue lipolysis liberating NEFA into the circulation. Circulating NEFA are directed towards skeletal muscle for oxidation, and the liver where they are either oxidised resulting in the production of ketone bodies or re-esterified into TAG and secreted in VLDL. Endogenous VLDL particles comprise the majority of the total TRL pool in the postabsorptive state. Insulin stimulates expression of adipose tissue LPL and suppresses skeletal muscle LPL, suggesting that skeletal muscle LPL-mediated clearance of TRL-TAG is greater in the postabsorptive state.
Chapter 2: Review of literature

After the ingestion of a meal, enhanced insulin secretion in response to hyperglycaemia suppresses adipose tissue lipolysis and stimulates re-esterification of NEFA, resulting in lower concentrations of circulating NEFA in the early postprandial period. Consequently, the reduced availability of NEFA as a fuel for skeletal muscle results in a shift towards glucose utilisation. Furthermore, suppression of hepatic NEFA flux reduces NEFA oxidation and formation of ketone bodies in addition to VLDL secretion. The postprandial increase in circulating TRL is largely accounted for by apolipoprotein B-100 containing lipoproteins (Schneeman et al., 1993), yet the increase in postprandial [TAG] largely reflects apolipoprotein B-48 containing lipoproteins (Cohn et al., 1993), suggesting that large quantities of TAG are transported by few chylomicron particles. Chylomicrons and VLDL compete for the same lipolytic pathway (Brunzell et al., 1973), but evidence suggests LPL preferentially hydrolyses larger chylomicron particles (Björkergen et al., 1996; Fisher et al., 1995; Schneeman et al., 1993). This suggests that reduced VLDL catabolism is responsible for the accumulation of predominantly endogenous VLDL particles in the postprandial period (Björkergen et al., 1996; Schneeman et al., 1993). The insulin-mediated up-regulation of adipose tissue LPL after a meal channels NEFA towards re-esterification and storage in adipocytes, supported by the peak in adipose tissue LPL approximately 3 to 5 h post-meal coinciding with the peak in plasma [TAG]. The postprandial uptake of NEFA by skeletal muscle is likely to be lower than adipose tissue, with fatty acids either oxidised for energy provision or re-esterified and stored as intra-muscular TAG.

2.2 Pathogenesis of atherosclerosis

The pathogenesis of atherosclerosis has been reviewed in detail previously (Falk, 2006; Hajjar and Nicholson, 1995; Libby et al., 2011; Ross, 1993) and a brief summary is presented in this section. Although the exact mechanisms are not fully established, the ‘response-to-injury’ hypothesis dating back to the 1970s has been pivotal in advancing our understanding of atherosclerosis (Ross and Glomset, 1973). In brief, endothelial injury or dysfunction is considered key in the early development of atherosclerosis, and precedes clinical manifestations of atherosclerotic disease. The endothelium forms the innermost cellular layer of the tunica intima in the blood vessel wall, and along with acting as a barrier between the vessel wall and the lumen, the endothelium plays a central role in regulating vasomotor function, inflammatory processes and haemostasis. Endothelial dysfunction provokes a cascade of inflammatory responses leading to the migration and retention of cholesterol-
containing LDL in the sub-endothelial space, which are susceptible to modification primarily through oxidation (de Graaf et al., 1991). The pro-inflammatory properties of modified LDL particles contribute to the up-regulation of adhesion molecules on the endothelium and release of chemokines to facilitate leukocyte (monocytes and T lymphocytes) migration into the sub-endothelial space. Monocyte-derived macrophages ingest oxidised LDL particles, mediated by scavenger receptors, and develop into foam cells which become the primary constituent of the ‘fatty streak’ recognised as the first visible atherosclerotic lesion. Lesion progression is characterised by the migration of smooth muscle cells from the tunica media to the tunica intima, proliferation of smooth muscle cells and the synthesis of a collagen-rich extracellular matrix which forms a fibrous cap to stabilise the core of macrophage-derived foam cells, extracellular lipid and necrotic cellular debris – this advanced lesion is known as the ‘fibrous plaque’. Clinical manifestations of atherosclerosis arise typically through impaired blood flow in the arterial lumen leading to tissue ischemia, or plaque instability and rupture, which may precipitate the formation of an occluding thrombus that promotes coronary events such as myocardial infarction or stroke.

2.3 Postprandial lipaemia: definition and measurement

Circulating concentrations of TAG have traditionally been measured in the postabsorptive state mainly due to the wide day-to-day variability in nonfasting concentrations and to allow the estimation of LDL cholesterol using the Friedewald equation (Friedewald et al., 1972). However, most people in Western society consume several meals a day, resulting in the majority of the daytime being spent in a postprandial state typically (14 to 16 h·24 h\(^{-1}\) for adults; Kolovou et al., 2011b). Therefore, studying TAG metabolism in the postprandial state may better represent the 24 h period.

Postprandial lipaemia is most often characterised by the elevation in circulating [TAG] following the consumption of a meal containing fat and is measured by quantifying the temporal change in the circulating [TAG] following a standardised meal. A large majority of postprandial studies have used a single meal, usually provided for breakfast, and although there is substantial heterogeneity in study design, a number of defining features are consistently adopted: a fasting blood sample is taken in the postabsorptive state before the standardised meal is consumed and subsequent blood samples are obtained throughout the postprandial rest period at pre-determined intervals, usually for 6 to 8 h. Typically, postprandial [TAG] will increase gradually following a single fat load, reaching a peak after
approximately 4 h (Nordestgaard et al., 2007), before returning to the baseline level after 6 to 8 h (Kolovou et al., 2011b). Consequently, the metabolic perturbations associated with a single meal are unlikely to subside before the consumption of subsequent meals. Some studies have introduced additional meals during the postprandial period to better reflect normal dietary practice, which appear to have an accumulative effect on the lipaemic response by further delaying the return of circulating TAG to the baseline level (Silva et al., 2005).

The temporal change in postprandial [TAG] can be represented graphically, and is most commonly reported as the total area under the concentration versus time curve (TAUC) calculated using the trapezium rule (Matthews et al., 1990). The incremental area under the concentration versus time curve (iAUC) can also be quantified using the same method after adjusting for fasting concentrations. In addition to the area under the curve, some authors may report mean postprandial [TAG], and possibly peak postprandial [TAG] which provides an indication of the magnitude of the lipaemic response.

2.4 Postprandial lipaemia and cardiovascular disease (CVD) risk

2.4.1 Evidence in adults

The relationship between lipid and lipoprotein particles and atherosclerosis was described over 60 years ago (Gofman et al., 1950; Moreton, 1947), with a subsequent study reporting that patients with coronary artery disease demonstrate a greater plasma lipid response to a fat-rich meal than healthy controls (Barritt, 1956). However, the atherogenic potential of postprandial TRL did not receive widespread attention until the pivotal work of Donald Zilversmit published in 1979. Zilversmit (1979) characterised atherosclerosis as a postprandial phenomenon and proposed that LPL-mediated hydrolysis of chylomicrons leads to the internalisation of cholesterol-enriched chylomicron remnants by arterial smooth muscle cells. Since this pivotal paper, a multitude of studies have been conducted that have advanced our understanding of the link between postprandial lipid metabolism and CVD risk.

The intima-media thickness of the common carotid artery is a commonly utilised surrogate marker of early atherosclerosis and has been positively correlated with postprandial [TAG], independent of other CVD risk factors including fasting [TAG] (Boquist et al., 1999; Karpe et al., 1998; Ryu et al., 1992; Teno et al., 2000). This relationship was found to be stronger in the early postprandial period between 1 to 4 h in one study (Boquist et al., 1999), whereas
others have reported a stronger relationship in the later postprandial period at 6 and 7 h (Karpe et al., 1998; Ryu et al., 1992). Nevertheless, although causality cannot be established, these studies support the positive association between postprandial lipaemia and early manifestations of atherosclerosis. Similarly, case-control studies have reported elevated postprandial lipaemia or delayed postprandial TAG clearance consistently in various adult population patient groups (Björkegren et al., 2000; Braun et al., 1997; Groot et al., 1991; Patsch et al., 1992; Potts et al., 1995; Schaefer et al., 2001; Weintraub et al., 1996). This relationship was still present in the studies reporting a similar concentration of fasting TAG between the patient and control groups (Björkegren et al., 2000; Braun et al., 1997; Groot et al., 1991). Furthermore, Patsch et al. (1992) demonstrated that postprandial [TAG] was more discriminatory than fasting levels, with the concentrations at 6 and 8 h after meal ingestion independently predicting the absence or presence of coronary artery disease in 68% of the 101 participants.

Several prospective cohort studies support the association between nonfasting [TAG] and coronary heart disease in men and women (Bansal et al., 2007; Eberly et al., 2003; Iso et al., 2001; Langsted et al., 2011; Lindman et al., 2010; Nordestgaard et al., 2007; Sarwar et al., 2007; Stampfer et al., 1996; Stensvold et al., 1993). In the Copenhagen City Heart Study involving 7,587 women and 6,394 men followed for 26 years, the age-adjusted hazard ratio for myocardial infarction was 16.8 in women and 4.6 in men whose nonfasting [TAG] was ≥5 mmol·L⁻¹ compared with <1.0 mmol·L⁻¹ (Nordestgaard et al., 2007). The hazard ratios were reduced to 5.4 and 2.4 respectively, but remained significant after multivariate adjustment for other established risk factors (Nordestgaard et al., 2007). Other studies have also reported that the relationship between nonfasting [TAG] and coronary heart disease is diminished after adjustment for confounding factors (Lindman et al., 2010; Sarwar et al., 2007); however, nonfasting [TAG] has emerged as a stronger predictor of coronary heart disease than traditional fasting levels (Bansal et al., 2007). Specifically, in the Women’s Health Study involving 26,509 initially healthy American women followed for 11 years, nonfasting [TAG] was associated with incident cardiovascular events independent of other cardiac risk factors, lipids and markers of insulin resistance, whereas fasting [TAG] demonstrated little independent relationship (Bansal et al., 2007). Collectively, these studies support the emergence of nonfasting [TAG] as a predictor of CVD risk in adults (Nordestgaard et al., 2009; Stalenhoef and de Graaf, 2008).
A number of potential mechanisms have been proposed to explain how metabolic perturbations in TRL metabolism accelerate the development of atherosclerosis. Zilversmit (1979) suggested that chylomicrons and their remnants directly infiltrate the arterial wall contributing to the formation of atherosclerotic plaques. Although the direct atherogenicity of chylomicron remnants has been supported (Proctor and Mamo, 1998), others have argued that the majority of circulating chylomicron remnants are removed before they are capable of penetrating the arterial wall (Karpe et al., 1997). An advancement of this hypothesis suggests modification of the lipoprotein profile promotes a more atherogenic lipid phenotype of elevated TRL and their remnants, low concentrations of HDL and a predominance of small, dense LDL (Cohn, 1998). Regular exposure to elevated postprandial [TAG] and impaired clearance of large VLDL particles exacerbates the movement of cholesteryl esters from HDL and LDL to TRL in exchange for TAG, a process facilitated by CETP (Frayn, 2010). The TAG-enriched LDL and HDL particles become substrates for hepatic lipase resulting in the formation of small, dense LDL particles and small, cholesteryl ester depleted HDL particles (Frayn, 2010). Small, dense LDL particles have a lower affinity for the LDL receptor (Galeano et al., 1994), which increases the residence time in the circulation and the opportunity to infiltrate the arterial intima where they become susceptible to the oxidative modification that contributes to the atherosclerotic process described in Section 2.2 (de Graaf et al., 1991; Griffin, 1999).

2.4.2 Evidence in children and adolescents

Although the clinical manifestations of atherosclerotic disease are not apparent typically until mid-adulthood, the process of atherosclerosis is initiated during childhood and adolescence and progresses over the lifespan (Froberg and Andersen, 2005; McGill et al., 2000a). Fatty streaks are considered the earliest lesion of atherosclerosis (McGill et al., 2000a), with the presence of aortic fatty streaks identified in the first decade of life (Holman et al., 1958) and coronary fatty streaks in the second decade of life (Strong and McGill, 1962). Although fatty streaks may be fairly innocuous, there is convincing evidence suggesting that they can progress gradually over time into clinically significant atherosclerotic lesions (Guyton and Klemp, 1993; Stary, 1990). Indeed, the prevalence and extent of fatty streaks and clinically significant atherosclerotic lesions increases rapidly in the arteries of adolescents and young adults (Strong et al., 1999).
Autopsy studies conducted as part of the Bogalusa Heart Study and the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Study have confirmed the presence of fatty streaks and fibrous plaques in the aorta and coronary arteries of adolescents and young adults following unexpected death (Berenson et al., 1998; McGill et al., 1997, 2000b). The extent of lesions was associated with a number of established risk factors including elevated concentrations of total cholesterol, LDL cholesterol, TAG, and low levels of HDL cholesterol, in addition to other modifiable risk factors such as obesity and hypertension (Berenson et al., 1998; McGill et al., 1997, 2000b). In fact, the presence of multiple antemortem risk factors augments the extent of atherosclerotic lesions in young people (Berenson et al., 1998).

Moreover, non-invasive imaging studies have demonstrated significant associations between adverse lipid and lipoprotein concentrations during childhood and adolescence and subclinical atherosclerosis in adulthood assessed by carotid artery calcification (Mahoney et al., 1996), elasticity (Juonala et al., 2005, 2008) and intima-media thickness (Davis et al., 2001; Freedman et al., 2008; Juonala et al., 2008, 2010; Li et al., 2003; Raitakari et al., 2003).

Longitudinal data with incident CVD as the primary outcome measure is confined to research conducted as part of the Princeton Lipid Research Clinics Follow-Up Study (Morrison et al., 2009, 2012). A cardiovascular risk factor profile was developed during childhood and adolescence, and fasting [TAG] emerged as an independent predictor of young adult CVD after a 22- to 31-year follow-up (Morrison et al., 2009, 2012). Considering CVD risk factors, including abnormal lipid and lipoprotein concentrations, during childhood and adolescence can track into adulthood (Bao et al., 1996; Eisenmann et al., 2004; Katzmarzyk et al., 2001; Nicklas et al., 2002), early identification of at-risk individuals may aid in the primary prevention of atherosclerosis and CVD.

Although the evidence linking adverse lipid and lipoprotein concentrations in childhood and adolescence with atherosclerotic progression is accumulating, the evidence-base is limited to studies quantifying lipids in the fasting state. However, substantial intra-individual variation is evident in fasting lipid and lipoprotein concentrations in children, with coefficients of variation ranging from 3.5% for HDL₃ to 25.4% for TAG (Tolfrey et al., 1999). In addition, the evidence in adults suggests that postprandial concentrations may be more informative of CVD risk than fasting measures (Bansal et al., 2007), highlighting the importance of studying [TAG] in the postprandial period. Nevertheless, these findings advocate the screening and regulation of CVD risk factors in childhood and adolescence to delay the progression of
atherosclerosis and incident CVD (Daniels and Greer, 2008). Accordingly, lifestyle interventions, including manipulations of exercise energy expenditure (EE) and dietary energy intake, that moderate postprandial lipaemia should be initiated during childhood and adolescence and require future research (Daniels et al., 2011; Froberg and Andersen, 2005; Kavey et al., 2003; McGill et al., 2000a). One facet of the European Youth Heart Study revealed that the odds ratio for clustering of CVD risk factors (systolic blood pressure, fasting [TAG], total cholesterol/HDL ratio, insulin resistance, sum of four skinfolds and aerobic fitness) was 3.29 in 9 to 15 year old children and adolescents classified in the lowest quintile of habitual physical activity compared with the most active quintile (Andersen et al., 2006). Subsequent reports support the association between objectively measured high physical activity levels and an improved cardiovascular health profile in children and adolescents (Brage et al., 2004; Ekelund et al., 2007, 2012), highlighting the importance of physical activity for health promotion (Daniels et al., 2011; Department of Health, Physical Activity, Health Improvement and Protection, 2011; Janssen and LeBlanc, 2010). Therefore, the following sections will review the effect of acute exercise and diet interventions on postprandial lipaemia in children and adolescents. Considering this field of study is in its infancy, evidence from adult studies will be presented first before highlighting the key findings to date and areas to explore in children and adolescents.

2.5 Exercise and postprandial lipaemia in adults

2.5.1 Exercise training

Regular endurance training promotes a myriad of non-transient adaptations in a range of performance, physiological and health markers (Hawley and Lessard, 2008; Holloszy and Coyle, 1984), which may contribute to the prevention and treatment of chronic diseases such as CVD and Type 2 diabetes mellitus (Hawley, 2004). Among these benefits, exercise has been shown to alter blood lipids and lipoproteins favourably (Durstine et al., 2001). Endurance-trained athletes demonstrate lower postprandial [TAG] and elevated TAG clearance (Cohen et al., 1991; Merrill et al., 1989), and endurance-training interventions in previously untrained individuals have reported reductions in postprandial [TAG] and TRL (Weintraub et al., 1989), along with increased TAG clearance (Thompson et al., 1988; Zmuda et al., 1998). However, the close proximity of the postprandial assessment to the cessation of training in these studies (~10 to 36 h) precludes the isolation of the acute and chronic effects of exercise training. Subsequent studies assessing participants > 48 h following the last
training session report no differences in postprandial lipaemia between trained and untrained participants (Herd et al., 2000; Tsetsonis et al., 1997), or in sedentary women undergoing a 12 week exercise programme (Aldred et al., 1995).

De-training studies provide further evidence that training-induced changes in postprandial TAG metabolism are short lived. Specifically, endurance-trained males refraining from exercise for a week experienced substantial increases in postprandial plasma TAG, chylomicron-TAG and VLDL-TAG concentrations by 53%, 68% and 51% respectively (Gill et al., 2003a). Other studies confirm rapid elevations in postprandial [TAG] occur in the early de-training period when the training stimulus is removed (37% to 45% after 60 h) (Hardman et al., 1998; Herd et al., 1998). Therefore, although the TAG-lowering effect of exercise appears short-lived, exercise should be performed on a regular basis to promote and maintain low concentrations of postprandial TAG and TRL.

2.5.2 Acute exercise

2.5.2.1 Study design

A wide variety of study designs have been implemented to examine the acute effect of exercise on postprandial lipaemia. Studies have included a wide range of participants, employing various exercise modes, volumes and intensities, and have introduced further variability in the timing of exercise in relation to the test meal, macronutrient content of the test meal and timing of blood samples for TAG assessment. The majority of exercise postprandial studies have employed a 2-day model (Figure 2.1) whereby the exercise session is performed in the late afternoon and the postprandial measures begin the following morning after an overnight fast, approximately 12 to 18 h following the cessation of exercise. In a few studies, the exercise session has been performed either immediately before or in the hours after the test meal on the postprandial day, but these findings appear less consistent (Hardman and Aldred, 1995; Katsanos et al., 2004; Pfeiffer et al., 2005; Zhang et al., 1998). A common feature of acute exercise postprandial studies is the completion of the conditions in a counterbalanced order separated by a standardised time interval (> 7 days). The pre-test conditions are standardised by implementing physical activity and dietary controls to minimise the potential confounding effects on the study outcome measures. Participants are usually asked to minimise their habitual physical activity and record their dietary intake for up to 3 days preceding the postprandial day of the first condition, and replicate this pattern before the subsequent conditions. In some studies, participants are provided with standardised
meals during this period to reduce between-subject variation. The postprandial measures have traditionally been quantified in response to a single, standardised breakfast meal, but more recent studies have introduced a lunch meal to better reflect normal dietary practice (e.g., Miyashita et al., 2008), which is likely to augment the postprandial lipaemic response (Silva et al., 2005). Although the majority of studies have used high-fat meals which may exaggerate postprandial lipaemia (> 50% of meal total energy from fat) (Maraki and Sidossis, 2013), a recent study reported similar postprandial [TAG] following the consumption of both moderate fat (59 g, 35%) and high fat (111 g, 66%) doses at rest and with an exercise stimulus (Hurren et al., 2011b).

![Figure 2.1](image-url)  
**Figure 2.1** Schematic of the 2-day experimental model adopted in the majority of exercise postprandial studies in adults and young people.

### 2.5.2.2 Acute moderate-intensity exercise

The literature is replete with adult-based studies examining the acute effects of exercise and dietary manipulations on postprandial lipaemia; these have been reviewed in detail elsewhere (e.g., Freese et al., 2014; Gill, 2004; Hardman, 1998; Katsanos, 2006; Maraki and Sidossis, 2010, 2013; Peddie et al., 2012). The consensus among these studies is that a single session of continuous, moderate-intensity exercise performed prior to a standardised meal reduces postprandial [TAG] in healthy, normolipidaemic men and women (Maraki and Sidossis, 2013), which appears independent of substrate metabolism during exercise (Malkova et al., 1999). This is reinforced by a recent meta-analysis reporting an overall moderate effect of acute aerobic exercise for the TAUC-TAG (-24%; Cohen’s d = -0.60, P < 0.05) and iAUC-TAG (-23%; Cohen’s d = -0.59, P < 0.05) (Freese et al., 2014). Elevated postprandial [TAG]
and delayed clearance of circulating [TAG] are frequently reported in adult patient groups (Björkegren et al., 2000; Braun et al., 1997; Groot et al., 1991; Patsch et al., 1992; Potts et al., 1995; Sarti and Gallagher, 2006; Schaefer et al., 2001; Weintraub et al., 1996). Exercise postprandial studies conducted in those demonstrating risk factors for CVD highlight the TAG-lowering effect of exercise in individuals who are overweight and obese (Burton et al., 2008; Farah et al., 2010; Gill et al., 2004; Miyashita, 2008; Miyashita et al., 2010), with metabolic syndrome (Mestek et al., 2008; Zhang et al., 2004) or Type 2 diabetes mellitus (Tobin et al., 2008), although these findings are not supported universally (Dalgaard et al., 2004; Gill et al., 2007). Furthermore, studies in adults have consistently demonstrated greater postprandial [TAG] in men than women (Couillard et al., 1999; Jackson et al., 2010; Kolovou et al., 2006; Koutsari et al., 2004). Although the mechanisms responsible for this sex difference are not well established, greater skeletal muscle uptake and retention of plasma [TAG] (Horton et al., 2002), lower abdominal visceral adipose tissue lipolysis (Couillard et al., 1999), greater suppression of upper-body subcutaneous adipose tissue lipolysis (Jensen, 1995) and/or the protective effect of oestrogen (Westerveld, 1998) in women have been implicated. Nevertheless, Gill et al. (2002b) compared 38 men and 43 women retrospectively and reported that the moderate-intensity exercise-induced reduction in postprandial [TAG] was not different between men and women (23.5% vs. 19.8% respectively). This finding has been supported by other studies demonstrating a similar magnitude of reduction in postprandial [TAG] in men and women following acute moderate-intensity exercise (Hardman and Aldred, 1995; Tsetsonis and Hardman, 1996).

An important determinant of the exercise-induced reduction in postprandial [TAG] is the associated exercise EE (Maraki and Sidossis, 2013), with manipulations of exercise intensity and duration supporting a dose-dependent response in adults (Gill et al., 2002a; Katsanos et al., 2004; Tsetsonis and Hardman, 1996). A recent review suggests that an EE threshold of ~30 kJ·kg$^{-1}$ body mass (~2-2.5 MJ) is required to elicit a reduction in postprandial [TAG] (Maraki and Sidossis, 2013); however, meaningful reductions in postprandial lipaemia are still evident following modest doses of moderate-intensity exercise EE (~1 MJ) (Miyashita, 2008; Miyashita et al., 2008, 2010). Nevertheless, aerobic exercise EE is negatively correlated with the magnitude of the postprandial lipaemic response ($r = -0.31$, $P = 0.009$) (Freese et al., 2014), suggesting that a greater aerobic exercise EE is associated with a greater reduction in postprandial [TAG].
Since 1995, the concept of exercise accumulation for health promotion has been advocated in international physical activity guidelines (Pate et al., 1995). A review of accumulated and continuous exercise intervention studies highlights the equivalent long-term benefits on cardiovascular fitness and blood pressure normalisation; however, the authors concluded that the effect on adiposity, fasting blood lipids and psychological well-being remains equivocal (Murphy et al., 2009). Several exercise postprandial studies have adopted acute exercise protocols that involve accumulating multiple short exercise bouts throughout the day, which may be easier to incorporate into a ‘normal’ day. A brief overview of these studies will be presented here but the reader is directed to a recent review for a more comprehensive insight (Miyashita et al., 2013).

Early studies reported that accumulating three equal bouts of moderate-intensity exercise (30 to 90 min in total duration) reduces postprandial [TAG] to a similar or even greater extent as one continuous exercise bout (Altena et al., 2004; Gill et al., 1998; Murphy et al., 2000). In contrast to these studies, single sessions of continuous low- and moderate-intensity walking (2.1 MJ, 500 kcal), but not two isoenergetic accumulated walking bouts, reduced postprandial [TAG] in middle-aged men with metabolic syndrome (Mestek et al., 2008). The reason for this difference is unclear, but the authors speculate that a greater exercise EE or number of exercise bouts may be required for accumulated exercise to reduce postprandial [TAG] (Mestek et al., 2008). A series of studies conducted by Miyashita and colleagues examined the acute effect of accumulating shorter (<10 min) bouts of moderate-intensity exercise which have demonstrated efficacy in reducing postprandial [TAG] compared with a non-exercise control (Miyashita et al., 2006a, 2009). These authors have also compared the effect of accumulating ten, 3 min exercise bouts with 30 min continuous exercise on postprandial lipaemia (Miyashita, 2008; Miyashita et al., 2006b, 2008). In each of these studies, accumulated exercise was equally efficacious in reducing postprandial [TAG] as a similar volume of continuous exercise in both young, healthy (Miyashita et al., 2006b, 2008) and obese men (Miyashita, 2008).

2.5.2.3 Acute high-intensity exercise

Adherence to the current international physical activity guidelines for health promotion is poor, with available evidence suggesting 31% of adults (28% men and 34% women) worldwide are insufficiently active (World Health Organisation, 2011b) and in England, 33% and 45% of men and women respectively do not meet the minimum recommendations for
physical activity (Health Survey for England, 2012a). Recent evidence suggests that adults associate high-intensity interval exercise with greater enjoyment compared with a similar duration of continuous exercise, despite higher ratings of perceived exertion (Bartlett et al., 2011). Considering lack of time and enjoyment are frequently cited barriers to exercise participation (Trost et al., 2002a), high-intensity exercise may be an attractive strategy to improve metabolic health. The effect of acute high-intensity exercise on postprandial lipaemia has received growing interest in recent years, but a brief overview of the evolution of high-intensity exercise in relation to exercise and health will be discussed first for completeness.

High-intensity exercise is characterised by repeated bursts of vigorous exercise interspersed with a standardised period of rest or active, low-intensity recovery. A multitude of high-intensity exercise models have been developed but the most common is the Wingate protocol, consisting of repeated (typically 4 to 7) 30 s ‘all-out’ cycle sprints against a high resistance with ~4 min recovery. Exercise training studies (2 to 7 weeks in duration) adopting the Wingate protocol, commonly referred to as ‘sprint interval training’, highlight the potency of this training method to improve cardiorespiratory fitness and induce favourable physiological adaptations in healthy and overweight adults (Babraj et al., 2009; Burgomaster et al., 2005, 2006; MacDougall et al., 1998; Whyte et al., 2010), which appear comparable to the effects elicited by traditional endurance training (Burgomaster et al., 2008; Gibala et al., 2006; Rakobowchuk et al., 2008). Moreover, this type of training has been shown to improve metabolic health outcomes, including endothelial function (Rakobowchuk et al., 2008), blood pressure (Whyte et al., 2010), resting fat oxidation (Whyte et al., 2010) and insulin sensitivity/glycaemic control (Babraj et al., 2009; Richards et al., 2010; Whyte et al., 2010).

However, the extremely demanding nature of ‘all-out’ sprinting has prompted more practical and tolerable models of high-intensity exercise (Gibala et al., 2012). A recently developed model is low volume, high-intensity interval training consisting of repeated (8 to 12) 1 min high-intensity efforts performed at 60 to 100% peak power output interspersed with 60 to 75 s recovery. This pattern of high-intensity training performed over 2 to 12 weeks appears an effective stimulus to improve a range of performance, physiological and health-related outcomes in healthy (Hood et al., 2011; Jacobs et al., 2013; Little et al., 2010) and patient populations (Currie et al., 2013; Little et al., 2011). The potential therapeutic effect of this exercise model is further demonstrated by the acute improvements seen in glycaemic control.
after a single exercise exposure (Gillen et al., 2012; Little et al., 2014). An adaptation of the low volume, high-intensity interval exercise regime adopted in these studies is pertinent to the experimental studies presented in Chapters 4, 7 and 8.

To the author’s knowledge, eight studies have considered the acute effect of high-intensity exercise on postprandial lipaemia in young, healthy men and women. Four studies have adopted the traditional Wingate protocol that dominates the training literature and involved performing either four or five ‘all-out’ cycle sprints with 4 to 4.5 min active recovery the day before the postprandial measurements (Freese et al., 2011; Gabriel et al., 2012, 2013; Tan et al., 2013). In the earliest of these studies, a single session of sprint interval cycling reduced postprandial [TAG] by 21%, an effect that was reduced to 10% when the exercise-induced energy deficit was replaced (Freese et al., 2011). Subsequent studies have reported similar reductions in postprandial [TAG] the day after the high-intensity exercise session (Gabriel et al., 2012, 2013), but the beneficial effect of sprint exercise dissipated after two days (Gabriel et al., 2013). An additional feature in the Gabriel et al. (2012) study was the inclusion of a moderate-intensity continuous walking condition (1.0 MJ, 241 kcal); however, in contrast to previous studies with a 30 min continuous exercise condition (Miyashita, 2008; Miyashita et al., 2006b, 2008, 2010), no effect was observed on the postprandial lipaemic response compared with the rest control condition. The reason for this discrepancy is unclear since the study design appears comparable to the study conducted by Miyashita et al. (2008), but the authors speculated that a lack of statistical power may be responsible. Alternatively, although the relative exercise intensity could not be established due to the absence of aerobic fitness assessment in the study by Gabriel et al. (2012), the brisk walk was performed at a slightly lower average oxygen uptake (V̇O₂) (Gabriel et al., 2012: 20 mL·kg·min⁻¹; Miyashita et al., 2008: 24 mL·kg·min⁻¹, 42% maximum V̇O₂). Therefore, it is possible that the exercise stimulus was insufficient to elicit a meaningful change in postprandial [TAG] in healthy, young men who were already regularly physically active. In a similar design, Tan et al. (2013) compared the Wingate protocol (0.27 MJ, 65 kcal), a single 20 min bout of continuous cycling at 70% maximum V̇O₂ (0.73 MJ, 173 kcal) and a rest control condition, but no differences in postprandial [TAG] were seen between the conditions. The authors emphasised the wide individual heterogeneity present in the postprandial responses and speculated that differences in participant characteristics, exercise timing, meal content and blood sampling may have contributed to the differing results (Tan et al., 2013).
Other postprandial studies with a high-intensity exercise component have adopted exercise protocols either with shorter sprint rest cycles (Allen et al., 2014; Tan et al., 2014) or longer, less intense efforts interspersed with shorter recovery intervals (Ferreira et al., 2011; Trombold et al., 2013). Specifically, Allen et al. (2014) reported no change in postprandial [TAG] in healthy males after performing $20 \times 6$ s maximal sprints with $24$ s recovery, but similar to Tan et al. (2013), the authors highlighted the substantial individual variation evident in the postprandial TAG response. In contrast, 20 min of repeated 8 s sprints with $12$ s recovery reduced postprandial [TAG] by $13\%$ in healthy, sedentary women (Tan et al., 2014). Longer duration, high-intensity interval running (alternating 3 min intervals at $98\%$ peak $\mathrm{VO}_2$ with $1.5$ min recovery for $40$ min) performed $30$ min before a high-fat meal reduced postprandial [TAG] to a similar extent compared with an isoenergetic (~$2.1$ MJ, $501$ kcal) bout of continuous running at $73\%$ peak $\mathrm{VO}_2$ (Ferreira et al., 2011). In the study by Trombold et al. (2013), high-intensity endurance cycling (alternating 2 min at $25\%$ peak $\mathrm{VO}_2$ and 2 min at $90\%$ peak $\mathrm{VO}_2$ for $42$ min) was more efficacious in reducing postprandial [TAG] than an isoenergetic $67$ min session of continuous, moderate-intensity cycling at $49\%$ peak $\mathrm{VO}_2$ ($-31\%$ vs. $-19\%$ respectively).

It is worth noting that estimating EE during high-intensity, non-steady state exercise based on indirect calorimetry is limited by the disturbances that occur in the bicarbonate pool; therefore, the gas composition of expired air is unlikely to reflect tissue metabolism (Jeukendrup and Wallis, 2005). Specifically, during increased glycolytic flux, lactate and hydrogen ions ($\mathrm{H}^+$) accumulate in the muscle and diffuse into the blood. The increase in $\mathrm{H}^+$ in the blood is buffered by sodium bicarbonate ($\mathrm{HCO}_3^-$) to form carbonic acid ($\mathrm{H}_2\mathrm{CO}_3$) which dissociates to form water ($\mathrm{H}_2\mathrm{O}$) and carbon dioxide ($\mathrm{CO}_2$) as follows:

$$\mathrm{H}^+ + \mathrm{HCO}_3^- \leftrightarrow \mathrm{H}_2\mathrm{CO}_3 \leftrightarrow \mathrm{H}_2\mathrm{O} + \mathrm{CO}_2$$

The production of excess, non-respiratory carbon dioxide is excreted at the lungs, which elevates carbon dioxide production and, therefore, is likely to overestimate carbohydrate oxidation and underestimate fat oxidation (Jeukendrup and Wallis, 2005; Rowlands, 2005). Romijn et al. (1992) reported that indirect calorimetry was a valid measurement of EE and substrate oxidation at exercise intensities up to $80$ to $85\%$ maximum $\mathrm{VO}_2$ when compared with a breath $^{13}\mathrm{C}/^{12}\mathrm{C}$ technique, although more recently, deviations have been demonstrated from $75\%$ maximum $\mathrm{VO}_2$ (Rowlands and Jeukendrup, 2004). Consequently, these limitations
can be problematic for investigators where indirect calorimetry is required to estimate EE (e.g., high-intensity treadmill exercise).

Although short-duration, high-intensity exercise has been proposed as a time-efficient strategy to improve cardiovascular health (Coyle, 2005), the total time of the exercise session is often comparable to traditional, continuous aerobic exercise due to the lengthy recovery time between the brief exercise intervals. Nevertheless, the evidence to date suggests high-intensity exercise may be an effective strategy to accrue potential health benefits. This is supported by a 16 year follow-up study showing that a single weekly bout of self-reported, high-intensity exercise is associated with a reduced risk of cardiovascular death compared to those reporting no activity in men (relative risk 0.61) and women (relative risk 0.49) (Wisløff et al., 2006). The potency of a single session of high-intensity exercise to reduce postprandial [TAG] is supported by several of the limited adult studies conducted to date and represents a potentially exciting avenue for future research. However, a few authors highlight the substantial heterogeneity in responses in the absence of an exercise-induced change in postprandial [TAG] which clearly requires further investigation.

2.5.3 Importance of the energy deficit

Despite the plethora of studies investigating the acute effects of exercise on postprandial lipaemia, only a small number of studies have examined whether the associated energy deficit or skeletal muscle contraction is responsible for the reduction in postprandial lipaemia. Gill and Hardman (2000) reported that the reduction in postprandial [TAG] following a 90 min session of moderate-intensity exercise was almost treble the attenuation seen when dietary energy intake was restricted in postmenopausal women the day before the postprandial measurements. Although a technical error in the exercise condition measurements resulted in a greater exercise energy deficit than the energy intake restriction condition (1.73 vs. 1.44 MJ respectively; ~17% difference), the authors indicated that the stark difference in outcome measures between the conditions meant that this did not affect the general interpretation of the results (Gill and Hardman, 2000). More recently, two studies have examined the efficacy of combining acute isoenergetic exercise and energy-intake restriction protocols to reduce postprandial [TAG] in healthy, young women (Maraki et al., 2009, 2010). The reduction in postprandial [TAG] was significantly greater for exercise than energy-intake restriction alone (23% vs. 12% respectively); however, the attenuation seen for exercise alone was matched when exercise was superimposed with energy-intake restriction (19%) (Maraki et al., 2009,
2010). The authors concluded that this combination may be attractive for individuals when regular, prolonged low- to moderate-intensity exercise is not feasible because of lifestyle constraints. Collectively, these studies suggest that although exercise EE may be a determinant in reducing postprandial lipaemia, the role of muscle contraction per se may need to be examined more thoroughly to determine its function when the exercise dose is low. The importance of the associated energy deficit represents a central theme to this thesis, and the role that exercise and energy-intake restriction play in modulating postprandial lipaemia will be explored experimentally in Chapter 6.

Further studies have examined the effect of exercise with dietary compensatory replacement (balance) on postprandial [TAG]. Burton et al. (2008) compared a single session of moderate intensity walking (~2.8 MJ), completed with and without energy replacement, with a resting control condition in overweight and obese middle-aged men. Although both exercise conditions induced metabolic changes in the postprandial period compared with a resting control, an exercise energy deficit was required to elicit reductions in fasting and postprandial [TAG] (Burton et al., 2008). Furthermore, a single session of high-intensity sprint interval cycling (4 × 30 s all-out sprints, 4 min recovery) reduced postprandial [TAG] by 21% in a group of healthy men and women (Freese et al., 2011). Although this reduction was diminished to 10% when the exercise-induced energy deficit was replaced, it remained significantly lower than a non-exercise control condition (Freese et al., 2011). The findings from these studies make an important contribution to the exercise postprandial literature and provide a platform for the work presented in Chapter 5 of this thesis.

Other studies have attempted to isolate the relative contribution of the exercise-induced energy and substrate deficit on postprandial lipaemia. Harrison et al. (2009) reported that the reduction in postprandial [TAG] was eliminated completely when high carbohydrate feedings at 0, 2 and 4 h post-exercise restored carbohydrate and energy balance in recreationally active men. A more recent study examined the effect of consuming an isoenergetic low- or high-carbohydrate meal immediately following 60 min cycling at 65% peak \(\text{V}O_2\) and ten, 1 min high-intensity intervals at ~95% peak \(\text{V}O_2\) (Trombold et al., 2014). In contrast to Harrison et al. (2009), consumption of a high-carbohydrate meal resulted in lower postprandial [TAG] compared with a non-exercise control; however, the reduction was augmented further with an isoenergetic low-carbohydrate meal and with no energy or carbohydrate replacement (Trombold et al., 2014). This finding has also been replicated in relation to fasting [TAG],
whereby a post-exercise low-carbohydrate meal that achieved energy balance significantly reduced fasting [TAG] the following morning compared with an isoenergetic high-carbohydrate meal (Newsom et al., 2010). Further exercise postprandial studies are clearly warranted to distinguish the effects of an energy and/or substrate deficit from that of exercise (Braun and Brooks, 2008). Nevertheless, maintenance of the exercise-induced energy and/or substrate deficit may be required to maximise the reduction in postprandial lipaemia, which has significant implications for individuals wanting to experience the ‘full’ benefit of exercise.

2.5.4 Potential mechanisms

Although the mechanisms underpinning the reduction in postprandial [TAG] following acute exercise are not fully understood, advances have been made in recent years. It is postulated that exercise evokes a transient increase in LPL expression and activity (Kiens and Richter, 1998; Seip et al., 1995, 1997; Seip and Semenkovich, 1998), which facilitates the hydrolysis and subsequent clearance of circulating lipoprotein TAG. The physiological site and temporal course of LPL upregulation in relation to exercise remains controversial; however, increases in skeletal muscle and post-heparin plasma LPL activity have been supported (Kiens and Richter, 1998; Seip et al., 1995, 1997; Zhang et al., 2002), and are likely to be elevated for up to 24 h post-exercise (Seip and Semenkovich, 1998). While exercise-induced changes in LPL are associated negatively with the acute reduction in postprandial [TAG] following exercise (Gill et al., 2003b; Herd et al., 2001), reductions in postprandial [TAG] have been reported without simultaneous increases in pre-heparin, post-heparin or skeletal muscle LPL activity (Gill et al., 2003b; Herd et al., 2001; Katsanos et al., 2004; Miyashita and Tokuyama, 2008), absolute leg TAG uptake (Malkova et al., 2000) or clearance of an intravenous lipid emulsion (Gill et al., 2001b). Consequently, it is unlikely that LPL-mediated clearance of circulating TAG is the sole mechanism responsible for the TAG-lowering effect of prior exercise.

The exercise-induced reduction in postprandial [TAG] is quantitatively greater in the VLDL fraction than the chylomicron fraction (Gill et al., 2001a, 2006; Malkova et al., 2000), suggesting that changes in VLDL synthesis and secretion may be implicated mechanistically. Indirect evidence for altered hepatic VLDL secretion can be drawn from studies reporting an exercise-induced increase in the circulating concentration of 3-hydroxybutyrate (3-OHB), a marker of hepatic fatty acid oxidation, alongside the reduction in postprandial [TAG] (Burton et al., 2008; Gill et al., 2001a, 2007; Malkova et al., 2000). This suggests a shift in hepatic VLDL kinetics from fatty acid re-esterification and VLDL synthesis towards hepatic fatty
acid oxidation. Accumulating evidence from basal kinetic studies suggests that VLDL-TAG clearance is enhanced the day following exercise in men and women, while hepatic VLDL-TAG secretion is unchanged in men (Bellou et al., 2013a; Magkos et al., 2006; Tsekouras et al., 2007), but decreased in women (Bellou et al., 2013c). It has been postulated that exercise promotes the secretion of fewer, TAG-richer VLDL apolipoprotein B-100 particles (Magkos et al., 2006). Although a more recent kinetic study did not report a change in VLDL$_1$-apo B production rates, exercise did increase TAG enrichment of VLDL$_1$ particles and tended to increase VLDL$_1$ particle size (Al-Shayji et al., 2012), which are likely to have a higher affinity for LPL (Fisher et al., 1995). Moreover, Gill et al. (2006) demonstrated a greater effect of prior exercise on VLDL$_1$ than chylomicron or VLDL$_2$ particles. A combination of enhanced TAG clearance and changes in VLDL kinetics are, therefore, likely to contribute to the TAG-lowering effect of acute exercise, which may be facilitated by increased hepatic and skeletal muscle blood flow promoting LPL-mediated hydrolysis of TAG (Hurren et al., 2011a; Malkova et al., 2000).

### 2.6 Exercise and postprandial whole-body fat oxidation in adults

#### 2.6.1 Acute exercise

Low resting fat oxidation has been implicated in the aetiology of obesity (Ellis et al., 2010; Kelley et al., 1999; Zurlo et al., 1990) and Type 2 diabetes mellitus (Blaak et al., 2001), and linked with the magnitude of postprandial lipaemia (Gill et al., 2007; Trombold et al., 2013). Beyond the well-established differences in substrate metabolism during exercise (Romijn et al., 1993), resting substrate metabolism is altered in the hours following an exercise session. In this regard, stable isotope tracer studies designed to track post-exercise fatty acid kinetics have reported elevated resting lipid oxidation for several hours in the postprandial state (Henderson et al., 2007; Votruba et al., 2002, 2003, 2005), which appears superior in men than women (Henderson et al., 2007), but independent of exercise intensity (Henderson et al., 2007; Votruba et al., 2002, 2003). Furthermore, respiratory exchange ratio values are significantly reduced up to 18 h following a single session of prolonged exercise in healthy men and women (Kiens and Richter, 1998; Kuo et al., 2005). These findings support a recent meta-analysis of 18 adult studies confirming the link between acute endurance exercise (60 to 120 min, 28 to 75% peak VO$_2$) and increased resting lipid oxidation (ES = 0.91) (Henderson and Alderman, 2014); this suggests exercise promotes the short-term partitioning of fatty acids towards oxidation rather than storage (Votruba et al., 2002, 2003, 2005).
Along with the assessment of postprandial lipaemia, several studies have quantified substrate oxidation in response to an acute exercise stimulus during the postprandial rest period. In the earliest studies in healthy men and women, performing 90 min moderate-intensity exercise the day before a high-fat meal reduced postprandial [TAG] and increased postprandial whole-body fat oxidation, whilst concomitantly reducing whole-body carbohydrate oxidation (Gill et al., 2001a; Herd et al., 2001; Tsetsonis et al., 1997). Moreover, Gill et al. (2001a) suggested that increases in both exogenous and endogenous fat oxidation mediated the exercise-induced increase in whole-body fat oxidation. More recently, Farah et al. (2010) have shown in sedentary overweight and obese men that exercising on three consecutive days does not augment the exercise-induced reduction in postprandial [TAG] and increase in resting whole-body fat oxidation seen after a single bout of moderate-intensity exercise. The similar reduction in postprandial [TAG] and increase in whole-body fat oxidation is likely to reflect the short-lived effect of exercise discussed previously (Section 2.5.1), and could point to a threshold attenuation above which additional increases in EE will not reduce postprandial [TAG] or increase whole-body fat oxidation further. Additionally, the authors postulate that sufficient dietary carbohydrate was consumed in both conditions to replace the liver and muscle glycogen stores depleted during the exercise sessions (discussed in Section 2.6.2), which may further contribute to the similar postprandial metabolic responses observed between the two conditions.

Another study has investigated the effect of exercise timing around meal ingestion and reported that whole-body fat oxidation was increased similarly during the subsequent 7 h postprandial period when moderate-intensity exercise was performed before or after a standardised breakfast meal (Farah and Gill, 2013). However, this effect was accounted for almost entirely by the net fat oxidation during the exercise session itself (Farah and Gill, 2013), which appears to contradict previous findings discussed above where elevations in whole-body fat oxidation persist the day following exercise despite the consumption of meals. This may reflect the lower exercise EE (~1.8 MJ vs. 2.3 to 4.5 MJ in previous studies) or perhaps the close proximity of the exercise session to the breakfast meal. Specifically, consuming a meal immediately post-exercise can attenuate the shift from carbohydrate to fat oxidation that normally occurs post-exercise (Dionne et al., 1999), and the elevated carbohydrate oxidation observed during exercise immediately following meal ingestion could reflect increased oxidation of exogenous carbohydrate (i.e., ingested at breakfast). This would reduce the degree of glycogen depletion from liver and muscle stores, which has been
implicated mechanistically to explain the post-exercise increase in fat oxidation (discussed in Section 2.6.2).

Other studies have investigated the postprandial metabolic responses following manipulations of the energy and carbohydrate deficit with mixed findings. A series of studies conducted by Melanson and colleagues (2009) suggested that exercise does not increase 24 h fat oxidation when energy balance is maintained. In contrast, replacing the exercise-induced energy deficit (~2.8 MJ) increased postprandial fat oxidation the following day by 14% compared with a non-exercise control, although an energy deficit was required to augment this effect (30% higher than control) (Burton et al., 2008). A more recent study manipulated the carbohydrate content of isoenergetic post-exercise meals achieving energy balance, and demonstrated that a low-, but not a high-carbohydrate meal promotes elevated whole-body fat oxidation the day following exercise (Trombold et al., 2014). This suggests that the carbohydrate deficit rather than the energy deficit may determine the increase in whole-body fat oxidation after exercise. However, a previous study demonstrated the contrary in that an exercise-induced energy deficit elicited a greater increase in fasting whole-body fat oxidation compared with maintaining energy balance, despite a similar dietary carbohydrate content of the post-exercise meal (Horowitz et al., 2005).

Two recent studies have compared the effect of different exercise modalities on postprandial substrate utilisation (Davitt et al., 2013; Trombold et al., 2013). In a stable isotope enrichment study, obese women completed a 60 min session of moderate-intensity treadmill exercise or an equivalent duration of resistance-type exercises 30 min before a liquid meal containing an isotope tracer was administered (Davitt et al., 2013). Both exercise modalities reduced postprandial [TAG] and increased whole-body and exogenous fat oxidation to a similar extent compared with a resting control condition (Davitt et al., 2013). Trombold et al. (2013) demonstrated that high-intensity intermittent cycling (42 min, alternating 2 min at 25% peak \( \dot{V}O_2 \) and 2 min at 90% peak \( \dot{V}O_2 \) performed the day before a standardised breakfast meal reduced postprandial [TAG] and increased whole-body fat oxidation to a greater extent than an isoenergetic bout of continuous moderate-intensity cycling (67 min, 49% peak \( \dot{V}O_2 \)) in healthy young men. Furthermore, postprandial whole-body fat oxidation was negatively correlated with the exercise-induced reduction in postprandial [TAG] (\( r = -0.67, P < 0.01 \)) (Trombold et al., 2013), which supports the findings of others (Burton et al., 2008; Farah et al., 2010; Trombold et al., 2014), and suggests that the exercise-induced
increase in postprandial whole-body fat oxidation at least partially augments the reduction in postprandial [TAG].

Collectively, these studies suggest that a single session of exercise transiently increases postprandial whole-body fat oxidation, which in turn may be associated with the reduction in postprandial [TAG]. However, careful consideration of the relative energy and/or macronutrient deficit and timing of exercise in relation to meal ingestion may be required to maximise the benefits of exercise.

2.6.2 Potential mechanisms

Elevated whole-body fat oxidation following exercise is thought to facilitate the restoration of glucose homeostasis and resynthesis of depleted skeletal muscle and/or hepatic glycogen stores (Kiens and Richter, 1998; Kimber et al., 2003), and may promote insulin sensitivity through partitioning excess fatty acids towards intramuscular TAG synthesis (Schenk and Horowitz, 2007). The contribution of intramuscular TAG to the increase in fat oxidation following exercise is debated, with reports of decreased (Kiens and Richter, 1998), unchanged (Kimber et al., 2003) and even increased concentrations (Schenk and Horowitz, 2007). Evidence of lipolysis and NEFA mobilisation from adipose tissue in the post-exercise recovery period supports NEFA as a potential source of fatty acids for oxidation (Henderson et al., 2007; Mulla et al., 2000). Furthermore, the commonly reported reduction in postprandial [TAG] following acute exercise supports the provision of fatty acids from TRL-TAG, mediated by increased TAG clearance to peripheral tissues and/or changes in VLDL synthesis and secretion (Section 2.5.4). Several regulatory mechanisms may be implicated in promoting the transient mobilisation and delivery of fatty acids for oxidation following prior exercise, including increased adipose tissue and skeletal muscle blood flow (Hurren et al., 2011a; Malkova et al., 2000; Mulla et al., 2000), increased skeletal muscle LPL activity (Kiens and Richter, 1998) and reduced pyruvate dehydrogenase activity (Kimber et al., 2003).

2.7 Exercise and postprandial lipaemia in children and adolescents

We have recently published a paper summarising the current studies investigating the effect of acute exercise on postprandial lipaemia in children and adolescents (Tolfrey et al., 2014b). Current international physical activity guidelines recommend that children and adolescents accumulate at least 60 min of moderate- to vigorous-intensity daily physical activity for health promotion (Department of Health, Physical Activity, Health Improvement and
Protection, 2011; Janssen and LeBlanc, 2010). A physically active lifestyle is associated with a multitude of health benefits in childhood and adolescence (Andersen et al., 2006; Brage et al., 2004; Ekelund et al., 2007, 2012; Janssen and LeBlanc, 2010), and improvements in important cardiovascular health outcomes are commonly reported after exercise training (Logan et al., 2014). Despite the well-established cardiovascular health benefits of regular physical activity, many children and adolescents, especially girls, fail to meet the guidelines (Hallal et al., 2012; Health Survey for England, 2012b; Verloigne et al., 2012), and physical activity participation has been shown to decline from childhood through adolescence (Health Survey for England, 2012b). Therefore, it is likely that exercise interventions in young people need to be engaging and sustainable in order for long-term benefits in metabolic health to emerge. This section will summarise the published acute exercise postprandial studies with young people to date.

2.7.1 Acute exercise

Eight studies have investigated the effect of acute exercise on postprandial lipaemia in children and adolescents (Barrett et al., 2007; Lee et al., 2013; MacEneaney et al., 2009; Sedgwick et al., 2013, 2014; Tolfrey et al., 2008, 2012, 2014a). A summary of these studies is presented in Table 2.2. In line with the adult literature, a single session of moderate- to vigorous-intensity exercise inducing an exercise EE ≥ 1.0 MJ (240 kcal) reduces postprandial [TAG] in boys and girls (Barrett et al., 2007; Lee et al., 2013; MacEneaney et al., 2009; Sedgwick et al., 2013, 2014; Tolfrey et al., 2008, 2012, 2014a). Although the magnitude of change in postprandial [TAG] after exercise varies in these studies (Table 2.2), on average the changes are moderate with estimated effect sizes (ES) ranging from 0.26 to 0.77 in those that reported statistically significant reductions compared to a resting control condition. Thus, an exercise-induced energy deficit is efficacious, but a closer examination of the exercise characteristics might be more enlightening and will be used to compare and contrast the main outcomes with the adult-based literature appraised above.
Table 2.2  Summary of studies examining the effect of acute exercise on postprandial lipaemia in children and adolescents.

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Intervention (INT)</th>
<th>EE (MJ)</th>
<th>INT to meal delay (h)</th>
<th>Amount of fat (g/kg BM)†</th>
<th>Effect size*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barrett et al. (2007)</td>
<td>10</td>
<td>M</td>
<td>15.3</td>
<td>4 × 15 min TMW @ 59% peak VO₂</td>
<td>2.0</td>
<td>16.0</td>
<td>1.3</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>M</td>
<td>15.4</td>
<td>4 × 18 min LIST @ 69% peak VO₂</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.66</td>
</tr>
<tr>
<td>Tolfrey et al. (2008)</td>
<td>8</td>
<td>M</td>
<td>12.9</td>
<td>6 × 10 min TMR @ 53% peak VO₂</td>
<td>1.5</td>
<td>14.7</td>
<td>1.5</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6 × 10 min TMR @ 75% peak VO₂</td>
<td>2.2</td>
<td></td>
<td></td>
<td>0.63</td>
</tr>
<tr>
<td>Tolfrey et al. (2012)</td>
<td>11</td>
<td>M</td>
<td>13.3</td>
<td>3 × 10 min TMR @ 55% peak VO₂</td>
<td>1.0</td>
<td>14.5</td>
<td>1.5</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6 × 10 min TMR @ 55% peak VO₂</td>
<td>2.0</td>
<td></td>
<td></td>
<td>0.32</td>
</tr>
<tr>
<td>Tolfrey et al. (2014a)</td>
<td>18</td>
<td>F</td>
<td>13.0</td>
<td>3 × 10 min TMW @ 55% peak VO₂</td>
<td>0.8</td>
<td>14.5</td>
<td>1.5</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6 × 10 min TMW @ 56% peak VO₂</td>
<td>1.5</td>
<td></td>
<td></td>
<td>0.36</td>
</tr>
<tr>
<td>Sedgwick et al. (2013)</td>
<td>13</td>
<td>M</td>
<td>13.6</td>
<td>60 min TMW @ 60% peak VO₂</td>
<td>1.9</td>
<td>15.0</td>
<td>Breakfast 1.5</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lunch 1.1</td>
<td></td>
</tr>
<tr>
<td>Sedgwick et al. (2014)</td>
<td>14</td>
<td>M</td>
<td>12.9</td>
<td>6 × 10 min TMR @ 72% peak VO₂</td>
<td>1.9</td>
<td>18.0</td>
<td>Breakfast 1.5</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>09:30 start (exo 10:50 rest) × 6</td>
<td></td>
<td></td>
<td>Lunch 1.1</td>
<td></td>
</tr>
<tr>
<td>MacEneaney et al. (2009)</td>
<td>10HW</td>
<td>M</td>
<td>15.6</td>
<td>59 min TM @ 65% peak VO₂</td>
<td>2.5</td>
<td>12.0 to 97</td>
<td>g/2 m² BSA</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>8OW</td>
<td>M</td>
<td>15.9</td>
<td>52 min TM @ 65% peak VO₂</td>
<td>2.5</td>
<td>14.0</td>
<td>g/2 m² BSA</td>
<td>0.56</td>
</tr>
<tr>
<td>Lee et al. (2013)</td>
<td>21B</td>
<td>9M/12F</td>
<td>15.4B</td>
<td>60 min cycling @ 50% peak VO₂</td>
<td>1.9B</td>
<td>14.0</td>
<td>64B &amp; 66W</td>
<td>0.16B</td>
</tr>
<tr>
<td></td>
<td>17W</td>
<td>12M/5F</td>
<td>14.5W</td>
<td></td>
<td>2.1W</td>
<td></td>
<td>g/2 m² BSA</td>
<td>0.40W</td>
</tr>
</tbody>
</table>

Adapted from Tolfrey et al. (2014b). Studies are arranged to match the critical appraisal in the text. †Unless indicated specifically, test meals were consumed as breakfast.

*An effect size of 0.2 represents the minimum important difference, 0.5 moderate and 0.8 large (Cohen, 1988); all values represent effects for exercise-induced total area under the concentration versus time curve for triacylglycerol (TAUC-TAG) compared with a non-exercise control condition - taken directly from published studies or calculated from \((\text{Exercise} - \text{Control}) / \text{Control standard deviation}) .

EE, energy expenditure; BM, body mass; M, male; TM, treadmill – W, walk, R, run; VO₂, oxygen uptake; LIST, Loughborough intermittent shuttle test; F, female; HW, healthy weight; OW, overweight; BSA, body surface area; B, black; W, white.
The majority of exercise postprandial studies in young people have adopted the standard 2-day model described previously (Section 2.5.2.1; Figure 2.1) and the level of standardisation for extraneous variables such as diet and physical activity has increased in recent years. In the first published study with young people, Barrett et al. (2007) demonstrated that single sessions of continuous moderate-intensity exercise and simulated intermittent games activity reduced postprandial [TAG] by ~14% and 26% respectively compared with a resting control in late adolescent boys. The intermittent games activity consisted of $4 \times 18$ min blocks of repeated walking, sprinting, cruising and jogging, with 3 min rest between each block. The greater reduction in the intermittent games activity condition was linked tentatively to the greater exercise EE; however, the evidence is limited by the indirect comparison of two different groups of boys in the between-measures design and absence of EE quantification during the intermittent games activity (Barrett et al., 2007). Nevertheless, it is very likely that the intermittent games arm of the study resulted in considerably higher EE given the total exercise time was 14 min longer and the average intensity was ~17% higher. In addition to being the first of its kind, this study is notable because the intermittent exercise was thought to better match the stop-start nature of young people’s activity habits compared with the long-duration continuous exercise that dominates the adult-based postprandial lipaemia literature. Recent evidence suggests that pre-pubertal boys prefer moderate-intensity exercise interspersed with short bursts of high-intensity effort compared with continuous, moderate-intensity exercise alone (Crisp et al., 2012). Considering children typically spend a greater percentage of time engaged in high-intensity activities than adults (Hoos et al., 2004), exercise with a high-intensity component may represent an attractive alternative to traditional moderate-intensity exercise to enable young people to acquire metabolic health benefits. Limited evidence suggests high-intensity interval training elicits meaningful improvements in CVD risk factors (Logan et al., 2014); however, the acute effect of high-intensity exercise on postprandial [TAG] in young people has yet to be clarified and warrants future investigation (Chapters 4, 7 and 8).

In contrast to the study conducted by Barrett and colleagues (2007), and studies with adults (Maraki and Sidossis, 2013), subsequent studies in healthy boys do not support an exercise EE dose-dependent response (Tolfrey et al., 2008, 2012). Specifically, performing 60 min of moderate and vigorous exercise were similarly efficacious in reducing postprandial [TAG] by ~24% and 21% respectively, despite the 45% greater exercise gross EE at the higher intensity (Tolfrey et al., 2008). Moreover, despite doubling the exercise gross EE in the 60 min (1967
kJ) compared with the 30 min condition (982 kJ), the additional attenuation in postprandial [TAG] was trivial (20% vs. 16% respectively) (Tolfrey et al., 2012). This led the authors to suggest that a ‘threshold’ attenuation may exist, above which additional increases in EE will not reduce postprandial [TAG] further in apparently healthy normolipidaemic boys (Tolfrey et al., 2012). Recently, when the same study design was conducted in healthy adolescent girls, only the 60 min exercise condition reduced postprandial [TAG] meaningfully and, therefore, does not provide direct support for a dose-response or ‘threshold’ attenuation (Tolfrey et al., 2014a). However, it is worth noting that 56% of the girls in this study experienced a lower postprandial TAG response after the 30 min exercise condition compared with a resting control, suggesting that on an individual level, some girls may benefit from a lower dose of moderate-intensity exercise (Tolfrey et al., 2014a). To the author’s knowledge, the study by Tolfrey et al. (2014a) represents the only study conducted in girls. In light of the lower physical activity levels in girls compared with similarly aged boys (Hallal et al., 2012; Health Survey for England, 2012b; Verloigne et al., 2012), and the decline in participation with age (Health Survey for England, 2012b), further research is required in this population and will be addressed in Chapters 6, 7 and 8.

Some postprandial lipaemia studies have included design features that provide insights beyond the EE focus that has dominated the majority of adult-based studies and the research with young people described above; these are now reviewed. Consumption of meals high in fat, and the associated lipaemic response, have been linked to endothelial dysfunction in adults (Vogel et al., 1997). Endothelial dysfunction is considered to be the first stage in atherogenesis (Ross, 1993), and is considered to be a pre-requisite for the development of atherosclerosis (Section 2.2; Juonala et al., 2004). In a study of 13 healthy adolescent boys, postprandial endothelial function, indicated by flow mediated dilation (FMD), was reduced by 32% following a high-fat breakfast and 24% after lunch compared with fasting in a non-exercise control condition (Sedgwick et al., 2013). In contrast, 60 min of treadmill walking at 60% peak VO$_2$ prevented the postprandial decline in FMD, once it had been normalised for the post-occlusion shear rate. It is noteworthy that the boys in this study did not demonstrate any of the risk factors for coronary heart disease, yet the consumption of the high-fat meals, increasingly common in westernised countries (Taveras et al., 2005), induced endothelial dysfunction to a similar extent seen in adults (Sedgwick et al., 2013). However, although this maintenance of normal endothelial function coincided with a reduction in postprandial plasma [TAG], FMD was not meaningfully related to [TAG] in either the control or exercise
conditions at any time point. Hence, the authors concluded that simultaneous changes in postprandial lipaemia and endothelial function were coincidental rather than causative.

When 60 min of exercise at 72% peak VO₂ was accumulated throughout the day in 6 × 10 min bouts in boys, this exercise was also found to prevent the postprandial decline in FMD (Sedgwick et al., 2014). However, although the total and incremental areas under the [TAG] versus time curve were reduced as a result of exercise by 11% and 16% respectively, these differences were not statistically significant. Despite these non-significant changes in postprandial [TAG], the reduction in the total and incremental areas under the [TAG] versus time curve are in line with previous research in adolescents and may still be meaningful physiologically. A significant attenuation of postprandial endothelial dysfunction was observed after the accumulated exercise; thus, providing additional support that exercise-induced alterations in endothelial function may be independent of changes in postprandial [TAG]. Accumulated, short-bouts of exercise may be particularly appealing to young people as they perceive prolonged activity to be more demanding than adults (Timmons and Bar-Or, 2003). In addition, short bouts of activity may be easier to incorporate into the school day than extended bouts of activity.

It would appear that only two studies have examined the postprandial lipaemic response to exercise in overweight young people (Lee et al., 2013; MacEneaney et al., 2009). MacEneaney et al. (2009) found overweight, late adolescent boys experienced a similar exercise-induced reduction in postprandial [TAG] (~20%) compared with similarly-aged, normal (healthy) weight boys. It should be noted, however, that the overweight boys in the MacEneaney et al. (2009) study had no history of diabetes, heart disease, or liver dysfunction, and were normo-tensive, -lipidaemic and -glycaemic. Despite the significant body size and composition differences between the two groups of boys, they were both considered to be healthy. Although the skinfold measures used to estimate body composition could not provide a more detailed pattern of fat distribution in the boys, there was a statistically significant positive association between the TAUC-TAG and sum of skinfolds in both the control (r = 0.49, P < 0.05) and exercise (r = 0.47, P < 0.05) conditions. Moreover, [TAG] returned to baseline after 6 h in the healthy weight boys, but not in the overweight boys. The authors suggested this may be due to delayed clearance, but recent evidence in adults may point to differences in hepatic release of fatty acids in TAG (Davitt et al., 2013).
In a study examining the effect of 60 min of moderate intensity cycling on postprandial lipaemia in overweight black and white adolescents (Lee et al., 2013), acute exercise reduced postprandial [TAG] in both groups, but the reduction was greater in white (19%) than in black (8%) adolescents. Interestingly, the authors found increased visceral fat was a major contributor to the greater reduction in [TAG] seen in the white, but not black adolescents. As in the MacEneaney et al. (2009) study, the overweight participants of the study were otherwise healthy. The results of the MacEneaney et al. (2009) and Lee et al. (2013) studies are supported by other non-exercise studies. Although obese and non-obese adolescents exhibit a similar postprandial TAG profile (Moreno et al., 2001; Umpaichitra et al., 2004), those with a central pattern of fat distribution displayed signs of impaired TAG clearance (Moreno et al., 2001). Consequently, the potential for exercise to improve this aspect of metabolic health in overweight children and adolescents is promising; however, the notorious difficulties in recruiting overweight and/or obese boys and girls to exercise-related studies are recognised.

2.7.2 Conclusion

Although from a small number of studies, the evidence from young people demonstrates consistently that a single session of moderate- to vigorous-intensity exercise promotes reductions in postprandial [TAG] in this population (Table 2.2). While it appears that a threshold of exercise EE may be required to promote a meaningful reduction in the postprandial TAG response, the evidence of a dose-dependent response is not yet supported directly in boys or girls (Tolfrey et al., 2008, 2012, 2014a). The mechanisms responsible for the reduction in postprandial [TAG] following acute exercise in young people have not been investigated currently, largely due to the invasive nature of the techniques required. Consequently, the proposed mechanisms in adults (Section 2.5.4) are drawn upon, although future examination of indirect and relatively non-invasive techniques (e.g., 3-OHB concentrations) may advance our understanding in paediatric populations. In the absence of a clinical endpoint, the relevance of these findings cannot be determined from a clinical perspective. Nevertheless, the evidence from these studies is encouraging and suggests exercise may be an effective strategy to reduce postprandial lipaemia and promote a healthier cardiovascular risk profile in children and adolescents, but additional work is required to increase the evidence base. Specifically, it has not been possible to identify published studies that have investigated the impact of high-intensity exercise or compared directly the effect of exercise and dietary manipulations on postprandial lipaemia in adolescent boys or girls,
which represents a time when many adverse health risk behaviours become established (Bao et al., 1996; Eisenmann et al., 2004; Katzmarzyk et al., 2001; Nicklas et al., 2002). Furthermore, the exercise-induced reduction in postprandial [TAG] and concomitant increase in whole-body fat oxidation reported in adults (Section 2.6.1) has not been investigated in young people and represents a potential avenue for future research. Finally, the postprandial lipaemic response to a single session of exercise has not been compared directly in boys and girls, highlighting a further gap in our understanding of this important marker of CVD risk.

2.8 Summary

Exaggerated postprandial [TAG] are implicated in the development and progression of atherosclerosis, and are established as an independent risk factor for future CVD, which remains a major public health concern in the United Kingdom and worldwide. The well-established paediatric origins of atherosclerosis highlight the need for interventions aimed at improving metabolic health early in life. In adults, acute exercise and diet interventions ameliorate the metabolic perturbations present in the postprandial period by reducing circulating [TAG] and increasing whole-body fat oxidation. Growing evidence in children and adolescents suggests acute moderate- to vigorous-intensity exercise interventions reduce postprandial [TAG]; however, little is known regarding the effect of novel exercise protocols or the relative importance of the energy deficit on postprandial [TAG] or whole-body fat oxidation. Therefore, this thesis aims to expand the evidence base in boys and girls by developing our understanding of the cardiovascular health benefits arising following acute exercise and diet manipulations. This was addressed in the proceeding chapters which aimed to answer the following research questions:

- Does a single session of high-intensity interval running reduce postprandial plasma [TAG] in boys (Chapter 4) and girls (Chapter 7)?
- To what extent does acute moderate-intensity exercise, with and without energy replacement, reduce postprandial plasma [TAG] and increase resting whole-body fat oxidation in boys (Chapter 5)?
- To what extent does an acute isoenergetic energy deficit induced by moderate-intensity exercise or energy-intake restriction reduce postprandial plasma [TAG] in girls (Chapter 6)?
• Does the combination of acute low volume, high-intensity interval running and energy-intake restriction reduce postprandial plasma [TAG] and increase resting whole-body fat oxidation in girls (Chapter 7)?

• Is the postprandial plasma TAG response to a single session of high-intensity interval running different in boys and girls (Chapter 8)?
CHAPTER 3
General methods

This chapter describes the general methods employed in the experimental studies presented in this thesis. All experimental studies were conducted at Loughborough University and were approved by the University Ethical Advisory Sub Committee. All researchers obtained enhanced clearance through the Criminal Records Bureau (replaced by the Disclosure and Barring Service, December 2012).

3.1 Participant recruitment

All participants were recruited from local secondary schools within the Loughborough area. The school head teacher was contacted and informed of the study purposes and main experimental procedures, and written confirmation of consent was obtained if they agreed to participate. Details of the experimental studies were explained to potential participants by the researchers during a brief presentation assembly at the school. Following the opportunity to raise any questions, potential participants interested in the study were provided with a written information pack to inform parents or guardians about the study. Before the study commenced, study volunteers were required to complete a health screen questionnaire with their parents or guardians (Appendix 1). Written informed consent was provided by a parent or guardian (Appendix 2) and study volunteers provided their written “willingness to participate” (Appendix 3). Participants and their parents or guardians were informed verbally and in writing that they were free to withdraw consent and discontinue participation in the study at any time without question.

The following inclusion criteria were used to recruit participants:

i. Boys or girls aged 11 to 13 years;
ii. A parent or guardian provided consent;
iii. Not taking any medication that may influence lipid or glucose metabolism;
iv. No known contraindications to exercise participation (e.g., congenital heart disease, uncontrolled exercise-induced asthma, chronic obstructive pulmonary disorder, musculoskeletal injury);
v. No diabetes;
vi. No coagulation or bleeding disorders;
vii. Tolerance for food and drink items provided during the study.

3.2 Anthropometry and physical maturation

Anthropometry was conducted with participants wearing shorts, T-shirt and socks. Stature was measured to the nearest 0.01 m using a fixed stadiometer (Holtain Ltd, Crosswell, UK) and body mass was quantified to the nearest 0.1 kg using a digital scale (Seca 770, Seca Ltd, Hamburg, Germany). Body mass index was calculated as body mass (kg) divided by stature squared (m$^2$). Triceps and subscapular skinfold thickness was measured by the same investigator to the nearest 0.2 mm on the right-hand side of the body using Harpenden callipers (Baty International, West Sussex, UK):

- **Triceps**: vertical fold on the posterior mid-line of the upper arm, mid-way between the acromion and olecranon;
- **Subscapular**: diagonal fold taken 2 cm below the inferior angle of the scapula at a 45° angle laterally and downward.

The median of three measurements at each site was used to estimate percent body fat (%BF) using the following maturation, race, and sex-specific equations (Slaughter et al., 1988):

Pubescent white males = $1.21 \times (\text{triceps} + \text{subscapular}) - .008 \times (\text{triceps} + \text{subscapular})^2 - 3.4$

Pubescent black males = $1.21 \times (\text{triceps} + \text{subscapular}) - .008 \times (\text{triceps} + \text{subscapular})^2 - 5.2$

All females = $1.33 \times (\text{triceps} + \text{subscapular}) - .013 \times (\text{triceps} + \text{subscapular})^2 - 2.5$

Lean body mass (LBM) was estimated using the following equation:

$$\text{LBM (kg)} = \text{body mass (kg)} \times (1-(\%BF / 100))$$

Participants were asked to provide a self-assessment of their level of physical maturity using drawings depicting the five stages of pubic hair development and either genital development in boys (Chapters 4, 5 and 8) or breast development in girls (Chapters 6, 7 and 8) (Appendix 4; Tanner, 1962). The scale ranges from 1 indicating pre-pubescence to 5 indicating full sexual maturity and participants identified the stage most closely resembling their current level of sexual development. Although this method of assessing biological maturation is widely used in paediatric exercise science because it is easy to administer, cost effective and
non-invasive (with self-assessment), it is important to consider potential limitations when interpreting these data. The stages are discrete categories imposed on a continuous process of biological maturation and provide no information on the age of entry or exit from the stage. There is considerable variability in the timing and tempo of maturation among adolescents and, therefore, the stages are not equivalent within sex (e.g., genital stage 3 is not equivalent to pubic hair stage 3 in boys) (Baxter-Jones et al., 2005). Furthermore, girls enter and end puberty approximately two years before boys and pubertal events do not occur in the same sequence between the sexes (Baxter-Jones et al., 2005); therefore, it is difficult to align girls and boys for pubertal status when making between sex-comparisons (Sherar et al., 2004). Self-assessment of sexual maturation shows moderate to high concordance with physician assessment (Matsudo and Matsudo, 1994; Schlossberger et al., 1992); however, young people tend to overestimate early stages and underestimate later stages of sexual development (Schlossberger et al., 1992).

3.3 Expired air measurements

3.3.1 Portable metabolic cart

Expired air samples were monitored continuously during exercise using an online breath-by-breath gas analysis system in Chapters 4, 7 and 8 (Metalyzer 3B, Cortex, Leipzig, Germany). The analyser was calibrated before each measurement using a bottled gas mixture containing 5.01% CO₂, 16.98% O₂, and balance N₂ (Cranlea and Company, Birmingham, UK) and a 3.0 L syringe (Hans Rudolph, Shawnee, USA). Participants wore an appropriate size facemask (Hans Rudolph, Shawnee, USA), which was checked for leaks and connected to the online system via a flowmeter before the expired air measurement began.

3.3.2 Douglas bags

Expired air samples were collected during rest or exercise into 100 L Douglas bags in Chapters 5, 6 and 7 (Cranlea and Company, Birmingham, UK). Oxygen uptake (\(\text{VO}_2\)) and carbon dioxide production were analysed using a paramagnetic oxygen analyser and an infrared carbon dioxide analyser (Servomex 1400, East Sussex, UK), which was calibrated with known reference gases prior to analysis (BOC Gases, Surrey, UK). The volume of expired air was quantified using a dry gas meter (Harvard Apparatus Ltd, Kent, UK) and the temperature of expired air was measured using a thermometer housed in the dry gas meter during evacuation (Edale Instruments (Cambridge) Ltd, UK). Ambient temperature and
barometric pressure were recorded using a wireless weather station (Oregon Scientific, Oregon, USA). All expired air measurements were corrected to standard room temperature and pressure for a dry gas (STPD), and \( \text{VO}_2 \), carbon dioxide production and respiratory exchange ratio were calculated for each sample.

### 3.3.3 Estimation of energy expenditure (EE) and substrate oxidation

Gross EE, carbohydrate oxidation and fat oxidation were estimated using the following stoichiometric equations during rest and steady-state exercise, assuming that the urinary nitrogen excretion rate was negligible (Frayn, 1983):

\[
\text{EE (kJ·min}^{-1}) = 4.1855 \times ((\text{fat (g·min}^{-1}) \times 9) + (\text{carbohydrate (g·min}^{-1}) \times 4))
\]

\[
\text{Fat oxidation (g·min}^{-1}) = 1.67 \times \dot{\text{V}}\text{O}_2 (\text{L·min}^{-1}) - 1.67 \times \dot{\text{V}}\text{CO}_2 (\text{L·min}^{-1})
\]

\[
\text{Carbohydrate oxidation (g·min}^{-1}) = 4.55 \times \dot{\text{V}}\text{CO}_2 (\text{L·min}^{-1}) - 3.21 \times \dot{\text{V}}\text{O}_2 (\text{L·min}^{-1})
\]

### 3.4 Heart rate and rating of perceived exertion (RPE)

Heart rate was monitored continuously during all exercise tests and interventions using short-range telemetry (Polar PE 4000, Kempele, Finland). The Borg scale was used to record RPE at pre-determined intervals during all exercise tests and interventions (Appendix 5; Borg, 1973). The scale ranges from 6 indicating rest to 20 indicating maximal exertion. Although several child-specific scales have been developed to measure effort perception during exercise in young people (see Appendix 6 for examples), Borg’s scale is considered a valid and reliable measure of perceived exertion in children as young as 9 years (Lamb, 1995).

### 3.5 Preliminary exercise tests

All exercise tests and interventions were conducted on a treadmill (Chapters 4, 6 and 8: TechnoGym RunRace, Gambettola, Italy; Chapters 5, 7 and 8: h/p/cosmos mercury med, Nussdorf-Traunstein, Germany). Participants were familiarised with walking, running and (dis)mounting the treadmill before the exercise tests commenced.

#### 3.5.1 Sub-maximal protocol

In Chapters 5 and 6, the participants completed a 16 min sub-maximal incremental treadmill protocol divided into four, 4 min stages starting at an initial speed of 4 km·h\(^{-1}\) (Chapter 6) or
5 km·h\(^{-1}\) (Chapter 5) and increasing 1 km·h\(^{-1}\) at the start of each subsequent stage, with the treadmill gradient set at 1% throughout (Jones and Doust, 1996). Using methods described above, individual steady-state relationships between treadmill speed, \(\text{VO}_2\) and heart rate were established. Expired air samples were collected during the final minute of each 4 minute stage into 100 L Douglas bags (Section 3.3.2), heart rate was monitored continuously and RPE was recorded in the final 10 s of each minute (Section 3.4).

### 3.5.2 Peak oxygen uptake (\(\text{VO}_2\))

#### 3.5.2.1 Gradient-based protocol

In Chapters 5 and 6, the sub-maximal protocol described above (Section 3.5.1) was followed by a standardised 10 min rest period before a gradient-based incremental treadmill protocol was completed to determine peak \(\text{VO}_2\). The participants ran at a fixed individual speed, identified from the sub-maximal protocol (Section 3.5.1), while the treadmill gradient was increased 1% each minute until volitional exhaustion. In Chapter 5, participants also completed a verification stage following a 10 min period of recovery (Barker et al., 2011). Participants ran at the same fixed individual speed, but the treadmill gradient was set 2% higher than the final gradient attained during the incremental protocol. Expired air measurements commenced after one minute and participants were required to run until volitional exhaustion (typically 2 to 3 minutes). Expired air samples were collected in one minute intervals using Douglas bags (Section 3.3.2), heart rate was monitored continuously and RPE was recorded in the final 10 s of each minute (Section 3.4).

#### 3.5.2.2 Speed-based protocol

In Chapters 4, 7 and 8, a speed-based incremental treadmill protocol was employed to determine peak \(\text{VO}_2\) and maximal aerobic speed (MAS), defined as the running speed eliciting the highest \(\text{VO}_2\) during an incremental test (Lacour et al., 1991). The protocol started at an initial speed of 5.0 km·h\(^{-1}\) (Chapters 7 and 8) or 6.0 km·h\(^{-1}\) (Chapters 4 and 8) with 0.5 km·h\(^{-1}\) increments every 30 s until volitional exhaustion, and the treadmill gradient was set at 1% throughout (Jones and Doust, 1996). Expired air samples were monitored continuously using a portable metabolic cart (Section 3.3.1), heart rate was monitored throughout and RPE was recorded in the final 10 s of each 30 s stage (Section 3.4). An average of the breath-by-breath \(\text{VO}_2\) data was taken every 10 s, and peak \(\text{VO}_2\) was defined as the highest 30 s rolling average; the treadmill speed corresponding to peak \(\text{VO}_2\) was recorded as MAS.
3.5.2.3 Criteria for attainment of peak oxygen uptake (\( \dot{V}O_2 \))

Attainment of maximal effort was confirmed based on the presence of a plateau in \( \dot{V}O_2 \) (\( \leq 3\% \)) with an increase in exercise intensity (i.e., speed or gradient). In the absence of a plateau in \( \dot{V}O_2 \), an exhaustive effort was verified based on the following secondary criteria: a peak heart rate \( \geq 95\% \) age-predicted maximum (220 - chronological age); a respiratory exchange ratio \( \geq 1.00 \); and clear subjective signs of fatigue (Armstrong et al., 1996).

3.6 Experimental design

The experimental studies in this thesis (Chapters 4 to 8) adopted the standard 2-day model employed in the majority of exercise postprandial studies in adults and young people (Chapter 2.5.2.1; Figure 2.1). Using a within-measures, counterbalanced, crossover design, participants completed the experimental intervention on day 1 (intervention day) prior to the assessment of the postprandial outcome measures on day 2 (postprandial day). Experimental conditions were separated by a standardised period of 14 days.

3.6.1 Standardisation of dietary intake

In each of the experimental chapters (Chapters 4 to 8), participants weighed and recorded their food and drink intake during the 48 h period (pre-intervention and intervention day) before day 2 of the first experimental condition (Appendix 7). Participants were asked to replicate this dietary pattern, including the amounts of food & drink and time of consumption, before the subsequent conditions. Two-day diet records were analysed using dietary analysis software (CompEat Pro Version 5.8.0, Nutrition Systems, Banbury, UK).

To standardise the overnight fasting period before day 2, participants consumed a small carbohydrate-rich cereal snack bar at 19:45 on the intervention day of each condition and arrived at the laboratory the next morning following a 12 h overnight fast. After 20:00, participants were allowed to drink plain water, but were asked not to consume any other drinks or food before arriving at the laboratory on day 2.

3.6.2 Standardisation of physical activity and sedentary time

Participants completed a self-reported diary of all physical activity categorised according to intensity level during the pre-intervention and intervention day of the first condition (Appendix 8). They were asked to minimise their physical activity during this period, and the
activity pattern was replicated before the remaining conditions. In Chapters 4 and 6, free-living physical activity and sedentary time was not quantified objectively so a comparison between the conditions is not available. In Chapters 5 and 7, an objective measurement of habitual physical activity and sedentary time was obtained using an ActiGraph GT1M accelerometer (ActiGraph, Pensacola, Florida, USA), which is a uniaxial device that detects vertical accelerations. Participants were asked to wear the ActiGraph on the right hip during waking hours (removed for water-based activities). During data processing, 5 s epoch data was re-integrated to 60 s epochs, 60 min of consecutive zeros, allowing for 2 min of non-zero interruptions was used to remove non-wear, and a minimum of 9 h of valid wear time was required for a valid day. Physical activity was expressed as average counts per minute (CPM), and intensity cut-points specified for 12 year olds were applied (Trost et al., 2002b): sedentary (< 100 counts·min\(^{-1}\)), light (100 – 1262 counts·min\(^{-1}\)), moderate (1262 – 4136 counts·min\(^{-1}\)) and vigorous (> 4136 counts·min\(^{-1}\)) activities.

### 3.6.3 Day 1: Intervention day

On day 1 at 15:30, participants arrived at the laboratory and all measures were completed by 16:30 (Chapter 4) or 17:30 (Chapters 5 to 8). Body mass was recorded at the start of each experimental condition to standardise the test meals provided on day 2.

### 3.6.4 Day 2: Postprandial day

In all experimental studies (Chapters 4 to 8), participants arrived at the laboratory at ~07:45 following a 12 h overnight fast. Participants consumed a standardised breakfast and lunch meal and blood samples were taken at pre-determined intervals in the fasting and postprandial state. Participants rested throughout the day and were able to read, watch DVD films and play non-active computer games. Participants consumed water *ad libitum* in the postprandial period of the first condition; the ingested volume was replicated in the subsequent conditions.

#### 3.6.4.1 Test meals

A breakfast meal consisting of croissants, chocolate spread, whole milk, double cream and milkshake powder was consumed following the completion of the fasting measures, marking the start of the postprandial period (~08:00). A lunch meal was eaten 4 h later at ~12:00 composed of white bread, butter, mild cheddar cheese, potato crisps, whole milk and milkshake powder. The meal quantity was prescribed relative to body mass and the amount
consumed is outlined in each experimental chapter (Chapters 4 to 8). During the first experimental condition, participants consumed the breakfast meal within 15 min and the lunch meal within 20 min; the time taken to consume each meal was recorded and replicated in the remaining conditions. To ensure consistency across participants and experimental conditions, participants consumed either chocolate or strawberry flavour milkshake powder on all visits.

### 3.6.4.2 Capillary blood sampling

Capillary blood samples were taken in the fasting state and then at 0.5, 1, 3, 4.5, 5 and 6.5 h following the consumption of the breakfast meal. To collect the capillary blood samples, the hand was pre-warmed for 5 min in water heated to 40°C. The fingertip was pierced (Unistik 3 Extra, Owen Mumford, UK) and up to 600 µL of whole capillary blood was collected into potassium-EDTA coated Microvette CB 300 tubes (Sarstedt Ltd, Leicester, UK). The whole blood samples were immediately centrifuged at 12,800 g for 15 min (Eppendorf 5415c, Hamburg, Germany). An automatic pipette was used to dispense 200 µL of plasma into a 0.5 ml Eppendorf tube (Eppendorf, Hamburg, Germany). The plasma samples were stored at -80°C for up to two months before subsequent analyses.

### 3.7 Analysis of capillary blood samples

#### 3.7.1 Estimation of plasma volume

In all experimental chapters (Chapters 4 to 8), haemoglobin concentration and haematocrit were quantified in duplicate in the fasting and final postprandial samples to estimate the acute change in plasma volume (Dill and Costill, 1974). Haemoglobin concentration was assessed using the cyanmethemoglobin method; 20 µL whole blood was added to 5 mL Drabkin’s solution and the absorbance was quantified photometrically at a wavelength of 546 nm using a spectrophotometer (Cecil CE1011, Cecil instruments, Cambridge, UK). Haematocrit was quantified using a microhaematocrit centrifuge and reader (Haematospin 1300 Microcentrifuge, Hawksley and Sons Ltd, Sussex, UK).

#### 3.7.2 Concentrations of plasma triacylglycerol (TAG), glucose, non-esterified fatty acids (NEFA) and insulin

Concentrations of plasma TAG and glucose were analysed in Chapters 4 to 8 by enzymatic colorimetric methods using a benchtop analyser (Pentra 400, HORIBA ABX Diagnostics,
Montpellier, France). The concentration of plasma NEFA (Randox Laboratories Ltd, County Antrim, UK) was analysed in Chapter 7 using the same method (Pentra 400, HORIBA ABX Diagnostics, Montpellier, France). Plasma insulin concentration was quantified in Chapters 4 and 6 using a commercially available enzyme-linked immunoassay (Mercodia Insulin ELISA, Mercodia AB, Uppsala, Sweden).

### 3.7.3 Precision of analysis

To eliminate inter-assay variation, the plasma samples for each participant were analysed in the same run. The within-batch coefficient of variation (CV) for each assay was completed for each run by repeating the measurement on the same plasma sample 10 times. Calculation of the CV was conducted on log-transformed data using the following formula:

\[
CV = 100 \times (e^{RMSE} - 1) \quad \text{(Batterham and George, 2003)}
\]

### 3.8 Statistical analyses

The statistical analysis procedures common to all experimental studies (Chapters 4 to 8) will be presented in this section and supplemented with specific details in the individual chapters. Data were analysed using the IBM SPSS Statistics Software for Windows version 20 (IBM Corporation, New York, USA). Descriptive statistics illustrating the physical and physiological characteristics of all participants and exercise responses were calculated. The trapezium rule was used to calculate the total area under the variable versus time curve (TAUC) and the incremental area under the variable versus time curve (iAUC) was calculated using the same method after correcting for fasting concentrations (Matthews et al., 1990). Normality of the data was checked by Shapiro Wilk tests. Homogeneity of variances was confirmed by Mauchley’s test of sphericity and a Greenhouse-Geisser correction was applied to the degrees of freedom if the sphericity assumption was violated. Energy and macronutrient intakes along with free-living physical activity and sedentary time (Chapters 5 and 7), estimated changes in plasma volume, fasting plasma concentrations and TAUC and iAUC responses were compared between experimental conditions using separate one-way within-measures analysis of variance (ANOVA). Differences in plasma concentrations over the total 6.5 h postprandial period were examined using separate 2 × 7 (condition by time; Chapter 4) or 3 × 7 (condition by time; Chapters 5 to 7) within-measures ANOVA. Temporal changes in TAUC-TAG between the experimental conditions were examined over sub-
sections of the total postprandial period (0 to 1 h, 1 to 4.5 h and 4.5 to 6.5 h) using separate one-way, within-measures ANOVA. *A priori* simple planned contrasts with CON as the reference category were conducted to follow up the effects of the omnibus ANOVAs. Bivariate correlations identifying possible determinants of exercise-induced changes in TAUC-TAG were quantified using Pearson’s product moment correlations or Spearman’s rank order correlation coefficients if the standardised residuals were not normally distributed. Normally distributed data are presented as mean (standard deviation (SD)). The 95% confidence intervals (CI) for mean absolute pairwise differences between experimental conditions were calculated using the t-distribution and degrees of freedom \((n – 1)\). Absolute standardised effect sizes (ES) were calculated for within-measures comparisons to supplement important findings as follows:

\[
ES = \frac{Mean_{v_2} - Mean_{v_1}}{CON SD}
\]

(Cumming and Finch, 2001)

In the absence of a clinical anchor, an ES of 0.2 was considered the minimum important difference for all outcome measures, 0.5 moderate and 0.8 large (Cohen, 1988). Statistical significance was accepted as \(P < 0.05\).
CHAPTER 4
Acute high-intensity interval running reduces postprandial lipaemia in boys

4.1 Abstract

Acute moderate-intensity exercise reduces postprandial lipaemia in boys. However, the effect of high-intensity exercise has not been investigated. This study examined the effect of low volume, high-intensity interval running (HIIR) on postprandial plasma triacylglycerol concentrations ([TAG]). Fifteen healthy, active boys (mean(SD): age 11.8(0.4) years; body mass 42.8(8.0) kg; peak oxygen uptake (VO₂) 55(6) mL·kg⁻¹·min⁻¹) completed two, 2-day conditions in a counterbalanced, crossover design separated by 14 days. On day 1, participants rested (CON) or completed 10 × 1 min running intervals at 100% maximal aerobic speed, determined from an incremental peak VO₂ test, with 1 min recovery between intervals (HIIR). On day 2, capillary blood samples were taken in the fasted state and at predetermined intervals throughout the 6.5 h postprandial period while participants rested. A standardised breakfast was consumed at 08:00 immediately after the fasting sample and a standardised lunch meal at 12:00. Differences in fasting plasma [TAG] were small to moderate (95% confidence interval (CI) -0.11 to 0.01 mmol·L⁻¹, effect size (ES) = 0.40, P = 0.10). Postprandial plasma [TAG] was lower during HIIR compared with CON (95% CI -0.19 to -0.02 mmol·L⁻¹, P = 0.02). The total area under the [TAG] versus time curve was lower following HIIR compared with CON (5.77(1.30) vs. 6.54(1.71) mmol·L⁻¹·6.5 h respectively; 95% CI -1.42 to -0.12 mmol·L⁻¹·6.5 h, ES = 0.51, P = 0.02). This is the first study to show that low volume, HIIR reduces postprandial plasma [TAG] in healthy, active 11 to 12 year old boys.
4.2 Introduction

Elevated plasma triacylglycerol concentrations ([TAG]), especially in the postprandial period, have been implicated in the development of atherosclerosis (Zilversmit, 1979). Impaired clearance of postprandial [TAG] is associated independently with an increased risk of future cardiovascular disease (CVD) (Bansal et al., 2007; Nordestgaard et al., 2007), which remain the leading cause of death in the United Kingdom (Townsend et al., 2012). Most people spend the majority of the daytime in a postprandial state typically; therefore, repeated daily exposure to elevated postprandial [TAG] promotes the development of an atherogenic lipid profile of small, dense low-density lipoprotein (LDL) and low concentrations of high-density lipoprotein (HDL) (Cohn, 1998). Considering the process of atherosclerosis is initiated during childhood, lifestyle interventions that moderate postprandial lipaemia by improving TAG metabolism may slow atherogenic progression, even during childhood and adolescence (McGill et al., 2000a).

Acute moderate-intensity exercise (30 min to 3 h in duration) performed up to 18 h before a standardised meal reduces postprandial lipaemia in adults (Peddie et al., 2012), although this effect is short-lived (Herd et al., 1998) and may be dependent on the exercise-induced energy expenditure (EE) (Gill et al., 2002a). Similar postprandial studies with boys have also reported acute reductions in postprandial lipaemia following a single moderate-intensity exercise session (Barrett et al., 2007; MacEneaney et al., 2009; Tolfrey et al., 2008, 2012). Barrett et al. (2007) suggested the larger reduction in postprandial [TAG] after intermittent-games activity compared with continuous exercise may reflect the higher exercise EE. However, the evidence is limited by the between-measures design and the inability to estimate the exercise EE during the intermittent-games activity (Barrett et al., 2007). Subsequent studies suggest a dose-dependent response is not supported in healthy young people (Tolfrey et al., 2008, 2012).

Recent findings have suggested that low volume, high-intensity interval training results in similar physiological and metabolic adaptations as higher volume, moderate- to vigorous-intensity continuous training (Hood et al., 2011; Little et al., 2011). Furthermore, this type of training may also improve insulin sensitivity and glycaemic control (Little et al., 2011). Acute intermittent high-intensity exercise has also been shown to reduce the postprandial lipaemic response in healthy adult men and women (Ferreira et al., 2011; Freese et al., 2011; Gabriel et al., 2012). However, it is not known whether a single session of low volume, high-
intensity exercise reduces the plasma TAG response to standardised meals in boys. Current international guidelines recommend that children and adolescents accumulate at least 60 min of moderate daily physical activity to promote and maintain health (Department of Health, Physical Activity, Health Improvement and Protection, 2011; Janssen and LeBlanc, 2010). Considering that many children and adolescents fail to meet these guidelines (Riddoch et al., 2007), low volume, high-intensity exercise may represent a viable alternative to help improve health and increase physical activity participation from a young age (Buchan et al., 2011).

Therefore, the aim of the present study was to examine the effect of a single bout of low volume, high-intensity interval running (HIIR) on postprandial lipaemia in healthy, active 11 to 12 year old boys. It was hypothesised that acute HIIR would effectively reduce postprandial [TAG] compared with a resting control despite the relatively low exercise EE.

4.3 Methods

4.3.1 Participants

Sixteen boys aged 11.3 to 12.9 years volunteered to participate in this study. Results are presented for 15 boys as one boy dropped out due to illness. All participants indicated they were actively participating in sport, but not specifically accustomed to high-intensity running. Physical and physiological characteristics of participants are presented in Table 4.1.

4.3.2 Preliminary anthropometric and exercise measurements

During the first visit to the laboratory, preliminary anthropometric measurements were recorded as described in Chapter 3.2. Following familiarisation with the treadmill, participants completed the speed-based incremental treadmill protocol to determine peak oxygen uptake (\( \dot{V}O_2 \)) and maximal aerobic speed (MAS) detailed in Chapter 3.5.2.2.

4.3.3 Experimental design

Participants completed two, 2-day experimental conditions: a resting control condition (CON) and a high-intensity interval running condition (HIIR). The study design is presented schematically in Figure 4.1.
Table 4.1  Physical and physiological characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.8 (0.4)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>42.8 (8.0)</td>
</tr>
<tr>
<td>Stature (m)</td>
<td>1.53 (0.09)</td>
</tr>
<tr>
<td>Body mass index (kg·m⁻²)</td>
<td>18.3 (2.8)</td>
</tr>
<tr>
<td>Percent body fat (%)</td>
<td>13.5 (5.2)</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>36.7 (5.4)</td>
</tr>
<tr>
<td>Genital development*</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Pubic hair development*</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Peak oxygen uptake (mL·kg⁻¹·min⁻¹)</td>
<td>55 (6)</td>
</tr>
</tbody>
</table>

Values are mean (SD) for $n = 15$. *Self-assessment – median (interquartile range).

Figure 4.1  Diagram of the 2-day study protocol. TAG, triacylglycerol. †Evening meal replicated from the first condition.

4.3.3.1  Standardisation of dietary intake

Participants weighed, recorded and replicated their habitual dietary intake on the pre-intervention and intervention day of all experimental conditions (Chapter 3.6.1). The macronutrient composition of the carbohydrate-rich cereal snack bar consumed at 19:45 on
the intervention day of each condition was 1.2 g fat, 16.1 g carbohydrate and 1.0 g protein, which provided 334 kJ energy.

4.3.3.2 Day 1: Intervention day

During HIIR, participants completed a 5 min warm-up at 60% MAS, followed immediately by the acute high-intensity running intervals and concluding with a 3 min cool-down at 40% MAS. The high-intensity exercise session involved 10 × 1 min treadmill runs at 100% MAS with 1 min active recovery between each interval. Several recent studies adopting a low volume, high-intensity exercise session (10 × 1 min high-intensity cycle sprints, 1 min recovery) reported that this protocol was well tolerated in sedentary men and women (Hood et al., 2011) and patients with Type 2 diabetes mellitus (Little et al., 2011). Following initial pilot work with 12 to 14 year old adolescents, this pattern of exercise on the treadmill was deemed suitable for this population. During the active recovery period, participants dismounted the treadmill and were encouraged to pace around the lab to avoid venous pooling and feeling light headed. Heart rate was monitored continuously and the participants provided a rating of perceived exertion (RPE) in the last 10 s of each interval as described previously (Chapter 3.4). During CON, participants rested in the laboratory to match the duration of HIIR.

4.3.3.3 Day 2: Postprandial day

Capillary blood samples were taken at pre-determined intervals throughout the 6.5 h postprandial rest period prior to, and following the consumption of standardised breakfast and lunch meals (Chapter 3.6.4). The breakfast meal provided 1.5 g fat (60% of meal total energy), 1.8 g carbohydrate (33%), 0.4 g protein (7%) and 93 kJ energy per kilogram body mass. The lunch meal provided 1.1 g fat (50%), 1.9 g carbohydrate (38%), 0.6 g protein (12%) and 86 kJ energy per kilogram body mass.

4.3.4 Analytical methods

Capillary blood samples were analysed for plasma [TAG], glucose concentration ([glucose]) and insulin concentration ([insulin]) (Chapter 3.7.2). The within-batch coefficient of variation for plasma [TAG], [glucose] and [insulin] were 1.0, 0.4 and 4.1% respectively.
Chapter 4: High-intensity exercise and postprandial metabolism

4.3.5 Statistical analyses

Data were analysed using the statistical methods presented in Chapter 3.8. Student’s paired t-tests were used to identify temporal changes in HIIR exercise responses between the first and final running interval.

4.4 Results

4.4.1 Dietary intake

Average energy intake was similar during the 48 h before day 2 of CON and HIIR (8.1(1.6) vs. 7.9(1.6) MJ·day\(^{-1}\) respectively; 95% confidence interval (CI) -1.3 to 0.9 MJ·day\(^{-1}\), \(P = 0.70\)). Average 2-day macronutrient intake did not differ between CON and HIIR for protein (69.0(15.7) vs. 68.8(14.3) g·day\(^{-1}\); 95% CI -8.4 to 8.1 g·day\(^{-1}\), \(P = 0.96\)), carbohydrate (270.0(48.4) vs. 253.1(55.4) g·day\(^{-1}\); 95% CI -55.0 to 21.2 g·day\(^{-1}\), \(P = 0.36\)) or fat (64.1(20.8) vs. 66.2(20.1) g·day\(^{-1}\); 95% CI -7.6 to 11.7 g·day\(^{-1}\), \(P = 0.65\)), respectively.

4.4.2 Responses to high-intensity interval running (HIIR)

The HIIR session was well tolerated by all participants and was performed at an average MAS of 12.5(1.6) km·h\(^{-1}\). Mean heart rate increased progressively from interval 1 to interval 10 (interval 1: 184(8) vs. interval 10: 194(8) beats·min\(^{-1}\); 95% CI 7 to 13 beats·min\(^{-1}\), effect size (ES) = 1.29, \(P < 0.001\)), which corresponded to 92(3)% and 97(2)% peak heart rate respectively (95% CI 4 to 6%, ES = 1.88, \(P < 0.001\)). Mean RPE during interval 1 was 10(3) (between very light and fairly light on the scale), but increased to 19(1) at the end of interval 10 (very, very hard) (95% CI 7 to 11, ES = 3.97, \(P < 0.001\)).

4.4.3 Plasma volume changes and fasting [TAG], [glucose] and [insulin]

Average changes in plasma volume between the fasting and 6.5 h postprandial samples were small and did not differ significantly between the two conditions (CON 0.46%, HIIR 0.85%; 95% CI -3.77 to 4.57%, \(P = 0.84\)). Therefore, the raw plasma [TAG], [glucose] and [insulin] were used in all statistical analyses without adjustment. The fasting plasma [TAG], [glucose] and [insulin] for each condition are shown in Table 4.2. Differences in fasting plasma [TAG] were small to moderate (95% CI -0.11 to 0.01 mmol·L\(^{-1}\), ES = 0.40, \(P = 0.10\)), with a slightly lower fasting [TAG] evident after HIIR. There were no differences in fasting plasma [glucose]
(95% CI -0.33 to 0.19 mmol·L\(^{-1}\), \(P = 0.56\)) or [insulin] (95% CI -19.0 to 16.9 mmol·L\(^{-1}\), \(P = 0.90\)) between CON and HIIR.

**Table 4.2** Fasting and postprandial plasma triacylglycerol, glucose and insulin concentrations in the control (CON) and high-intensity interval running (HIIR) conditions.

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>HIIR</th>
<th>Mean Difference</th>
<th>95% CI*</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Triacylglycerol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (mmol·L(^{-1}))</td>
<td>0.58 (0.14)</td>
<td>0.53 (0.11)</td>
<td>-0.05</td>
<td>-0.11 to 0.01</td>
<td>0.40</td>
</tr>
<tr>
<td>TAUC (mmol·L(^{-1}) 6.5 h)</td>
<td>6.54 (1.71)</td>
<td>5.77 (1.30)</td>
<td>-0.77</td>
<td>-1.42 to -0.12(^{a})</td>
<td>0.51</td>
</tr>
<tr>
<td>iAUC (mmol·L(^{-1}) 6.5 h)</td>
<td>3.08 (1.26)</td>
<td>2.61 (0.87)</td>
<td>-0.47</td>
<td>-1.04 to 0.10</td>
<td>0.43</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (mmol·L(^{-1}))</td>
<td>5.52 (0.33)</td>
<td>5.45 (0.63)</td>
<td>-0.07</td>
<td>-0.33 to 0.19</td>
<td>0.14</td>
</tr>
<tr>
<td>TAUC (mmol·L(^{-1}) 6.5 h)</td>
<td>42.7 (2.9)</td>
<td>42.4 (2.0)</td>
<td>-0.3</td>
<td>-1.9 to 1.3</td>
<td>0.13</td>
</tr>
<tr>
<td>iAUC (mmol·L(^{-1}) 6.5 h)</td>
<td>9.53 (4.21)</td>
<td>9.64 (4.75)</td>
<td>0.11</td>
<td>-1.89 to 2.11</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Insulin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (pmol·L(^{-1}))</td>
<td>44.4 (17.9)</td>
<td>43.4 (39.9)</td>
<td>-1.0</td>
<td>-19.0 to 16.9</td>
<td>0.03</td>
</tr>
<tr>
<td>TAUC (pmol·L(^{-1}) 6.5 h)</td>
<td>1297 (302)</td>
<td>1224 (366)</td>
<td>-73</td>
<td>-224 to 79</td>
<td>0.22</td>
</tr>
<tr>
<td>iAUC (pmol·L(^{-1}) 6.5 h)</td>
<td>1030 (270)</td>
<td>963 (320)</td>
<td>-67</td>
<td>-170 to 37</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Values are mean (SD) for \(n = 15\). *95% confidence interval of the mean absolute difference between CON and HIIR.

TAUC, total area under the concentration versus time curve; iAUC, incremental area under the concentration versus time curve.

\(^{a}\) Significant difference between HIIR and CON (\(P < 0.05\))
Figure 4.2  Fasting (F) and postprandial plasma triacylglycerol concentrations ([TAG]) in the control (CON) and high-intensity interval running (HIIR) conditions (n = 15). Values are mean (SD). Black rectangles denote breakfast and lunch meals consumed at 08:00 and 12:00, respectively. Main effect condition $P = 0.02$; main effect time $P < 0.001$; condition by time interaction $P = 0.11$.

4.4.4 Plasma [TAG], [glucose] and [insulin] in the postprandial period

Plasma TAG responses over the postprandial period for the experimental conditions are shown in Figure 4.2. Postprandial plasma [TAG] was lower during HIIR compared with CON (main effect condition 95% CI -0.19 to -0.02 mmol∙L$^{-1}$, $P = 0.02$; main effect time $P < 0.001$; condition by time interaction $P = 0.11$). The peak in plasma [TAG], occurring at 5 h in both conditions, was lower following HIIR compared with CON (95% CI -0.36 to -0.06 mmol∙L$^{-1}$, ES = 0.70, $P = 0.01$). In addition, the total area under the concentration versus time curve (TAUC) for TAG was lower following HIIR compared with CON (95% CI -1.42 to -0.12 mmol∙L$^{-1}$ 6.5 h, ES = 0.51, $P = 0.02$) (Table 4.2). Differences in sub-sections of the TAUC-TAG between CON and HIIR were identified (main effect condition 95% CI -0.47 to -0.04, $P = 0.02$). Specifically, TAUC-TAG was lower after HIIR compared with CON between 0 to 1 h (95% CI -0.10 to 0.00, ES = 0.29, $P = 0.05$), 1 to 4.5 h (95% CI -0.85 to 0.03, ES = 0.46, $P = 0.07$) and 4.5 to 6.5 h (95% CI -0.54 to -0.08, ES = 0.58, $P = 0.01$).
Individual changes (delta) in TAUC-TAG between CON and HIIR are shown in Figure 4.3. Ten boys responded to the interval running session (i.e., the reductions in TAUC-TAG following the high-intensity interval running exceeded the control). Percent peak heart rate during the interval runs was the only measured variable demonstrating a meaningful relationship with TAUC-TAG ($r = -0.69; 95\% CI -0.89 to -0.27, P = 0.005$), explaining 48% of the variance (Figure 4.4). Differences in the incremental area under the concentration versus time curve (iAUC) for TAG between CON and HIIR were small to moderate (95% CI -1.04 to 0.10 mmol\( \cdot \)L\(^{-1}\) 6.5 h, ES = 0.43, $P = 0.10$) (Table 4.2).

**Figure 4.3** Individual changes (delta) in the total area under the plasma triacylglycerol (TAG) concentration versus time curve (TAUC) between the high-intensity interval running and control conditions (HIIR minus CON). Participant data are organised according to the size of the delta change in TAUC-TAG. Negative values indicate a reduction in TAUC-TAG in HIIR compared with CON.

Postprandial plasma [glucose] did not differ significantly between CON and HIIR (main effect condition 95% CI -0.30 to 0.15 mmol\( \cdot \)L\(^{-1}\), $P = 0.48$; main effect time $P < 0.001$; condition by time interaction $P = 0.29$). No meaningful difference in TAUC-glucose was evident between CON and HIIR (95% CI -1.9 to 1.3 mmol\( \cdot \)L\(^{-1}\) 6.5 h, $P = 0.68$) (Table 4.2).
The iAUC-glucose did not differ meaningfully between CON and HIIR (95% CI -1.89 to 2.11 mmol·L⁻¹·6.5 h, \( P = 0.91 \)) (Table 4.2).

There was no significant difference in postprandial plasma [insulin] between CON and HIIR (main effect condition 95% CI -35 to 13 mmol·L⁻¹, \( P = 0.35 \); main effect time \( P < 0.001 \); condition by time interaction \( P = 0.69 \)). In addition, TAUC-insulin was similar across the experimental conditions (95% CI -224 to 79 mmol·L⁻¹·6.5 h, \( P = 0.32 \)) (Table 4.2). No meaningful difference in iAUC-insulin was evident between CON and HIIR (95% CI -170 to 37 mmol·L⁻¹·6.5 h, \( P = 0.19 \)) (Table 4.2).

**Figure 4.4**  The relationship between individual changes (delta; Figure 4.3) in the total area under the plasma triacylglycerol (TAG) concentration versus time curve (TAUC) and average percent peak heart rate during the high-intensity interval running (HIIR) condition. CON, control condition.

### 4.5 Discussion

The main finding of the present study was that a single session of low volume HIIR performed the day before standardised test meals reduced postprandial plasma [TAG] in healthy, active 11 to 12 year old boys. To our knowledge, this is the first study to investigate the effect of HIIR on postprandial lipaemia in boys. The exercise protocol was well tolerated.
by all participants and, therefore, may have practical applications for health in similar populations.

Changes in fasting plasma [TAG] were small to moderate following the exercise intervention, consistent with previous findings in young people involving moderate and vigorous intensity exercise (Barrett et al., 2007; Tolfrey et al., 2008, 2012). However, fasting [TAG] is typically less predictive of CVD risk than postprandial [TAG] (Bansal et al., 2007). In addition, substantial variation is evident in fasting [TAG] in children (Tolfrey et al., 1999), highlighting the importance of studying plasma [TAG] in the postprandial period.

Along with the plethora of studies supporting the TAG-lowering effect of moderate-intensity exercise in adults (Peddie et al., 2012), there is growing evidence that moderate and vigorous intensity exercise promote reductions in postprandial lipaemia in boys (Barrett et al., 2007; MacEneaney et al., 2009; Tolfrey et al., 2008, 2012). The current study extends these findings by demonstrating for the first time that low volume HIIR reduces postprandial plasma [TAG] in 11 to 12 year old boys (Table 4.2, Figure 4.2). Several recent studies with adults also support the reduction in postprandial [TAG] following intermittent high-intensity interval running (Ferreira et al., 2011) and all-out cycle sprints (Freese et al., 2011; Gabriel et al., 2012). Interestingly, a small to moderate reduction in iAUC-TAG was evident after HIIR suggesting that the TAG-lowering effect of HIIR is influenced, in part, by the change in fasting [TAG] (i.e. endogenous very low-density lipoprotein (VLDL) metabolism). Nevertheless, the iAUC-TAG was lower after HIIR compared with CON indicating that differences in the metabolism of exogenous TAG may contribute somewhat to the lower postprandial [TAG] evident after HIIR.

Currently, the change in postprandial lipaemia after exercise in young people varies, with estimated ES ranging from 0.26 to 0.77 (Table 2.2; Barrett et al., 2007; MacEneaney et al., 2009; Tolfrey et al., 2008, 2012). However, on average, the changes are moderate and comparable to the attenuation evident after HIIR in our study. Although comparing different groups of boys indirectly may be confounded by differences in participant characteristics, it provides an important insight into the extent HIIR reduces postprandial lipaemia in this population. Previous studies with young boys demonstrate that the peak in postprandial [TAG] occurs 2 to 4 h following the consumption of a single standardised meal (Barrett et al., 2007; MacEneaney et al., 2009; Tolfrey et al., 2008, 2012). The later peak in the present study (~5 h in both conditions) reflects the additional effect of the lunch meal on the postprandial
lipaemic response. Differences in postprandial plasma [TAG] between CON and HIIR were observed throughout the 6.5 h postprandial period. Although other studies with boys have not examined differences in TAUC-TAG over sub-sections of the total postprandial period, the TAG-lowering effect of moderate and vigorous intensity exercise appears to persist throughout the postprandial period (Barrett et al., 2007; MacEneaney et al., 2009; Tolfrey et al., 2008, 2012).

The effect of exercise on postprandial TAG metabolism has traditionally been linked with the exercise EE in adults (Gill et al., 2002a). A recent review suggests that an EE threshold of 2 MJ is required to elicit acute reductions in postprandial [TAG] (Maraki and Sidossis, 2010). However, the evidence of a dose-dependent response in young people is not supported (Tolfrey et al., 2008, 2012). Although it was not possible to measure the exercise EE directly, it is reasonable to assume that the short duration of HIIR (10 minutes in total) would incur a lower EE than that reported in other studies investigating the effect of longer duration, moderate or vigorous intensity exercise on postprandial [TAG] in boys (Barrett et al., 2007; MacEneaney et al., 2009; Tolfrey et al., 2008, 2012), and below the 2 MJ threshold suggested in adults (Maraki and Sidossis, 2010). Estimating EE during high-intensity exercise based on indirect calorimetry is limited by the disturbances in the bicarbonate pool that occur during non-steady state exercise and, therefore, the gas composition of expired air is unlikely to reflect tissue metabolism (Chapter 2.5.2.3; Jeukendrup and Wallis, 2005). Indeed, Gabriel et al. (2012) reported that in adults, low volume, sprint interval cycling reduced postprandial lipaemia, and yet, no effect of brisk walking was observed on postprandial [TAG] despite a 57% greater exercise EE estimated from the average power output and mechanical efficiency. Consequently, the capacity for high-intensity exercise to reduce postprandial [TAG] suggests that the exercise intensity is important in modifying the postprandial lipaemic response.

Clear inter-individual variability is evident in the exercise-induced changes in plasma [TAG] (Figure 4.3), which is consistent with previous work in boys (Tolfrey et al., 2012) and adults (Gill et al., 2007). Average percent peak heart rate during HIIR was the only physical or exercise response variable that demonstrated a significant correlation with exercise-induced TAUC-TAG (Figure 4.4), suggesting that exercising at a higher relative intensity augments the attenuation in postprandial [TAG]. Although \( \dot{V}O_2 \) was not measured during HIIR, neither \( \dot{V}O_2 \), substrate utilisation nor exercise EE have been found to contribute meaningfully to the individual heterogeneity in postprandial lipaemia following moderate-intensity exercise in
adults and boys previously (Gill et al., 2007; Tolfrey et al., 2012). While the majority of boys in the current study were classified as early pubescent, a range of self-assessed sexual maturity ratings were identified (pubic hair development stage 1: \( n = 2 \); stage 2: \( n = 11 \); stage 3: \( n = 2 \)). Subsequent analyses revealed no discernible effect of maturity status on the postprandial TAG response (data not shown). However, the influence of maturity status on postprandial lipaemia cannot be determined with confidence from our findings due to the limitations associated with self-report data and stages of sexual maturation (Chapter 3.2), in addition to the relatively small sample size.

It is widely accepted that nonfasting [TAG] is an independent risk factor for future cardiovascular events (Bansal et al., 2007; Nordestgaard et al., 2007), and efforts to reduce cardiovascular risk factors should begin from a young age (McGill et al., 2000a). However, the clinical relevance of the exercise-induced reduction in postprandial [TAG] cannot be determined from our findings. Currently, it has not been possible to identify a pre-defined postprandial lipaemic response in young people or adults beyond which further reductions will confer improved health. Consequently, it is not possible to identify how many participants experienced meaningful reductions in postprandial [TAG] following HIIR. Nevertheless, all participants in the present study demonstrated a healthy postprandial TAG profile independent of the experimental condition and the time of TAG measurement and, therefore, the potential for HIIR to elicit reductions in postprandial lipaemia in individuals with normal postprandial TAG metabolism is encouraging.

The potential for low volume HIIR to reduce the postprandial lipaemic response is promising considering many young people fall short of the current physical activity recommendations (Riddoch et al., 2007). A perceived lack of time and enjoyment are highlighted frequently as barriers to exercise participation in adolescents (Butt et al., 2011). The total exercise time commitment (including warm-up, recovery and cool-down) in the present study was 27 min, highlighting the time-efficiency of our HIIR session. It has been found that combining moderate-intensity exercise with periods of high-intensity effort is associated with greater perceived enjoyment than performing a similar duration of continuous, moderate-intensity exercise alone in prepubertal boys (Crisp et al., 2012). Moreover, children typically spend a lower percentage of time engaged in low-intensity activities and more time on high-intensity activities compared with adults (Hoos et al., 2004). Consequently, HIIR may represent an effective strategy in boys to improve health that is practical, time-efficient and enjoyable, but further research is required to support this.
In the present study, no differences in postprandial plasma [glucose] or [insulin] were evident, and therefore, are unlikely to contribute to the diminished postprandial plasma TAG response following HIIR. The evidence for acute moderate-intensity exercise-induced changes in postprandial [glucose] following high-fat meals are generally not well supported in young people (Barrett et al., 2007; MacEneaney et al., 2009; Tolfrey et al., 2008, 2012). Furthermore, despite the paucity of research, no effect of moderate-intensity exercise was evident on the postprandial insulin profile (MacEneaney et al., 2009). Insulin is known to play a pivotal role in TAG metabolism, regulating the uptake of TAG to skeletal muscle and adipose tissue, along with the release of VLDL from hepatic tissue. However, exercise-induced changes in postprandial lipaemia appear independent of the postprandial insulin response (Gill et al., 2002b).

The mechanisms responsible for the acute attenuation in postprandial [TAG] following exercise are not known currently in young people and cannot be elucidated from our findings. In adults, it is proposed that enhanced removal of TAG from the blood mediated by increased lipoprotein lipase (LPL) activity in the plasma or muscle (Gill et al., 2003b; Herd et al., 2001) and/or a reduction in hepatic VLDL-TAG synthesis and secretion (Magkos, 2009) is responsible. However, it is likely that enhanced muscle LPL activity is mediated by a reduction in plasma [insulin] (Kiens et al., 1989), which was not observed in the present study. In support of the latter mechanism, Gill et al. (2007) reported 3-hydroxybutyrate (3-OHB) concentration, a marker of hepatic fatty acid oxidation, was associated with moderate-intensity exercise-induced changes in postprandial [TAG]. However, no effect of high-intensity exercise was observed on plasma 3-OHB concentrations in adults (Gabriel et al., 2012).

A possible limitation of this study is the accuracy of physical activity replication between the experimental conditions. Participants were asked to subjectively record their physical activities 48 h before day 2 of the first experimental condition and then replicate this during the same period before the second condition. Although this procedure was verified verbally and by comparing the diaries, in the absence of an objective measure to quantify free-living physical activity and sedentary time, discrepancies between the conditions would introduce variability between the conditions that may influence the postprandial measures. A further limitation concerns the fact that participants did not complete a session of similar duration moderate-intensity exercise for comparison with HIIR.
In conclusion, the present study is the first to our knowledge to show that low volume HIIR performed ~15.5 h before a standardised breakfast reduces postprandial plasma [TAG] in healthy, active 11 to 12 year old boys. Low volume, high-intensity exercise may be a time-efficient strategy to improve health in boys, but further work is required to examine this chronically.
CHAPTER 5

Influence of acute moderate-intensity exercise with and without energy replacement on postprandial lipaemia and whole-body fat oxidation in boys

5.1 Abstract

Acute bouts of exercise reduce postprandial triacylglycerol concentrations ([TAG]) in healthy boys and girls; however, it is not known whether this effect is mediated by the energy deficit. This study examined whether the exercise-induced reduction in postprandial plasma [TAG] persists after immediate dietary replacement of the exercise energy expenditure (EE) in eighteen healthy boys (mean(SD): age 12.3(0.5) years; body mass 41.3(8.4) kg; peak oxygen uptake (\(\dot{V}O_2\)) 55(5) mL·kg\(^{-1}\)·min\(^{-1}\)). Participants completed three, 2-day conditions in a counterbalanced, crossover design separated by 14 days. On day 1, participants rested (CON), exercised at 60% peak \(\dot{V}O_2\) inducing a net EE of 32 kJ·kg\(^{-1}\)·body mass (EX-DEF) or completed the same exercise with the net EE replaced immediately (EX-REP). On day 2, capillary blood and expired air samples were taken in the fasted state and at predetermined intervals throughout the 6.5 h postprandial period. A standardised breakfast and lunch meal were consumed immediately and 4 h, respectively, after the fasting sample. Based on ratios of the geometric means (95% confidence intervals (CI) for ratios), EX-DEF fasting plasma [TAG] was 19% and 15% lower than CON (-31 to -5%, effect size (ES) = 1.15, \(P = 0.01\)) and EX-REP (-32 to 4%, \(ES = 0.91, P = 0.11\)) respectively; CON and EX-REP were similar (-4%; -19 to 12%, \(P = 0.56\)). The EX-DEF total area under the [TAG] versus time curve was 15% and 16% lower than CON (-27 to -1%, \(ES = 0.55, P = 0.04\)) and EX-REP (-31 to 1%, \(ES = 0.62, P = 0.07\)) respectively; CON and EX-REP were not different (2%; -13 to 20%, \(P = 0.80\)). Based on the arithmetic means (95% CI), the relative contribution of fat oxidation to total resting EE was greater than CON (56(13)%) in EX-DEF (63(11)%); 0 to 15%, \(ES = 0.60, P = 0.05\)), but EX-REP (57(15)%) was similar to EX-DEF (-16 to 4%, \(P = 0.25\)) and CON (-7 to 10%, \(P = 0.67\)). Immediate replacement of the exercise-induced energy deficit negates the reduction in postprandial plasma [TAG] in healthy boys.
5.2 Introduction

Elevated postprandial triacylglycerol concentrations ([TAG]) are predisposed to the development and progression of atherosclerosis (Zilversmit, 1979), and independently predict future cardiovascular disease (CVD) risk in men and women (Bansal et al., 2007; Nordestgaard et al., 2007). Although the clinical manifestations of atherosclerotic disease emerge in adulthood typically, the paediatric origins of atherosclerosis are well established (Froberg and Andersen, 2005; McGill et al., 2000a). Furthermore, childhood fasting [TAG] predicts young adult CVD risk (Morrison et al., 2009, 2012). Most people spend the majority of waking hours in a postprandial state typically, resulting in extended periods of elevated postprandial [TAG]. Considering CVD remains the leading cause of mortality in the United Kingdom (Townsend et al., 2012) and worldwide (World Health Organisation, 2011a), prevention by targeting modifiable risk factors is a high priority on the public health agenda (World Health Organisation, 2011a). Therefore, lifestyle modifications that reduce postprandial [TAG] from a young age may delay precursors of atherosclerotic disease leading to important long-term metabolic health benefits (Froberg and Andersen, 2005; McGill et al., 2000a).

Previous research highlights the potency of acute moderate- to vigorous-intensity exercise interventions completed up to 18 h before a standardised meal to reduce postprandial [TAG] in adults (Freese et al., 2014; Maraki and Sidossis, 2013) and young people (Table 2.2; Tolfrey et al., 2014b). Furthermore, acute exercise has been shown to increase resting fat oxidation in the postprandial period in adults (Davitt et al., 2013; Trombold et al., 2013), which is associated with the magnitude of the postprandial lipaemic response (Trombold et al., 2013). Considering energy status can have profound effects on metabolism (Braun and Brooks, 2008), the acute exercise-evoked changes in postprandial TAG metabolism may be mediated by the associated energy deficit. In this regard, an exercise-induced energy deficit appears more potent than an isoenergetic diet-induced deficit in reducing postprandial [TAG] in girls (Chapter 6) and pre- and post-menopausal women (Gill and Hardman, 2000; Maraki et al., 2010). Moreover, replacement of the exercise-induced energy deficit in adults diminishes or even eliminates the reduction in postprandial [TAG] (Burton et al., 2008; Freese et al., 2011; Harrison et al., 2009; Trombold et al., 2014), and concomitant increase in resting whole-body fat oxidation (Burton et al., 2008; Trombold et al., 2014). However, it is not known whether the acute exercise-induced reduction in postprandial [TAG] and increase
in resting whole-body fat oxidation persists after replacing the exercise energy expenditure (EE) in boys, which may have important implications regarding acute exercise, dietary compensation behaviours and metabolic health.

Therefore, the aim of the present study was to examine the effect of acute moderate-intensity exercise, with and without immediate dietary replacement of the exercise-induced energy deficit, on postprandial plasma [TAG] and resting whole-body fat oxidation in healthy, recreationally active boys.

5.3 Methods

5.3.1 Participants

Eighteen recreationally active boys aged 11.4 to 13.2 years volunteered to participate in this study. Physical and physiological characteristics of participants are presented in Table 5.1.

<table>
<thead>
<tr>
<th>Table 5.1</th>
<th>Physical and physiological characteristics.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>12.3 (0.5)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>41.3 (8.4)</td>
</tr>
<tr>
<td>Stature (m)</td>
<td>1.50 (0.07)</td>
</tr>
<tr>
<td>Body mass index (kg·m(^{-2}))</td>
<td>18.1 (2.4)</td>
</tr>
<tr>
<td>Percent body fat (%)</td>
<td>16.3 (5.5)</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>34.1 (4.7)</td>
</tr>
<tr>
<td>Genital development*</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Pubic hair development*</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Peak oxygen uptake (mL·kg(^{-1})·min(^{-1}))</td>
<td>55 (5)</td>
</tr>
</tbody>
</table>

Values are mean (SD) for \(n = 18\). *Self-assessment – median (interquartile range).

5.3.2 Preliminary exercise measurements

During the first visit to the laboratory, preliminary anthropometry was completed as described in Chapter 3.2. Following familiarisation with the treadmill, participants completed two preliminary exercise tests: 1) 16 min sub-maximal incremental treadmill protocol
(Chapter 3.5.1); 2) incremental uphill treadmill protocol and verification stage to determine peak oxygen uptake ($\dot{VO}_2$) performed at a fixed individual speed (8.0 to 10.0 km·h$^{-1}$) (Chapter 3.5.2.1). Data from the 16 min sub-maximal incremental and peak $\dot{VO}_2$ tests were used to determine the treadmill speed required to elicit 60% peak $\dot{VO}_2$ during the experimental exercise conditions.

### 5.3.3 Experimental design

Participants completed three, 2-day experimental conditions: rest control (CON), exercise with energy deficit (EX-DEF) and exercise with energy replacement (EX-REP). The study design is presented schematically in Figure 5.1.

![Figure 5.1 Diagram of the 2-day study protocol. TM, treadmill; $\dot{VO}_2$, oxygen uptake; TAG, triacylglycerol. *Evening meal replicated from the first condition.](image)

#### 5.3.3.1 Standardisation of dietary intake

Participants weighed, recorded and replicated their habitual dietary intake on the pre-intervention and intervention day of all experimental conditions (Chapter 3.6.1). The macronutrient composition of the carbohydrate-rich cereal snack bar consumed at 19:45 on the intervention day of each condition was 1.4 g fat, 12.3 g carbohydrate and 1.0 g protein, which provided 313 kJ energy.
5.3.3.2 Standardisation of free-living physical activity and sedentary time

An ActiGraph GT1M accelerometer was worn on the pre-intervention and intervention day of all experimental conditions as described previously (Chapter 3.6.2).

5.3.3.3 Day 1: Intervention day

During CON, participants rested in the laboratory for the duration of the visit. During EX-DEF and EX-REP, participants exercised on the treadmill at 60% peak VO\textsubscript{2} in 20 min intervals separated by a standardised period of 5 min seated rest, and was designed to induce a net EE of 32 kJ·kg\textsuperscript{-1} body mass. Expired air samples were collected and analysed as described in Chapter 3.3.2 during the third, tenth and seventeenth minute of each 20 min block to calculate the relative exercise intensity. The treadmill speed was adjusted occasionally to ensure the target exercise intensity was met. The exercise EE and the oxidation of carbohydrate and fat were estimated via indirect calorimetry (Chapter 3.3.3). The net EE of exercise was calculated as the exercise gross EE minus resting EE, where resting EE was estimated using age- and sex-specific equations (FAO/WHO/UNU, 2004). Heart rate was monitored throughout and the participants provided a rating of perceived exertion (RPE) during the last 10 s of each expired air sampling period (Chapter 3.4). Immediately following the cessation of exercise in EX-REP, the exercise-induced EE was replaced using a milkshake drink composed of strawberry or chocolate milkshake powder and whole milk which provided 12.9(2.7) g fat (36(2)% of drink total energy), 39.0(8.3) g carbohydrate (49(3)%), 11.7(2.5) g protein (15(1)%) and 1333(271) kJ energy. Perceptions of hunger, satiety, fullness and prospective food consumption were assessed pre- and post-milkshake consumption using a 100 mm visual analogue scale (Flint et al., 2000), and participants were also asked to rate the palatability of the milkshake (Appendix 9). Appetite sensations were similar before and after the milkshake and the milkshake palatability score was high, confirming the acceptability of the dietary intervention (data not shown).

5.3.3.4 Day 2: Postprandial day

Capillary blood samples were taken at pre-determined intervals throughout the 6.5 h rest period, prior to and following the consumption of standardised breakfast and lunch meals (Chapter 3.6.4). A 5 min resting expired air sample was collected after each capillary blood sample using Douglas bags (Chapter 3.3.2), and EE, fat oxidation and carbohydrate oxidation were estimated (Chapter 3.3.3). The postprandial expired air data for one boy were spurious
so results are presented for 17 boys. The breakfast meal provided 1.5 g fat (61.1% of meal total energy), 1.8 g carbohydrate (32.5%), 0.4 g protein (6.3%) and 95 kJ energy per kilogram body mass. The lunch meal provided 1.0 g fat (47.4%), 2.0 g carbohydrate (40.4%), 0.6 g protein (12.2%) and 81 kJ energy per kilogram body mass.

5.3.4 Analytical methods

Capillary blood samples were analysed for plasma [TAG] and glucose concentration ([glucose]) (Chapter 3.7.2). The within-batch coefficient of variation for plasma [TAG] and [glucose] were 2.6 and 0.5% respectively.

5.3.5 Statistical analyses

Data were analysed using the statistical methods presented in Chapter 3.8. Differences between EX-DEF and EX-REP exercise responses were examined using Student’s paired t-tests. Postprandial resting whole-body EE and substrate oxidation were calculated as total area under the variable versus time curve (TAUC) and, where appropriate, divided by the total duration of the postprandial period (6.5 h). Data for free-living physical activity and sedentary time, and concentrations of plasma TAG and glucose were natural log transformed prior to analysis. These data are presented as median (interquartile range) and analysis is based on the ratios of the geometric means and 95% confidence intervals (CI) for ratios. All analysis of variance (ANOVA) analyses were adjusted appropriately for the period effect (Senn, 2002).

5.4 Results

5.4.1 Dietary intake

Energy and macronutrient intakes were not different between CON, EX-DEF and EX-REP on the pre-intervention day ($P \geq 0.25$). Average daily energy intake was 7.9(1.8) MJ, and dietary intake of protein, carbohydrate and fat was 69.2(21.7) g, 254(62) g and 66.6(24.5) g respectively. Energy and macronutrient intakes during the intervention day are displayed in Table 5.2. Energy and absolute protein, carbohydrate and fat intake on the intervention day were higher in EX-REP compared with CON and EX-DEF (effect size (ES) = 0.60 to 1.22, $P < 0.001$); CON and EX-DEF were similar ($P \geq 0.27$). However, no differences were observed across the conditions when accounting for the additional energy and macronutrients consumed in the post-exercise milkshake drink (ES = 0.01 to 0.12, $P \geq 0.52$). The
contribution of protein, carbohydrate and fat to total energy intake was not different across the conditions ($P \geq 0.42$).

5.4.2 Free-living physical activity and sedentary time

On the pre-intervention day, one-way ANOVA identified a trend for differences in counts per minute (CPM) across the conditions ($P = 0.09$), with simple planned contrasts revealing EX-REP CPM was 70 counts·min$^{-1}$ lower compared with CON (-25 to -2%, $ES = 0.40, P = 0.02$). No other differences were seen in physical activity levels or sedentary time across the conditions on the pre-intervention day ($P \geq 0.10$). Physical activity levels and sedentary time on the intervention day are displayed in Table 5.3. No significant differences were seen across the conditions for daily wear time ($P = 0.45$), sedentary time ($P = 0.52$) or time spent in moderate-intensity activities ($P = 0.76$). Average CPM was higher than CON by 363 counts·min$^{-1}$ in EX-DEF ($ES = 2.35, P < 0.001$) and by 344 counts·min$^{-1}$ in EX-REP ($ES = 2.26, P < 0.001$); EX-REP and EX-DEF were similar (19 counts·min$^{-1}$; $P = 0.74$). Time spent in light-intensity activities was lower than CON by 40 min in EX-DEF ($ES = 0.87, P = 0.01$) and by 24 min in EX-REP ($ES = 0.50, P = 0.05$); EX-REP and EX-DEF were not different from each other (-16 min; $P = 0.26$). Time spent in vigorous-intensity activities was higher than CON by 44 min in EX-DEF ($ES = 2.09, P < 0.001$) and by 47 min in EX-REP ($ES = 2.16, P < 0.001$); EX-REP and EX-DEF were similar (-3 min; $P = 0.36$). No differences were seen in physical activity levels or sedentary time across the conditions when accounting for the time resting or exercising in the laboratory ($P \geq 0.16$).
Table 5.2 Energy and macronutrient intakes during the intervention day in the control (CON), exercise with energy deficit (EX-DEF) and exercise with energy replacement (EX-REP) conditions.

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>EX-DEF</th>
<th>EX-REP</th>
<th>CON vs. EX-DEF 95% CI*</th>
<th>CON vs. EX-REP 95% CI*</th>
<th>EX-REP vs. EX-DEF 95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (MJ·day⁻¹)</td>
<td>7.0 (1.4)</td>
<td>7.0 (1.6)</td>
<td>8.4 (1.4)</td>
<td>-0.2 to 0.3</td>
<td>1.1 to 1.7ᵇ</td>
<td>-1.6 to -1.0ᶜ</td>
</tr>
<tr>
<td>Protein (g·day⁻¹)</td>
<td>55.6 (14.0)</td>
<td>56.5 (14.5)</td>
<td>67.5 (13.4)</td>
<td>-0.7 to 2.3</td>
<td>9.7 to 13.8ᵇ</td>
<td>-12.9 to -9.1ᶜ</td>
</tr>
<tr>
<td>CHO (g·day⁻¹)</td>
<td>224 (36)</td>
<td>225 (45)</td>
<td>268 (41)</td>
<td>-10 to 10</td>
<td>33 to 53ᵇ</td>
<td>-52 to -33ᶜ</td>
</tr>
<tr>
<td>Fat (g·day⁻¹)</td>
<td>60.4 (19.6)</td>
<td>61.0 (21.0)</td>
<td>72.8 (18.4)</td>
<td>-1.5 to 3.0</td>
<td>8.3 to 16.4ᵇ</td>
<td>-15.9 to -7.3ᶜ</td>
</tr>
<tr>
<td>% energy intake from protein</td>
<td>13 (3)</td>
<td>14 (3)</td>
<td>14 (2)</td>
<td>-0.1 to 0.5</td>
<td>-0.2 to 0.5</td>
<td>-0.5 to 0.4</td>
</tr>
<tr>
<td>% energy intake from CHO</td>
<td>55(5)</td>
<td>54 (6)</td>
<td>54 (4)</td>
<td>-1.1 to 0.4</td>
<td>-1.6 to 0.5</td>
<td>-0.8 to 1.3</td>
</tr>
<tr>
<td>% energy intake from fat</td>
<td>32 (6)</td>
<td>32 (6)</td>
<td>32 (5)</td>
<td>-0.4 to 0.8</td>
<td>-0.6 to 1.4</td>
<td>-1.2 to 0.8</td>
</tr>
</tbody>
</table>

Values are mean (SD) for n = 18. Values for EX-REP include the post-exercise milkshake drink. *95% confidence interval of the mean absolute difference between the experimental conditions.

CHO, carbohydrate.

ᵇ Significant difference between EX-REP and CON (P < 0.001)

ᶜ Significant difference between EX-DEF and EX-REP (P < 0.001)
Table 5.3  Physical activity levels and sedentary time during the intervention day in the control (CON), exercise with energy deficit (EX-DEF) and exercise with energy replacement (EX-REP) conditions.

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>EX-DEF</th>
<th>EX-REP</th>
<th>CON vs. EX-DEF 95% CI*</th>
<th>CON vs. EX-REP 95% CI*</th>
<th>EX-REP vs. EX-DEF 95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily wear time (min)</td>
<td>819 (766-870)</td>
<td>795 (773-865)</td>
<td>853 (802-885)</td>
<td>-9 to 4%</td>
<td>-5 to 7%</td>
<td>-10 to 4%</td>
</tr>
<tr>
<td>Counts per minute</td>
<td>429 (349-568)</td>
<td>835 (745-940)</td>
<td>839 (666-901)</td>
<td>56 to 109%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53 to 103%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-12 to 19%</td>
</tr>
<tr>
<td>Sedentary activity (min)</td>
<td>432 (375-472)</td>
<td>378 (320-404)</td>
<td>384 (350-450)</td>
<td>-23 to 9%</td>
<td>-17 to 11%</td>
<td>-21 to 15%</td>
</tr>
<tr>
<td>Light activity (min)</td>
<td>296 (268-343)</td>
<td>264 (234-294)</td>
<td>286 (254-314)</td>
<td>-22 to -4%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-15 to 0%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-16 to 5%</td>
</tr>
<tr>
<td>Moderate activity (min)</td>
<td>88 (70-122)</td>
<td>93 (74-119)</td>
<td>91 (77-107)</td>
<td>-39 to 29%</td>
<td>-29 to 18%</td>
<td>-38 to 52%</td>
</tr>
<tr>
<td>Vigorous activity (min)</td>
<td>7 (1-10)</td>
<td>56 (50-62)</td>
<td>56 (52-65)</td>
<td>357 to 1487%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>420 to 1485%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-19 to 9%</td>
</tr>
</tbody>
</table>

Values are median (interquartile range) for n = 18. Statistical analyses are based on natural log transformed data. *95% confidence interval for the ratio of geometric means.

<sup>a</sup> Significant difference between EX-DEF and CON (P < 0.05)

<sup>b</sup> Significant difference between EX-REP and CON (P < 0.05)
5.4.3 Treadmill exercise responses

The treadmill exercise responses for EX-DEF and EX-REP are displayed in Table 5.4. The boys exercised at an average intensity of 60% peak $\dot{V}O_2$ and expended 32 kJ·kg$^{-1}$ body mass in both exercise conditions. No significant differences were seen in any of the treadmill exercise responses between EX-DEF and EX-REP ($P \geq 0.15$).

Table 5.4 Intermittent treadmill responses in the exercise with energy deficit (EX-DEF) and exercise with energy replacement (EX-REP) conditions.

<table>
<thead>
<tr>
<th></th>
<th>EX-DEF</th>
<th>EX-REP</th>
<th>EX-REP vs. EX-DEF 95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise time (min)</td>
<td>56.4 (5.6)</td>
<td>56.0 (5.5)</td>
<td>-0.6 to 1.5</td>
</tr>
<tr>
<td>Treadmill speed (km·h$^{-1}$)</td>
<td>7.1 (0.6)</td>
<td>7.2 (0.6)</td>
<td>-0.3 to 0.1</td>
</tr>
<tr>
<td>Heart rate (beats·min$^{-1}$)</td>
<td>160 (12)</td>
<td>160 (14)</td>
<td>-6 to 6</td>
</tr>
<tr>
<td>Percent peak heart rate (%)</td>
<td>79 (6)</td>
<td>79 (6)</td>
<td>-3 to 3</td>
</tr>
<tr>
<td>Rating of perceived exertion</td>
<td>11 (3)</td>
<td>11 (2)</td>
<td>-1 to 1</td>
</tr>
<tr>
<td>Oxygen uptake (L·min$^{-1}$)</td>
<td>1.35 (0.18)</td>
<td>1.35 (0.19)</td>
<td>-0.01 to 0.01</td>
</tr>
<tr>
<td>Percent peak oxygen uptake (%)</td>
<td>60 (1)</td>
<td>60 (1)</td>
<td>-0.3 to 0.5</td>
</tr>
<tr>
<td>Respiratory exchange ratio</td>
<td>0.85 (0.03)</td>
<td>0.86 (0.04)</td>
<td>-0.04 to 0.01</td>
</tr>
<tr>
<td>Fat oxidation (g)</td>
<td>20.2 (7.4)</td>
<td>17.6 (4.9)</td>
<td>-1.0 to 6.2</td>
</tr>
<tr>
<td>Carbohydrate oxidation (g)</td>
<td>47.6 (10.4)</td>
<td>53.5 (19.4)</td>
<td>-14.2 to 2.5</td>
</tr>
<tr>
<td>Net energy expenditure (kJ)</td>
<td>1327 (260)</td>
<td>1329 (272)</td>
<td>-11 to 9</td>
</tr>
<tr>
<td>Net energy expenditure (kJ·kg$^{-1}$)</td>
<td>32.0 (0.2)</td>
<td>31.9 (0.3)</td>
<td>-0.1 to 0.2</td>
</tr>
</tbody>
</table>

Values are mean (SD) for $n = 18$. *95% confidence interval of the mean absolute difference between the experimental conditions.
Figure 5.2 Postprandial whole-body fat and carbohydrate oxidation expressed as a percentage of the total energy expenditure (EE) in the control (CON), exercise with energy deficit (EX-DEF) and exercise with energy replacement (EX-REP) conditions \((n = 17)\). Fat and carbohydrate oxidation were estimated using stoichiometric equations, assuming that the contribution from protein was negligible (Chapter 3.3.3; Frayn, 1983). Values represent the total area under the substrate oxidation versus time curve divided by the duration of the postprandial period (6.5 h).

5.4.4 Resting whole-body energy expenditure (EE) and substrate oxidation

Total resting EE over the 6.5 h postprandial period was not different across the conditions (CON 2.2(0.3) MJ, EX-DEF 2.1(0.3) MJ, EX-REP 2.2(0.3) MJ; \(P = 0.30\)). The relative contribution of fat oxidation to total resting EE was greater than CON (56(13)%) in EX-DEF (63(11)%; 95% CI 0 to 15%, \(ES = 0.60, P = 0.05\)), but EX-REP (57(15)%) was similar to EX-DEF (95% CI -16 to 4%, \(P = 0.25\)) and CON (95% CI -7 to 10%, \(P = 0.67\)) (Figure 5.2). Reciprocally, the relative contribution of carbohydrate oxidation to total resting EE was lower than CON (44(13)%) in EX-DEF (37(11)%; 95% CI -15 to 0%, \(ES = 0.60, P = 0.05\)), but EX-REP (43(15)%) was similar to EX-DEF (95% CI -4 to 16%, \(P = 0.25\)) and CON (95% CI -10 to 7%, \(P = 0.67\)) (Figure 5.2).
5.4.5 Plasma volume changes and fasting [TAG] and [glucose]

Average changes in plasma volume between the fasting and 6.5 h postprandial samples were not different across the conditions (CON 0.9%, EX-DEF 1.4%, EX-REP 0.5%; \( P = 0.90 \)). Therefore, the raw plasma [TAG] and [glucose] were not adjusted prior to statistical analyses. The fasting plasma [TAG] and [glucose] are displayed in Table 5.5. One-way ANOVA identified differences in fasting plasma [TAG] across the conditions (\( P = 0.04 \)). Specifically, EX-DEF fasting plasma [TAG] was 19% and 15% lower than CON (ES = 1.15, \( P = 0.01 \)) and EX-REP (ES = 0.91, \( P = 0.11 \)) respectively; CON and EX-REP were similar (-4%; \( P = 0.56 \)). No differences were observed in fasting plasma [glucose] across the conditions (\( P = 0.14 \)).

5.4.6 Plasma [TAG] and [glucose] in the postprandial period

Plasma TAG responses over the postprandial period for the experimental conditions are shown in Figure 5.3. Two-way ANOVA revealed a trend for differences in postprandial plasma [TAG] over time across conditions (main effect condition \( P = 0.06 \); main effect time \( P < 0.001 \); condition by time interaction \( P = 0.06 \)). Mean EX-DEF postprandial plasma [TAG] was 14% and 16% lower than CON (-26 to -1%, ES = 0.40, \( P = 0.04 \)) and EX-REP (-31 to 2%, ES = 0.45, \( P = 0.07 \)) respectively; CON and EX-REP were similar (2%; -13 to 20%, \( P = 0.79 \)). The alpha value for the interaction reflected the higher [TAG] in EX-REP at 6.5 h compared with CON and EX-DEF. The EX-DEF TAUC-TAG was 15% and 16% lower than CON (ES = 0.55, \( P = 0.04 \)) and EX-REP (ES = 0.62, \( P = 0.07 \)) respectively; CON and EX-REP were not different (2%; \( P = 0.80 \)) (Table 5.5). Specifically, EX-DEF was lower than CON between 0 to 1 h by 13% (-23 to -2%, ES = 0.56, \( P = 0.03 \)), 1 to 4.5 h by 17% (-29 to -4%, ES = 0.63, \( P = 0.02 \)) and 4.5 to 6.5 h by 12% (-25 to 4%, ES = 0.40, \( P = 0.13 \)); EX-DEF was lower than EX-REP between 0 to 1 h by 13% (-27 to 5%, ES = 0.54, \( P = 0.13 \)), 1 to 4.5 h by 16% (-32 to 2%, ES = 0.60, \( P = 0.08 \)) and 4.5 to 6.5 h by 18% (-32 to 0%, ES = 0.62, \( P = 0.05 \)). No differences in TAUC-TAG subsections were observed between CON and EX-REP (\( P \geq 0.37 \)). The incremental area under the concentration versus time curve (iAUC) for TAG was similar across the conditions (\( P = 0.14 \)) (Table 5.5).
Table 5.5  Fasting and postprandial plasma triacylglycerol and glucose concentrations in the control (CON), exercise with energy deficit (EX-DEF) and exercise with energy replacement (EX-REP) conditions.

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>EX-DEF</th>
<th>EX-REP</th>
<th>CON vs. EX-DEF 95% CI*</th>
<th>CON vs. EX-REP 95% CI*</th>
<th>EX-REP vs. EX-DEF 95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Triacylglycerol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (mmol·L⁻¹)</td>
<td>0.61 (0.54-0.68)</td>
<td>0.47 (0.41-0.61)</td>
<td>0.57 (0.45-0.76)</td>
<td>-31 to -5%²</td>
<td>-19 to 12%</td>
<td>-32 to 4%</td>
</tr>
<tr>
<td>TAUC (mmol·L⁻¹ 6.5 h)</td>
<td>6.09 (5.67-7.73)</td>
<td>5.23 (4.33-6.92)</td>
<td>6.31 (5.36-7.98)</td>
<td>-27 to -1%²</td>
<td>-13 to 20%</td>
<td>-31 to 1%</td>
</tr>
<tr>
<td>iAUC (mmol·L⁻¹ 6.5 h)</td>
<td>2.61 (1.89-3.71)</td>
<td>2.58 (1.64-3.29)</td>
<td>2.93 (2.28-3.84)</td>
<td>-23 to 11%</td>
<td>-10 to 41%</td>
<td>-34 to 2%</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (mmol·L⁻¹)</td>
<td>5.90 (5.61-6.14)</td>
<td>5.72 (5.58-6.00)</td>
<td>6.02 (5.79-6.18)</td>
<td>-3 to 1%</td>
<td>-1 to 3%</td>
<td>-5 to 0%</td>
</tr>
<tr>
<td>TAUC (mmol·L⁻¹ 6.5 h)</td>
<td>44.4 (43.1-45.6)</td>
<td>44.4 (42.5-46.1)</td>
<td>44.4 (42.2-47.4)</td>
<td>-4 to 2%</td>
<td>-5 to 3%</td>
<td>-4 to 4%</td>
</tr>
<tr>
<td>iAUC (mmol·L⁻¹ 6.5 h)</td>
<td>9.64 (7.32-10.94)</td>
<td>9.89 (7.27-12.79)</td>
<td>8.30 (6.26-12.17)</td>
<td>-34 to 22%</td>
<td>-23 to 9%</td>
<td>-31 to 39%</td>
</tr>
</tbody>
</table>

Values are median (interquartile range) for n = 18. Statistical analyses are based on natural log transformed data. *95% confidence interval for the ratio of geometric means.

TAUC, total area under the concentration versus time curve; iAUC, incremental area under the concentration versus time curve.

² Significant difference between EX-DEF and CON (P < 0.05)
Figure 5.3  Fasting (F) and postprandial plasma triacylglycerol concentrations ([TAG]) in the exercise with energy-replacement (EX-REP), control (CON) and exercise with energy deficit (EX-DEF) conditions \((n = 18)\). Values are mean (SD). Black rectangles denote consumption of breakfast and lunch meals at 08:00 and 12:00, respectively. Main effect condition \(P = 0.06\); main effect time \(P < 0.001\); condition by time interaction \(P = 0.06\).

Individual changes (delta) in TAUC-TAG for EX-DEF and EX-REP relative to CON are shown in Figure 5.4. The reductions in TAUC-TAG were greater than changes in CON for fourteen (78%) boys in EX-DEF and ten (56%) boys in EX-REP. Strong positive correlations were observed between the intervention-induced change in fasting plasma [TAG] and the change in TAUC-TAG relative to CON for EX-DEF \((r = 0.88, P < 0.001)\) and EX-REP \((r = 0.88, P < 0.001)\). The measured physical and physiological characteristics (Table 5.1), dietary intake (Table 5.2), free-living physical activity and sedentary time (Table 5.3), exercise responses (Table 5.4) and resting whole-body EE and substrate oxidation (Section 5.4.4, Figure 5.2) did not account for any of the inter-individual variability in delta TAUC-TAG for EX-DEF or EX-REP. The Pearson’s product moment correlation for the individual changes in TAUC-TAG between EX-DEF and EX-REP was small \((r = 0.38, P = 0.12)\).
Figure 5.4  Individual changes (delta) in the total area under the plasma triacylglycerol (TAG) concentration versus time curve (TAUC) between the exercise with energy deficit (EX-DEF) and exercise with energy replacement (EX-REP) conditions compared with the control condition (CON): A) EX-DEF minus CON; B) EX-REP minus CON. Participant data are organised according to the
size of the intervention-induced change in TAUC-TAG; thus, the order of the individual participants is not identical in A and B. A negative response indicates a reduction in TAUC-TAG in the intervention compared with CON.

No differences in postprandial plasma [glucose] were seen across the conditions (main effect condition $P = 0.94$; main effect time $P < 0.001$; condition by time interaction $P = 0.73$). No meaningful differences were observed in TAUC-glucose ($P = 0.80$) or iAUC-glucose ($P = 0.70$) across the conditions (Table 5.5).

### 5.5 Discussion

The primary finding from the present study was that immediate replacement of the acute exercise-induced energy deficit negates the reduction in fasting and postprandial plasma [TAG] in healthy boys. Furthermore, an exercise-induced energy deficit was required to promote an increase in postprandial whole-body fat oxidation. Therefore, judicious use of energy replacement practices immediately post-exercise may be required in boys to maximise the metabolic health benefits of exercise.

The reduction in fasting plasma [TAG] in EX-DEF compared with CON and EX-REP supports the majority of previous findings in young people where the exercise-induced energy deficit was maintained in the post-exercise period (Chapters 6 and 7; Barrett et al., 2007; Lee et al., 2013; Tolfrey et al., 2008, 2012, 2014a). Although elevated fasting [TAG] are associated with impaired postprandial metabolism in young people (Couch et al., 2000), fasting [TAG] are highly variable in children (Tolfrey et al., 1999), and are less predictive typically of CVD risk than nonfasting concentrations in adults (Bansal et al., 2007; Nordestgaard et al., 2007). Therefore, studying postprandial [TAG] may be more informative of cardiovascular health in young people.

The reduction in postprandial plasma [TAG] after EX-DEF supports previous studies employing acute moderate- to vigorous-intensity exercise interventions in young people (1.0 to 2.5 MJ) (Table 2.2; Tolfrey et al., 2014b), and has been linked to the total EE of the exercise session in adults (Maraki and Sidossis, 2013). An exercise-induced energy deficit elicits a greater reduction in postprandial [TAG] than an isoenergetic diet-induced energy deficit in girls (Chapter 6) and pre- and post-menopausal women (Gill and Hardman, 2000; Maraki et al., 2010). A further avenue of research demonstrates that replacing the exercise-induced energy deficit diminishes or eliminates the reduction in postprandial [TAG]
following acute moderate- and high-intensity exercise interventions in men and women (Burton et al., 2008; Freese et al., 2011; Harrison et al., 2009; Trombold et al., 2014). Furthermore, the carbohydrate composition of the post-exercise replacement meal has also been shown to contribute to the subsequent postprandial TAG response the following day in healthy, young men (Trombold et al., 2014). The current study is the first to extend these findings to young people by showing that immediate replacement of the exercise net EE counter-acts the exercise-evoked reduction in postprandial plasma [TAG] in healthy boys (Table 5.5, Figure 5.3).

The increase in resting whole-body fat oxidation the day after EX-DEF is in agreement with adult studies reporting accompanying reductions in postprandial [TAG] (Burton et al., 2008; Davitt et al., 2013; Trombold et al., 2013, 2014), and a recent study in girls demonstrating reduced postprandial plasma [TAG] and elevated whole-body fat oxidation the day following acute manipulations of high-intensity interval running and energy-intake restriction (Chapter 7). Previous exercise postprandial studies in adults have shown that replacing the exercise-induced energy deficit attenuates the increase in postprandial whole-body fat oxidation (Burton et al., 2008; Trombold et al., 2014), but this effect may be dependent on the carbohydrate content of the post-exercise meal (Trombold et al., 2014). Resting whole-body fat oxidation in EX-REP was not statistically different to EX-DEF or CON; however, a thorough appraisal of the mean differences and absolute standardised ES revealed that EX-REP was 2% higher than CON (ES = 0.14, P = 0.67) but 6% lower than EX-DEF (ES = 0.46, P = 0.25). Therefore, immediate replacement of the exercise-induced energy deficit appears to diminish the increase in resting whole-body fat oxidation the following day, suggesting that an energy deficit may be required to elicit a meaningful increase in resting whole-body fat oxidation.

The mechanisms underlying the exercise-induced changes in postprandial lipid metabolism and the interaction with energy deficit in young people are unclear, but might be inferred from the adult literature. Elevated whole-body fat oxidation following exercise with energy deficit may facilitate the resynthesis of depleted skeletal muscle and/or hepatic glycogen stores through a number of regulatory mechanisms (Kiens and Richter, 1998; Kimber et al., 2003), including enhanced skeletal muscle lipoprotein lipase (LPL) activity promoting increased clearance of circulating TAG (Gill et al., 2003b; Kiens and Richter, 1998). Furthermore, hepatic fatty acid flux may be shifted towards oxidation and away from re-esterification resulting in the secretion of fewer, possibly TAG-richer very low-density
lipoproteins (VLDL) with a higher affinity for LPL (Magkos et al., 2006). Immediate replacement of the exercise-induced energy deficit presumably accelerates hepatic and muscle glycogen replenishment (Casey et al., 2000; Wallis et al., 2008), and attenuates the reduction in postprandial [TAG] and increase in resting whole-body fat oxidation that normally follows exercise with energy deficit. A recent stable isotope enrichment study suggested that exercise reduces postprandial [TAG] from endogenously derived fatty acids in plasma TAG and not the increased clearance of exogenous dietary fat (Davitt et al., 2013). The present study supports this contention indirectly shown by the small differences in iAUC-TAG, and the strong positive relationship observed between the intervention-induced changes in fasting plasma [TAG] and TAUC-TAG for EX-DEF (r = 0.88, P < 0.001) and EX-REP (r = 0.88, P < 0.001).

Completing the exercise session on the intervention day of EX-DEF and EX-REP resulted in the boys spending substantially more time engaged in vigorous-intensity physical activities, and less time engaged in light-intensity physical activities (Table 5.3). However, there were no differences between conditions after accounting for the time spent resting or exercising in the laboratory, suggesting that the implemented between condition control of free-living physical activity and sedentary time was effective. The exercise protocol adopted in EX-DEF and EX-REP meets the current international physical activity guidelines for health promotion in children and adolescents (Department of Health, Physical Activity, Health Improvement and Protection, 2011; Janssen and LeBlanc, 2010). While the clinical significance of our findings cannot be determined, high childhood fasting [TAG] is an independent predictor of young adult CVD (Morrison et al., 2009, 2012), and physical inactivity is associated independently with the clustering of CVD risk factors in childhood and adolescence (Ekelund et al., 2012). Furthermore, low rates of fat oxidation in adults have been implicated in the pathology of obesity (Ellis et al., 2010; Kelley et al., 1999; Zurlo et al., 1990) and Type 2 diabetes mellitus (Blaak et al., 2001). Considering childhood CVD risk factors, including physical inactivity, track into adulthood (Eisenmann et al., 2004; Telama et al., 2005), interventions that improve the CVD risk factor profile should be paediatric orientated (Froberg and Andersen, 2005; McGill et al., 2000a). The majority of the postprandial TAG samples (98%) in the current study were below the 2.3 mmol·L⁻¹ threshold proposed as a desirable concentration in children and adolescents (Kolovou et al., 2011a). Although it is encouraging that EX-DEF resulted in lower postprandial plasma [TAG] and increased resting whole-body fat oxidation in boys with a predominantly healthy postprandial TAG profile, the
impact of post-exercise energy intake should be carefully considered to optimise the metabolic health benefits of exercise.

Similar to previous exercise postprandial studies in adults (Gill et al., 2007) and young people (Tolfrey et al., 2012, 2014a), considerable inter-individual variation was present in the postprandial TAG response following EX-DEF and EX-REP (Figure 5.4). This suggests that some boys may still experience reductions in postprandial plasma [TAG] when the exercise-induced energy deficit is replaced; however, an energy deficit may be required to maximise the health benefits of exercise in the majority of boys. In line with the current study, previous studies in young people have been unable to elucidate the underlying factors explaining the substantial individual heterogeneity in postprandial plasma [TAG] (Tolfrey et al., 2012, 2014a). It has been demonstrated in adults that the exercise-induced increase in postprandial whole-body fat oxidation is associated negatively with the postprandial lipaemic response (Burton et al., 2008; Trombold et al., 2013, 2014); however, intervention-induced changes in whole-body fat oxidation were not associated with any index of lipaemia in the current study or in a recent study in girls presented in this thesis (Chapter 7). This suggests that exercise-induced changes in postprandial plasma [TAG] and whole-body fat oxidation may occur independently in boys.

Fasting and postprandial plasma [glucose] were not different across the conditions in the current study. This supports the majority of previous exercise postprandial studies in young people whereby a single session of moderate- to vigorous-intensity exercise did not change the fasting or postprandial plasma glucose response (Chapters 4 and 6; Barrett et al., 2007; Lee et al., 2013; MacEneaney et al., 2009; Sedgwick et al., 2013, 2014; Tolfrey et al., 2008, 2014a).

The present study is limited by the absence of EE quantification during the 5 min rest periods between exercise intervals in EX-DEF and EX-REP, and excess post-exercise oxygen consumption was not measured following the exercise sessions. Although the contribution to the net EE is likely to be relatively small (Børsheim and Bahr, 2003), an underestimation of the net EE and incomplete replacement of the energy deficit in EX-REP cannot be ruled out.

In conclusion, immediate replacement of the acute exercise-induced energy deficit completed approximately ~14.5 h before a standardised meal eliminates the reduction in fasting and postprandial plasma [TAG] in healthy boys. In addition, meaningful increases in postprandial whole-body fat oxidation appear dependent on the presence of an energy deficit.
Consequently, avoiding regular post-exercise energy intake should be advocated to maintain a negative energy balance in healthy, 11 to 13 year old boys, which could have important implications in terms of maximising the beneficial effect of exercise on postprandial lipaemia and substrate oxidation early in life.
CHAPTER 6

Acute effects of energy deficit induced by moderate-intensity exercise or energy-intake restriction on postprandial lipaemia in healthy girls

6.1 Abstract

The exercise-induced reduction in postprandial triacylglycerol concentrations ([TAG]) in young people may reflect an ensuing energy deficit. This study examined the effect of an isoenergetic acute energy deficit, induced by moderate-intensity exercise or energy-intake restriction on postprandial plasma [TAG] in girls. Eleven healthy girls (mean(SD): age 12.1(0.6) years; body mass 42.1(5.8) kg; peak oxygen uptake (VO₂) 47(6) mL·kg⁻¹·min⁻¹) completed three, 2-day conditions in a counterbalanced, crossover design. On day 1, participants either walked at 60(2)% peak VO₂ (energy deficit 1.55(0.20) MJ) (EX), restricted food energy intake (energy deficit 1.51(0.25) MJ) (ER) or rested (CON). On day 2, capillary blood samples were taken at predetermined intervals throughout the 6.5 h postprandial period prior to, and following, the ingestion of standardised breakfast and lunch meals. Based on ratios of the geometric means (95% confidence intervals (CI) for ratios), fasting plasma [TAG] was 29% and 13% lower than CON in EX (-42 to -13%, effect size (ES) = 1.39, P = 0.01) and ER (-22 to -4%, ES = 0.57, P = 0.02) respectively; EX was 19% lower than ER (-35 to 1%, ES = 0.82, P = 0.06). The EX total area under the [TAG] versus time curve was 21% and 13% lower than CON (-30 to -11%, ES = 0.71, P = 0.004) and ER (-24 to 1%, ES = 0.39, P = 0.06) respectively; ER was marginally lower than CON (-10%; -22 to 4%, ES = 0.32, P = 0.12). An exercise-induced energy deficit elicited a greater reduction in fasting plasma [TAG] with a trend for a larger attenuation in postprandial plasma [TAG] than an isoenergetic diet-induced energy deficit in healthy girls.
6.2 Introduction

Elevated postprandial triacylglycerol concentrations ([TAG]) have been implicated in the development of atherosclerosis (Zilversmit, 1979), and are established as an independent risk factor for future cardiovascular disease (CVD) in adults (Bansal et al., 2007; Nordestgaard et al., 2007). Although the metabolic perturbations present following the ingestion of a meal appear short-lived, most people spend the majority of the daytime in a postprandial state typically. Therefore, repeated episodes of exaggerated postprandial [TAG] contribute to the atherogenic lipid phenotype of TAG-rich lipoprotein remnants, small, dense low-density lipoprotein (LDL) and low concentrations of high-density lipoprotein (HDL) (Cohn, 1998). The process of atherosclerosis is initiated during childhood and progresses over the lifespan, prompting preventive lifestyle interventions, such as exercise and diet that may delay precursors of atherosclerotic progression early in life (McGill et al., 2000a).

Acute aerobic exercise (30 min to 3 h in duration) performed up to 18 h before a test meal reduces postprandial [TAG] in adults (Maraki and Sidossis, 2013). Furthermore, accumulating evidence in boys and girls demonstrates the postprandial TAG-lowering effect of moderate- to vigorous-intensity exercise interventions (Barrett et al., 2007; Lee et al., 2013; MacEneaney et al., 2009; Sedgwick et al., 2013, 2014; Tolfrey et al., 2008, 2012, 2014a). It is possible that the energy deficit associated with a single session of exercise may be responsible for the reduction in postprandial [TAG]. Replacing the exercise-induced energy deficit has been shown to diminish or even eliminate the reduction in postprandial [TAG] in men and women (Burton et al., 2008; Freese et al., 2011; Harrison et al., 2009). Studies manipulating the origin of the energy deficit through exercise energy expenditure (EE) and dietary energy intake suggest that an exercise-induced energy deficit reduces postprandial [TAG] to a greater extent than a diet-induced energy deficit in women (Gill and Hardman, 2000; Maraki et al., 2010). Nevertheless, reductions in postprandial [TAG] have been reported following a single day of energy-intake restriction in young, healthy women (Maraki et al., 2010). However, we are not aware of studies that have examined the acute effect of energy-intake restriction on postprandial [TAG] in young people, highlighting an important gap in our current knowledge which may have important implications in terms of providing opportunities to improve metabolic health early in life.

Current international guidelines recommend that children and adolescents accumulate at least 60 min of moderate-intensity daily physical activity for health promotion (Department of
Health, Physical Activity, Health Improvement and Protection, 2011; Janssen and LeBlanc, 2010); however, many young people fail to meet these guidelines, and girls are less active typically than their male peers (Riddoch et al., 2007). To our knowledge, only one study has examined the acute effect of exercise on postprandial plasma [TAG] in girls, reporting that 60, but not 30 min of moderate-intensity exercise reduces postprandial plasma [TAG] in 10 to 14 year old girls (Tolfrey et al., 2014a). Consequently, additional work is required to expand the evidence base in girls to identify engaging and sustainable lifestyle interventions that improve this CVD risk factor from a young age. Therefore, the aim of the present study was to compare the effect of an isoenergetic energy deficit, induced by acute moderate-intensity exercise or energy-intake restriction on postprandial plasma [TAG] in healthy, recreationally active girls.

6.3 Methods

6.3.1 Participants

Thirteen girls volunteered to participate in this study. Results are presented for 11 girls (11.4 to 13.1 years) as one girl’s habitual daily energy intake was too low to justify energy-intake restriction and another dropped out for personal reasons unrelated to the study. Physical and physiological characteristics are presented in Table 6.1.

6.3.2 Preliminary exercise measurements

During the first visit to the laboratory, preliminary anthropometric measurements were obtained as described in Chapter 3.2. Following familiarisation with the treadmill, participants completed two preliminary exercise tests: 1) 16 min sub-maximal incremental treadmill protocol (Chapter 3.5.1); 2) incremental uphill treadmill protocol to determine peak oxygen uptake (\( \dot{VO}_2 \)) performed at a fixed individual speed (7.0 to 8.5 km·h\(^{-1} \)) (Chapter 3.5.2.1). Data from the 16 min sub-maximal incremental and peak \( \dot{VO}_2 \) tests were used to determine the treadmill speed required to elicit 60% peak \( \dot{VO}_2 \) during the experimental exercise condition.
Table 6.1  Physical and physiological characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>12.1 (0.6)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>42.1 (5.8)</td>
</tr>
<tr>
<td>Stature (m)</td>
<td>1.52 (0.06)</td>
</tr>
<tr>
<td>Body mass index (kg·m^{-2})</td>
<td>18.1 (1.8)</td>
</tr>
<tr>
<td>Percent body fat (%)</td>
<td>19.7 (3.6)</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>33.7 (4.3)</td>
</tr>
<tr>
<td>Breast development*</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Pubic hair development*</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Peak oxygen uptake (mL·kg^{-1}·min^{-1})</td>
<td>47 (6)</td>
</tr>
</tbody>
</table>

Values are mean (SD) for n = 11. *Self-assessment – median (interquartile range).

6.3.3  Experimental design

Participants completed three, 2-day experimental conditions: moderate-intensity exercise (EX), energy-intake restriction (ER) and rest control (CON). The study design is presented schematically in Figure 6.1.

Figure 6.1  Diagram of the 2-day study protocol. TAG, triacylglycerol. *Lunch and evening meal replicated across conditions, but with a small reduction in energy intake in ER.
6.3.3.1 Standardisation of dietary intake

Participants weighed, recorded and replicated their habitual dietary intake on the pre-intervention and intervention day of all experimental conditions (Chapter 3.6.1), but with a controlled reduction in energy intake on the intervention day of ER. Participants completing ER as the first condition were asked to record their usual dietary intake for two consecutive days at least one week in advance so that the prescribed energy-intake restriction could be calculated and standardised. The macronutrient composition of the carbohydrate-rich cereal snack bar consumed at 19:45 on the intervention day of each condition was 1.3 g fat, 17.2 g carbohydrate and 1.0 g protein, which provided 357 kJ energy.

6.3.3.2 Day 1: Intervention day

During EX, participants exercised on the treadmill at 60% peak $\dot{VO}_2$ in 20 min intervals, with a standardised 5 min period of seated rest between each interval. Expired air samples were collected and analysed as described previously in Chapter 3.3.2 during the third, tenth and seventeenth minute to calculate the relative exercise intensity. The treadmill speed was adjusted occasionally to ensure the target exercise intensity was met. The exercise EE and the oxidation of carbohydrate and fat were estimated via indirect calorimetry (Chapter 3.3.3). The net EE of exercise was calculated as the exercise gross EE minus resting EE, where resting EE was estimated using age- and sex-specific equations (FAO/WHO/UNU, 2004). Heart rate was monitored throughout and the participants provided a rating of perceived exertion (RPE) during the last 10 s of each expired air sampling period (Chapter 3.4). Participants maintained their habitual dietary intake throughout the day. During ER, the girls rested in the laboratory for the duration of the visit and reduced their habitual food energy intake by the net EE of exercise, with the energy intakes at lunch and evening meal reduced by 43% and 57% of the total net EE of exercise respectively. During CON, participants rested in the laboratory for the duration of the visit and maintained their habitual dietary intake throughout the day.

6.3.3.3 Day 2: Postprandial day

Capillary blood samples were taken at pre-determined intervals throughout the 6.5 h postprandial rest period prior to, and following the consumption of standardised breakfast and lunch meals (Chapter 3.6.4). The breakfast meal provided 1.5 g fat (60% of meal total energy), 1.8 g carbohydrate (33%), 0.4 g protein (7%) and 93 kJ energy per kilogram body
mass. The lunch meal provided 1.0 g fat (48%), 1.9 g carbohydrate (40%), 0.6 g protein (12%) and 79 kJ energy per kilogram body mass.

6.3.4 Analytical methods

Capillary blood samples were analysed for plasma [TAG], glucose concentration ([glucose]) and insulin concentration ([insulin]) (Chapter 3.7.2). The within-batch coefficient of variation for plasma [TAG], [glucose] and [insulin] were 1.1, 0.5 and 6.2% respectively.

6.3.5 Statistical analyses

Data were analysed using the statistical methods presented in Chapter 3.8. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated (Matthews et al., 1985). Concentrations of plasma TAG, glucose and insulin and HOMA-IR were natural log transformed prior to analysis. These data are presented as median (interquartile range) and analysis is based on the ratios of the geometric means and 95% confidence intervals (CI) for ratios. HOMA-IR was compared between experimental conditions using one-way within-measures analysis of variance (ANOVA). All ANOVA analyses were adjusted appropriately for the period effect (Senn, 2002).

6.4 Results

6.4.1 Dietary intake

Energy and macronutrient intakes were not significantly different on the pre-intervention day across the three conditions (\(P \geq 0.47\)). Average daily energy intake was 6.9(2.1) MJ, and dietary intake of protein, carbohydrate and fat was 58.4(13.5) g, 248(92) g and 46.8(13.6) g respectively. Energy and macronutrient intakes during the intervention day are displayed in Table 6.2. As anticipated, two-way ANOVA identified differences in energy and macronutrient intakes on the intervention day across the conditions (\(P < 0.001\)). Energy intake was considerably lower in ER compared with CON (effect size (ES) = 1.12, \(P < 0.001\)) and EX (ES = 1.06, \(P < 0.001\)); CON and EX were not significantly different (\(P = 0.27\)). Absolute protein, carbohydrate and fat intake were considerably lower in ER compared with CON and EX (ES = 0.83 to 1.07, \(P < 0.05\)), with no significant difference between CON and EX (\(P \geq 0.20\)). Changes in the contribution of protein, carbohydrate and fat to total energy intake across the conditions were not significantly different (\(P \geq 0.22\)).
Table 6.2  Energy and macronutrient intakes during the intervention day of the moderate-intensity exercise (EX), energy-intake restriction (ER) and control (CON) conditions.

<table>
<thead>
<tr>
<th></th>
<th>EX</th>
<th>ER</th>
<th>CON</th>
<th>CON vs. EX 95% CI*</th>
<th>CON vs. ER 95% CI*</th>
<th>ER vs. EX 95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (MJ·day(^{-1}))</td>
<td>7.2 (1.3)</td>
<td>5.8 (1.3)</td>
<td>7.3 (1.3)</td>
<td>-0.2 to 0.1</td>
<td>-1.7 to -1.3(^{b})</td>
<td>1.3 to 1.6(^{c})</td>
</tr>
<tr>
<td>Protein (g·day(^{-1}))</td>
<td>59.1 (11.5)</td>
<td>48.7 (12.8)</td>
<td>60.5 (12.5)</td>
<td>-3.9 to 1.2</td>
<td>-14.6 to -8.9(^{b})</td>
<td>8.5 to 12.4(^{c})</td>
</tr>
<tr>
<td>CHO (g·day(^{-1}))</td>
<td>249 (51)</td>
<td>199 (50)</td>
<td>249 (53)</td>
<td>-6 to 6</td>
<td>-61 to -39(^{b})</td>
<td>39 to 61(^{c})</td>
</tr>
<tr>
<td>Fat (g·day(^{-1}))</td>
<td>55.4 (12.7)</td>
<td>44.2 (10.1)</td>
<td>57.1 (12.1)</td>
<td>-4.2 to 1.1</td>
<td>-15.7 to -9.6(^{b})</td>
<td>6.1 to 16.2(^{c})</td>
</tr>
<tr>
<td>% energy intake from protein</td>
<td>14 (2)</td>
<td>14 (2)</td>
<td>14 (2)</td>
<td>-0.4 to 0.1</td>
<td>-0.3 to 0.5</td>
<td>-0.7 to 0.1</td>
</tr>
<tr>
<td>% energy intake from CHO</td>
<td>57 (5)</td>
<td>57 (4)</td>
<td>57 (4)</td>
<td>-0.6 to 2.0</td>
<td>-0.5 to 1.3</td>
<td>-1.8 to 2.4</td>
</tr>
<tr>
<td>% energy intake from fat</td>
<td>29 (5)</td>
<td>29 (4)</td>
<td>29 (4)</td>
<td>-1.9 to 0.8</td>
<td>-1.5 to 0.4</td>
<td>-2.2 to 2.2</td>
</tr>
</tbody>
</table>

Values are mean (SD) for \(n = 11\). *95% confidence interval of the mean absolute difference between the experimental conditions.

CHO, carbohydrate.

\(^{b}\) Significant difference between ER and CON \((P < 0.001)\)

\(^{c}\) Significant difference between EX and ER \((P < 0.05)\)
6.4.2 Exercise responses

Mean \( \text{VO}_2 \) during EX was 1.18(0.16) L·min\(^{-1} \), corresponding to 60(2)% peak \( \text{VO}_2 \), and the average respiratory exchange ratio was 0.85(0.03). Mean heart rate was 161(6) beats·min\(^{-1} \), which represented 80(3)% of peak heart rate, and the average RPE was 13(1) (‘somewhat hard’ on the scale). The estimated exercise net EE was 1.46(0.01) MJ.

6.4.3 Exercise- and diet-induced energy deficits

Accounting for the exercise net EE (Section 6.4.2) and energy intake (Table 6.2) on the intervention day, the resulting energy deficit relative to CON was 1.55(0.20) MJ in EX and 1.51(0.25) MJ in ER. The exercise- and diet-induced energy deficits were not significantly different from each other (95% CI -0.07 to 0.14 MJ, \( P = 0.49 \)).

6.4.4 Plasma volume changes and fasting [TAG], [glucose] and [insulin]

Average changes in plasma volume between the fasting and 6.5 h postprandial samples were small and did not differ significantly between the three conditions (EX 0.6%, ER 1.0%, CON 1.2%; \( P = 0.67 \)). Therefore, the raw plasma [TAG], [glucose] and [insulin] were used in all statistical analyses without adjustment. The fasting plasma [TAG], [glucose] and [insulin] for each condition are shown in Table 6.3. One-way ANOVA revealed differences across the conditions in fasting plasma [TAG] (\( P = 0.002 \)). Specifically, fasting plasma [TAG] was 29% and 13% lower than CON in EX (ES = 1.39, \( P = 0.01 \)) and ER (ES = 0.57, \( P = 0.02 \)) respectively; EX was 19% lower than ER (ES = 0.82, \( P = 0.06 \)). One-way ANOVA revealed a tendency for differences in fasting plasma [glucose] across the conditions (\( P = 0.08 \)), with simple planned contrasts revealing a trend for lower fasting plasma [glucose] in EX than CON (-3%; ES = 0.67, \( P = 0.07 \)). One-way ANOVA revealed a tendency for differences in fasting plasma [insulin] across the conditions (\( P = 0.07 \)). Fasting plasma [insulin] was 26% and 18% lower than CON in EX (ES = 0.69, \( P = 0.02 \)) and ER (ES = 0.46, \( P = 0.10 \)) respectively; ER and EX were not significantly different (-10%; \( P = 0.52 \)). One-way ANOVA revealed a tendency for differences in fasting HOMA-IR across the conditions (\( P = 0.05 \)). Fasting HOMA-IR was lower compared with CON (2.35(1.71-2.97)) by 29% in EX (1.59(1.18-2.29); -46 to -6%, ES = 0.71, \( P = 0.03 \)) and by 20% in ER (1.58(1.42-2.81); -39 to 3%, ES = 0.47, \( P = 0.07 \)); ER and EX were not significantly different (-11%; -39 to 32%, \( P = 0.49 \)).
Table 6.3  Fasting and postprandial plasma triacylglycerol, glucose and insulin concentrations in the moderate-intensity exercise (EX), energy-intake restriction (ER) and control (CON) conditions.

<table>
<thead>
<tr>
<th></th>
<th>EX</th>
<th>ER</th>
<th>CON</th>
<th>CON vs. EX 95% CI*</th>
<th>CON vs. ER 95% CI*</th>
<th>ER vs. EX 95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Triacylglycerol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (mmol·L⁻¹)</td>
<td>0.69 (0.53-0.82)</td>
<td>0.76 (0.62-0.92)</td>
<td>0.92 (0.87-1.20)</td>
<td>-42 to -13%ᵃ</td>
<td>-22 to -4%ᵇ</td>
<td>-35 to 1%</td>
</tr>
<tr>
<td>TAUC (mmol·L⁻¹ 6.5 h)</td>
<td>6.97 (6.35-9.16)</td>
<td>7.71 (6.60-8.54)</td>
<td>8.89 (6.77-12.23)</td>
<td>-30 to -11%ᵃ</td>
<td>-22 to 4%</td>
<td>-24 to 1%</td>
</tr>
<tr>
<td>iAUC (mmol·L⁻¹ 6.5 h)</td>
<td>3.30 (2.94-4.13)</td>
<td>2.98 (2.26-3.62)</td>
<td>3.37 (2.37-5.03)</td>
<td>-28 to 28%</td>
<td>-36 to 48%</td>
<td>-17 to 17%</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (mmol·L⁻¹)</td>
<td>5.36 (5.18-5.52)</td>
<td>5.39 (5.23-5.57)</td>
<td>5.55 (5.33-5.63)</td>
<td>-7 to 0%</td>
<td>-5 to 1%</td>
<td>-4 to 2%</td>
</tr>
<tr>
<td>TAUC (mmol·L⁻¹ 6.5 h)</td>
<td>44.1 (42.1-47.3)</td>
<td>43.8 (41.2-44.9)</td>
<td>43.8 (41.7-47.2)</td>
<td>-1 to 3%</td>
<td>-7 to 3%</td>
<td>-2 to 8%</td>
</tr>
<tr>
<td>iAUC (mmol·L⁻¹ 6.5 h)</td>
<td>13.07 (9.77-15.48)</td>
<td>11.27 (9.42-13.49)</td>
<td>10.55 (7.78-12.70)</td>
<td>0 to 38%ᵃ</td>
<td>-30 to 24%</td>
<td>3 to 54%ᶜ</td>
</tr>
<tr>
<td><strong>Insulin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (pmol·L⁻¹)</td>
<td>41.6 (31.4-56.1)</td>
<td>39.2 (36.0-72.4)</td>
<td>54.9 (41.5-73.8)</td>
<td>-43 to -6%ᵃ</td>
<td>-37 to 6%</td>
<td>-38 to 32%</td>
</tr>
<tr>
<td>TAUC (pmol·L⁻¹ 6.5 h)</td>
<td>1474 (1208-1889)</td>
<td>1406 (1224-1589)</td>
<td>1818 (1341-1944)</td>
<td>-20 to 11%</td>
<td>-21 to 8%</td>
<td>-21 to 32%</td>
</tr>
<tr>
<td>iAUC (pmol·L⁻¹ 6.5 h)</td>
<td>1262 (952-1587)</td>
<td>1126 (974-1371)</td>
<td>1503 (967-1613)</td>
<td>-13 to 16%</td>
<td>-20 to 13%</td>
<td>-19 to 37%</td>
</tr>
</tbody>
</table>

Values are median (interquartile range) for n = 11. Statistical analyses are based on natural log transformed data. *95% confidence interval for the ratio of geometric means.

TAUC, total area under the concentration versus time curve; iAUC, incremental area under the concentration versus time curve.
Significant difference between EX and CON (\(P < 0.05\))

Significant difference between ER and CON (\(P < 0.05\))

Significant difference between EX and ER (\(P < 0.05\))

6.4.5 Plasma [TAG], [glucose] and [insulin] in the postprandial period

Plasma TAG responses over the postprandial period for EX, ER and CON are shown in Figure 6.2. Two-way ANOVA revealed differences in postprandial plasma [TAG] over time across conditions (main effect condition \(P = 0.003\); main effect time \(P < 0.001\); condition by time interaction \(P = 0.20\)). Mean postprandial plasma [TAG] was 22% and 14% lower in EX than CON (-32 to -12%, ES = 0.66, \(P = 0.004\)) and ER (-27 to 0%, ES = 0.40, \(P = 0.05\)) respectively; ER was marginally lower than CON (-9%; -21 to 3%, ES = 0.26, \(P = 0.11\)). The total area under the concentration versus time curve (TAUC) for TAG was 21% lower after EX than CON (ES = 0.71, \(P = 0.004\)), with small, but statistically insignificant, differences seen between ER and CON (-10%; ES = 0.32, \(P = 0.12\)) and EX and ER (-13%; ES = 0.39, \(P = 0.06\)) (Table 6.3). The TAUC-TAG was lower after EX compared with CON between 0 to 1 h by 26% (-39 to -11%, ES = 1.12, \(P = 0.01\)), 1 to 4.5 h by 22% (-33 to -10%, ES = 0.67, \(P = 0.01\)) and 4.5 to 6.5 h by 19% (-26 to -10%, ES = 0.64, \(P = 0.003\)); ER was lower than CON between 0 to 1 h by 11% (-20 to -2%, ES = 0.43, \(P = 0.03\)) and 1 to 4.5 h by 13% (-23 to -1%, ES = 0.37, \(P = 0.04\)). The TAUC-TAG was lower following EX than ER between 0 to 1 h by 17% (-34 to 4%, ES = 0.69, \(P = 0.08\)) and 4.5 to 6.5 h by 14% (-25 to -1%, ES = 0.47, \(P = 0.04\)). No significant differences were observed in the incremental area under the concentration versus time curve (iAUC) for TAG across the conditions (\(P = 0.84\)) (Table 6.3).

Meaningful positive correlations were identified between the intervention-induced change in fasting [TAG] and the change in TAUC-TAG relative to CON for EX (\(r = 0.65, P = 0.03\)) and ER (\(r = 0.57, P = 0.07\)). Individual changes (delta) in TAUC-TAG for EX and ER relative to CON are shown in Figure 6.3. The reductions in TAUC-TAG following EX and ER were greater than changes in CON for ten (91%) and eight (73%) girls respectively. The measured physical and physiological characteristics (Table 6.1), dietary intake (Table 6.2), exercise responses (Section 6.4.2) and fasting plasma [glucose] and [insulin] (Table 6.3) did not account for any of the inter-individual variability in delta TAUC-TAG for EX or ER. The Pearson’s product moment correlation for the individual changes in TAUC-TAG between EX and ER was trivial (\(r = 0.01, P = 0.98\)).
Figure 6.2  Fasting (F) and postprandial plasma triacylglycerol concentrations ([TAG]) in the control (CON), energy-intake restriction (ER) and moderate-intensity exercise (EX) conditions (n = 11). Values are mean (SD). Black rectangles denote consumption of breakfast and lunch meals at 08:00 and 12:00, respectively. Main effect condition P = 0.003; main effect time P < 0.001; condition by time interaction P = 0.20.

No differences in postprandial plasma [glucose] were seen across the conditions (main effect condition P = 0.13; main effect time P < 0.001; condition by time interaction P = 0.20). No significant differences were observed in TAUC-glucose across the conditions (P = 0.27) (Table 6.3). The EX iAUC-glucose was higher by 17% and 26% than CON (ES = 0.43, P = 0.05) and ER (ES = 0.62, P = 0.03) respectively; CON and ER were not significantly different (-7%; P = 0.55) (Table 6.3).

No differences in postprandial plasma [insulin] were seen across the conditions (main effect condition P = 0.63; main effect time P < 0.001; condition by time interaction P = 0.08). No significant differences were evident in TAUC-insulin (P = 0.56) or iAUC-insulin (P = 0.74) across the conditions (Table 6.3).
Figure 6.3  Individual changes (delta) in the total area under the plasma triacylglycerol (TAG) concentration versus time curve (TAUC) between the moderate-intensity exercise (EX) and energy-intake restriction (ER) conditions compared with the control condition (CON): A) EX minus CON; B) ER minus CON. Participant data are organised according to the size of the intervention-
induced change in TAUC-TAG; thus, the order of the individual participants is not identical in A and B. A negative response indicates a reduction in TAUC-TAG in the intervention compared with CON.

6.5 Discussion

The main novel finding from the present study was that an acute exercise-induced energy deficit elicits a greater reduction in fasting plasma [TAG] with a trend for a larger attenuation in postprandial plasma [TAG] than an isoenergetic diet-induced energy deficit in healthy, recreationally active girls. This suggests that the physiological origin of the energy deficit influences the magnitude of change in fasting and postprandial plasma [TAG] in girls.

The magnitude of reduction in fasting plasma [TAG] in ER (Table 6.3) supports the majority of previous findings in young people following moderate- to vigorous-intensity exercise (1.0 to 2.2 MJ) (Barrett et al., 2007; Lee et al., 2013; Tolfrey et al., 2008, 2012, 2014a). However, the considerable attenuation seen after EX (Table 6.3) is greater than the reductions reported previously (Barrett et al., 2007; Lee et al., 2013; Tolfrey et al., 2008, 2012, 2014a). It is likely that the lower fasting plasma [TAG] in EX and ER contributed to the lower TAG response evident over the postprandial period (Couch et al., 2000). Nevertheless, fasting [TAG] vary considerably in children (Tolfrey et al., 1999), and are typically less predictive of future cardiovascular events than postprandial [TAG] in women (Bansal et al., 2007), suggesting that postprandial [TAG] may provide a better insight into metabolic health in young people.

The reduction in postprandial plasma [TAG] after EX supports previous studies with boys and girls demonstrating that acute moderate- to vigorous-intensity exercise (1.0 to 2.5 MJ) reduces postprandial [TAG] (Barrett et al., 2007; Lee et al., 2013; MacEneaney et al., 2009; Sedgwick et al., 2013, 2014; Tolfrey et al., 2008, 2012, 2014a), but the small attenuation seen due to ER is a novel finding in this population (Table 6.3, Figure 6.2). Although the magnitude of reduction in postprandial [TAG] after ~60 min exercise varies in young people with estimated ES ranging from 0.26 to 0.77, on average, the changes are moderate (Table 2.2; Barrett et al., 2007; Lee et al., 2013; MacEneaney et al., 2009; Sedgwick et al., 2013, 2014; Tolfrey et al., 2008, 2012, 2014a), and greater than the small attenuation seen in ER. However, the magnitude of reduction in postprandial plasma [TAG] after EX is greater than the reductions reported in the majority of exercise postprandial studies in young people (Table 2.2; Barrett et al., 2007; Lee et al., 2013; MacEneaney et al., 2009; Sedgwick et al.,
2013, 2014; Tolfrey et al., 2008, 2012, 2014a). While the clinical significance of our findings cannot be established, 96% of the postprandial TAG samples in the present study were below the 2.3 mmol·L⁻¹ threshold suggested as a desirable concentration in young people (Kolovou et al., 2011a); therefore, the potential for girls with healthy postprandial TAG metabolism to benefit from exercise and diet interventions is promising.

The mechanisms responsible for the reduction in postprandial [TAG] in young people following exercise and diet interventions are not known currently, and cannot be inferred from our findings. In adults, increased clearance of circulating TAG facilitated by enhanced skeletal muscle lipoprotein lipase (LPL) has been implicated following acute exercise (Gill et al., 2003b). Furthermore, acute exercise-induced reductions in the circulating concentration of fasting and postprandial very low-density lipoprotein (VLDL)-TAG have been reported (Malkova et al., 2000), possibly due to the secretion of fewer, TAG-richer VLDL particles which are likely to have a higher affinity for LPL (Magkos et al., 2006). In the present study, the small differences identified in iAUC-TAG between EX, ER and CON suggests that changes in fasting [TAG], reflecting hepatic VLDL metabolism, contributes to the reduction in postprandial [TAG] after EX and ER. Indeed, the greatest difference in [TAG] between the three conditions was evident in the early postprandial period (0 to 1 h). This is further supported by the meaningful relationship seen between the intervention-induced changes in fasting plasma [TAG] and TAUC-TAG for EX (r = 0.65, P = 0.03) and ER (r = 0.57, P = 0.07). A recent stable isotope enrichment study reported that the exercise-evoked reduction in postprandial plasma [TAG] is achieved by a reduction in endogenous plasma [TAG] and not the concentration of meal-derived fatty acids in circulating TAG (Davitt et al., 2013). Therefore, changes in exogenous TAG metabolism may elicit a smaller influence on the postprandial TAG response.

The present study demonstrates for the first time in 11 to 13 year old girls that an exercise-induced energy deficit tended to reduce postprandial plasma [TAG] to a greater extent than an equivalent diet-induced energy deficit, supporting the studies conducted to date in healthy women (Gill and Hardman, 2000; Maraki et al., 2010). In the earliest of these studies, the reduction in postprandial [TAG] following a single exercise session was treble that caused by a diet-induced energy deficit (Gill and Hardman, 2000); although, it is worth noting that the energy deficit induced by intake restriction was approximately 17% lower than exercise. More recently, Maraki et al. (2010) demonstrated that exercise was superior to an equivalent energy deficit from energy-intake restriction, reducing postprandial [TAG] by 23% and 12%
respectively. Consequently, while reducing habitual energy intake may elicit a small reduction in postprandial [TAG], an exercise-induced energy deficit may be required to maximise the reduction in this important marker of atherogenic disease risk in girls. Nevertheless, the present study contributes to providing young people with an array of lifestyle options that may reduce postprandial plasma [TAG]. Mild, carefully managed reductions in dietary energy intake may be an attractive alternative in young people who find it difficult to accumulate sufficient physical activity for health.

The contrasting effect of EX and ER on postprandial plasma [TAG] may be attributable to the physiological origin of the energy deficit. Energy provision during moderate-intensity exercise is primarily met by the utilisation of skeletal muscle glycogen, intramuscular TAG, circulating free fatty acids and plasma glucose (Romijn et al., 1993), although the contribution of lipid to the exercise EE is greater in children than adults at a given relative exercise intensity (Riddell, 2008). In contrast, energy-intake restriction shifts the body towards the postabsorptive state leading to the breakdown of liver glycogen and the release of free fatty acids from adipose tissue (Frayn et al., 1994). Therefore, it is possible that the effect of EX and ER on postprandial plasma [TAG] is mediated by a different mechanism. However, a series of basal VLDL kinetic studies have demonstrated recently that exercise- and diet-induced attenuations in fasting VLDL-[TAG] manifest through a reduction in hepatic VLDL-TAG secretion and increased plasma clearance of VLDL-TAG in healthy, young women (Bellou et al., 2013b, 2013c); although, a lower energy deficit from moderate-intensity exercise compared with energy-intake restriction (~2 vs. 3 MJ respectively) was required to reveal these effects (Bellou et al., 2013b, 2013c). Consequently, the mitigating effect of EX and ER on postprandial plasma [TAG] may not be mediated solely by the ensuing energy deficit, but further exercise postprandial studies are required to support this in young people.

In line with previous studies in young people (Tolfrey et al., 2012, 2014a), substantial inter-individual variability is evident in the fasting and postprandial plasma [TAG] after EX and ER (Figure 6.3), which could not be accounted for by any of the measured variables included in the present study. Similar heterogeneity has been reported in adults previously, with exercise-induced changes in 3-hydroxybutyrate (3-OHB), a marker of hepatic fatty acid oxidation, identified as a strong predictor of the exercise-induced reduction in fasting and postprandial [TAG] (Gill et al., 2007). This marker may explain some of the variance in the present study, but further work is required to examine this systematically. A range of self-assessed maturity ratings were identified in the present study. Although a possible
maturational effect cannot be eliminated completely due to the relatively low sample size and limitations associated with self-assessed secondary sex characteristics (Chapter 3.2), we found no discernible effect of maturity status on any of the outcome measures, including the inter-individual variability evident in fasting and postprandial plasma [TAG].

The tendency for lower fasting plasma [glucose] in EX compared with CON is likely to contribute to the greater iAUC-glucose observed; however, a similar change in fasting plasma [glucose] was not seen between ER and EX despite the higher iAUC-glucose in EX (Table 6.3). The reason for these differences in glucose metabolism are unclear and appear inconsistent with the exercise postprandial studies in young people, with the majority reporting no exercise-induced changes in either fasting or postprandial [glucose] (Barrett et al., 2007; Lee et al., 2013; MacEneaney et al., 2009; Sedgwick et al., 2013, 2014; Tolfrey et al., 2008, 2014a). In the absence of a change in postprandial [glucose], Sedgwick et al. (2013) suggest the lower TAUC-insulin response following moderate-intensity exercise indicates an acute improvement in insulin sensitivity. Although we observed lower fasting plasma [insulin] in EX and ER compared with CON, this difference did not persist into the postprandial period (Table 6.3). The lower fasting HOMA-IR in EX and ER compared with CON points to an acute improvement in insulin resistance. Nevertheless, the change in HOMA-IR was not associated with the change in postprandial plasma [TAG] in EX or ER, and the TAG-lowering effect of exercise has been shown to occur independent of changes in insulin sensitivity previously (Gill et al., 2002b).

A limitation of the present study is that EE was not quantified during the short rest periods of EX, and excess post-exercise oxygen consumption was not measured following the exercise session. Although this omission may have underestimated the energy deficit in EX resulting in a higher energy deficit compared with ER, it is likely that the contribution to the total exercise EE was relatively small (Børsheim and Bahr, 2003). Furthermore, this study investigated healthy, recreationally active girls. Further research should be conducted to examine if similar responses are observed in young people with CVD risk factors, such as fasting hypertriglyceridaemia, obesity and insulin resistance. Future studies investigating the effect of replacing the exercise EE on postprandial lipaemia in young people would also be timely (Chapter 5).

In conclusion, this study shows for the first time in healthy, recreationally active girls that an exercise-induced energy deficit elicits a greater reduction in fasting plasma [TAG] with a
trend for a larger attenuation in postprandial plasma [TAG] than an isoenergetic diet-induced energy deficit. Therefore, exercise prescription may promote greater acute benefits in TAG metabolism than dietary restriction alone in girls.
CHAPTER 7
Effect of high-intensity interval running and energy-intake restriction on postprandial lipaemia and whole-body fat oxidation in girls

7.1 Abstract

The potency of combining acute exercise and diet manipulations on postprandial triacylglycerol concentrations ([TAG]) is not known in young people. This study compared the efficacy of combining acute high-intensity interval running (HIIR) with mild energy-intake restriction to augment the total energy deficit on postprandial plasma [TAG] in sixteen healthy girls (mean(SD): age 12.1(0.7) years; body mass 45.1(7.6) kg; peak oxygen uptake (\(\text{VO}_2\)) 43(6) mL·kg\(^{-1}\)·min\(^{-1}\)). Participants completed three, 2-day conditions in a counterbalanced, crossover design separated by 14 days. On day 1, participants completed 10 × 1 min interval runs (HIIR), 5 × 1 min interval runs and restricted food energy intake by 0.82(0.19) MJ (195(46) kcal; HIIR-ER) or rested (CON). Exercise was completed at 100% maximal aerobic speed, determined from an incremental peak\(\text{VO}_2\) test, with 1 min recovery between intervals. On day 2, capillary blood samples were taken in the fasted state and at predetermined intervals throughout the 6.5 h postprandial period. A standardised breakfast and lunch were consumed immediately and 4 h, respectively, after the fasting sample. Based on ratios of the geometric means (95% confidence intervals (CI) for ratios), fasting plasma [TAG] was 16% and 8% lower than CON in HIIR (-25 to -5%, effect size (ES) = 0.49, \(P = 0.01\)) and HIIR-ER (-18 to 3%, ES = 0.24, \(P = 0.14\)) respectively; HIIR was 8% lower than HIIR-ER (-16 to -1%, ES = 0.25, \(P = 0.04\)). The total area under the [TAG] versus time curve was 10% and 9% lower than CON in HIIR (-16 to -4%, ES = 0.30, \(P = 0.01\)) and HIIR-ER (-16 to -2%, ES = 0.28, \(P = 0.02\)) respectively; HIIR-ER and HIIR were similar (-1%; -9 to 8%, \(P = 0.82\)). Based on the arithmetic means (95% CI), the relative contribution of fat oxidation to total resting energy expenditure was greater than CON (44(17)% in HIIR (53(17)%; -4 to 22%, ES = 0.50, \(P = 0.14\)) and HIIR-ER (51(13)%; 0 to 14%, ES = 0.39, \(P = 0.05\)); HIIR-ER and HIIR were similar (-11 to 15%, \(P = 0.74\)). Manipulations of HIIR and ER reduce postprandial plasma [TAG] and increase resting whole-body fat oxidation in girls. The magnitude of effect was marginally, though not meaningfully, greater following HIIR than HIIR-ER.
7.2 Introduction

Elevated postprandial plasma triacylglycerol concentrations ([TAG]) are implicated in atherogenic development and progression (Zilversmit, 1979), and are established as an independent predictor of cardiovascular disease (CVD) incidence in women (Bansal et al., 2007). Although the clinical manifestations of atherosclerotic disease are not apparent until adulthood typically, the process of atherosclerosis originates in childhood (Froberg and Andersen, 2005; McGill et al., 2000a), and childhood fasting [TAG] is an independent predictor of young adult CVD (Morrison et al., 2009, 2012). Therefore, interventions that reduce postprandial [TAG] and delay precursors of atherosclerotic disease should be initiated early in life (Froberg and Andersen, 2005; McGill et al., 2000a).

Adult studies have shown consistently that acute aerobic exercise (30 min to 3 h in duration) performed the day before a standardised meal reduces postprandial [TAG] (Freese et al., 2014; Maraki and Sidossis, 2013), and increases resting whole-body fat oxidation (Burton et al., 2008; Davitt et al., 2013; Trombold et al., 2013, 2014). Similar reductions in postprandial [TAG] have been reported following acute moderate- to vigorous-intensity exercise in young people (Table 2.2; Tolfrey et al., 2014b). Several recent studies in adults highlight the potential efficacy of acute, intermittent high-intensity exercise to elicit reductions in postprandial [TAG] (Gabriel et al., 2012; Trombold et al., 2013), in addition to improvements in insulin sensitivity and resting whole-body fat oxidation (Trombold et al., 2013; Whyte et al., 2013). Similarly, we have demonstrated moderate reductions in postprandial [TAG] following a single session of high-intensity interval running (HIIR) in healthy 11 to 12 year old boys (Chapter 4). The majority of young people, particularly girls, fail to meet the current international guidelines of 60 min of daily moderate- to vigorous-intensity exercise for health promotion (Hallal et al., 2012; Health Survey for England, 2012b; Verloigne et al., 2012). Considering lack of time and enjoyment are frequently highlighted as barriers to exercise participation in adolescent girls (Butt et al., 2011; Kimm et al., 2006), the effect of different strategies that reduce the total exercise commitment and promote enjoyment on metabolic health markers should be investigated in girls. Therefore, the first aim of the present study was to examine the effect of a single session of HIIR on postprandial plasma [TAG] and resting whole-body fat oxidation in healthy girls.

A small number of studies have compared manipulations in exercise and dietary intake on postprandial [TAG] to investigate whether the exercise-evoked reduction in postprandial
[TAG] is a consequence of the associated energy deficit or skeletal muscle contraction. Acute moderate-intensity exercise appears more efficacious in reducing postprandial [TAG] than isoenergetic mild energy-intake restriction in healthy 11 to 13 year old girls (Chapter 6) and pre- and post-menopausal women (Gill and Hardman, 2000; Maraki et al., 2010). Although the combination of moderate-intensity exercise and energy-intake restriction did not exceed the reduction seen for exercise alone in healthy pre-menopausal women, it did at least match it (Maraki et al., 2010). To the author’s knowledge, however, no study has examined whether the combination of exercise and energy-intake restriction reduces postprandial [TAG] in young people. Therefore, the second aim of the present study was to compare the effect of a smaller dose of HIIR combined with energy-intake restriction (HIIR-ER) with the full HIIR protocol (undertaken previously in boys; Chapter 4) and a rest control condition on postprandial plasma [TAG] and whole-body fat oxidation in healthy, recreationally active girls.

7.3 Methods

7.3.1 Participants

A total of 19 recreationally active girls volunteered to participate in this study, with results presented for 16 girls (11.3 to 13.3 years) as one girl did not adhere to the required dietary replication and two girls dropped out for personal reasons unrelated to the study. All participants indicated that they were generally physically active, but not specifically accustomed to high-intensity running. Physical and physiological characteristics of participants are presented in Table 7.1.

7.3.2 Preliminary exercise measurements

During the first visit to the laboratory, preliminary anthropometry was completed as described in Chapter 3.2. Following familiarisation with the treadmill, participants completed the speed-based incremental treadmill protocol to determine peak oxygen uptake (VO$_2$) and maximal aerobic speed (MAS) detailed in Chapter 3.5.2.2.
Chapter 7: Combined exercise, energy restriction and postprandial metabolism

Table 7.1  Physical and physiological characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>12.1 (0.7)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>45.1 (7.6)</td>
</tr>
<tr>
<td>Stature (m)</td>
<td>1.55 (0.09)</td>
</tr>
<tr>
<td>Body mass index (kg·m⁻²)</td>
<td>18.7 (2.1)</td>
</tr>
<tr>
<td>Percent body fat (%)</td>
<td>21.4 (3.6)</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>35.4 (5.5)</td>
</tr>
<tr>
<td>Breast development*</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Pubic hair development*</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Peak oxygen uptake (mL·kg⁻¹·min⁻¹)</td>
<td>43 (6)</td>
</tr>
</tbody>
</table>

Values are mean (SD) for n = 16. *Self-assessment – median (interquartile range).

7.3.3 Experimental design

Participants completed three, 2-day experimental conditions: high-intensity interval running (HIIR), high-intensity interval running and energy-intake restriction (HIIR-ER) and rest control (CON). The study design is presented schematically in Figure 7.1.

7.3.3.1 Standardisation of dietary intake

Participants weighed, recorded and replicated their habitual dietary intake on the pre-intervention and intervention day of all experimental conditions (Chapter 3.6.1), but with a controlled reduction in energy intake on the intervention day of HIIR-ER. Participants completing HIIR-ER as the first condition were asked to record their usual dietary intake for two consecutive days at least one week in advance so that the prescribed energy-intake restriction could be calculated and standardised. The macronutrient composition of the carbohydrate-rich cereal snack bar consumed at 19:45 on the intervention day of each condition was 1.1 g fat, 15.7 g carbohydrate and 1.0 g protein, which provided 337 kJ energy.
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Figure 7.1 Diagram of the 2-day study protocol. TAG, triacylglycerol; NEFA, non-esterified fatty acids. *Evening meal replicated from the first condition but with a small reduction in energy intake in HIIR-ER.

7.3.3.2 Standardisation of free-living physical activity and sedentary time

An ActiGraph GT1M accelerometer was worn on the pre-intervention and intervention day of all experimental conditions as described previously (Chapter 3.6.2).

7.3.3.3 Day 1: Intervention day

During HIIR and HIIR-ER, the girls completed a 5 min warm-up at 60% MAS followed immediately by the acute high-intensity running intervals. The high-intensity running comprised either 10 (HIIR) or 5 (HIIR-ER) × 1 min treadmill runs at 100% MAS, with 1 min active recovery between each interval. Participants dismounted the treadmill during the active recovery periods and were encouraged to pace around the lab to avoid venous pooling and feeling light headed. Heart rate was monitored continuously and the participants provided a rating of perceived exertion (RPE) in the last 10 s of each running interval as described previously (Chapter 3.4), and affective valence was quantified at the end of each running interval using a validated feeling scale (FS) (Appendix 10; Hardy and Rejeski, 1989). Within 5 min of exercise completion, participants completed the modified Physical Activity Enjoyment Scale (PACES; Appendix 11; Motl et al., 2001), and total enjoyment was calculated by summing the 16 responses after eight items were reversed scored. During CON, participants rested in the laboratory for the duration of the visit. Participants maintained and replicated their habitual dietary intake throughout the day in all three conditions, but with a
controlled reduction in habitual food energy intake at the evening meal in HIIR-ER by 0.82(0.19) MJ (195(46) kcal).

7.3.3.4 Day 2: Postprandial day

Capillary blood samples were taken at pre-determined intervals throughout the 6.5 h postprandial rest period prior to, and following the consumption of standardised breakfast and lunch meals (Chapter 3.6.4). A 5 min resting expired air sample was collected after each capillary blood sample using Douglas bags (Chapter 3.3.2), and energy expenditure (EE), fat oxidation and carbohydrate oxidation were estimated (Chapter 3.3.3). The postprandial expired air data for one girl were spurious so results are presented for 15 girls. The breakfast meal provided 1.5 g fat (61.3% of meal total energy), 1.8 g carbohydrate (32.3%), 0.4 g protein (6.4%) and 94 kJ energy per kilogram body mass. The lunch meal provided 1.3 g fat (53.5%), 1.9 g carbohydrate (35.5%), 0.6 g protein (11.0%) and 92 kJ energy per kilogram body mass.

7.3.4 Analytical methods

Capillary blood samples were analysed for plasma [TAG], non-esterified fatty acid concentration ([NEFA]) and glucose concentration ([glucose]) (Chapter 3.7.2). Plasma samples were also analysed for the concentration of 3-hydroxybutyrate (3-OHB) (Randox Laboratories Limited, County Antrim, UK); however, the assay was unsuccessful in quantifying the concentration of 3-OHB in the majority of fasting and postprandial samples and, therefore, these data are not presented. The within-batch coefficient of variation for plasma [TAG], [NEFA] and [glucose] were 1.6, 1.5 and 0.8% respectively.

7.3.5 Statistical analyses

Data were analysed using the statistical methods presented in Chapter 3.8. Differences between HIIR and HIIR-ER exercise responses for running intervals 1 to 5 were compared using separate $2 \times 5$ (condition by interval) within-measures analysis of variance (ANOVA) and Student’s paired t-tests were used to identify temporal changes between the first and final running interval. Data for PACES were analysed using Student’s paired t-tests. Postprandial resting whole-body EE and substrate oxidation were calculated as total area under the variable versus time curve (TAUC) and, where appropriate, divided by the total duration of the postprandial period (6.5 h). Data for free-living physical activity and sedentary time, and concentrations of plasma TAG, NEFA and glucose were natural log transformed prior to
analysis. These data are presented as median (interquartile range) and analysis is based on the ratios of the geometric means and 95% confidence intervals (CI) for ratios. The incremental area under the concentration versus time curve (iAUC) for NEFA is negative due to the decrease in postprandial [NEFA] from the fasting concentration. All ANOVA analyses were adjusted appropriately for the period effect (Senn, 2002).

7.4 Results

7.4.1 Dietary intake

Energy and macronutrient intakes were similar on the pre-intervention day across the three conditions ($P \geq 0.14$). Average daily energy intake was 7.0(1.8) MJ, and dietary intake of protein, carbohydrate and fat was 59.8(19.0) g, 231(70) g and 56.5(14.7) g respectively. Energy and macronutrient intakes during the intervention day are displayed in Table 7.2. Energy intake on the intervention day of HIIR-ER was lower compared with CON (effect size (ES) = 0.60, $P < 0.001$) and HIIR (ES = 0.54, $P < 0.001$); HIIR was significantly, but not meaningfully, lower than CON (ES = 0.06, $P = 0.01$). Absolute protein, carbohydrate and fat intake were lower in HIIR-ER compared with CON and HIIR (ES = 0.35 to 0.63, $P < 0.001$). Absolute protein intake was not different between CON and HIIR ($P = 0.62$), but intakes of carbohydrate (ES = 0.09, $P = 0.01$) and fat (ES = 0.04, $P < 0.001$) were statistically, although not meaningfully, lower in HIIR than CON. The only statistical difference in the contribution of protein, carbohydrate and fat to total energy intake was a marginally greater contribution of protein in HIIR than CON (ES = 0.07, $P = 0.03$), and a marginally lower contribution of carbohydrate in HIIR than HIIR-ER (ES = 0.31, $P = 0.05$).
Table 7.2  Energy and macronutrient intakes during the intervention day of the high-intensity interval running (HIIR), high-intensity interval running and energy-intake restriction (HIIR-ER) and control (CON) conditions.

<table>
<thead>
<tr>
<th></th>
<th>HIIR (MJ·day⁻¹)</th>
<th>HIIR-ER (MJ·day⁻¹)</th>
<th>CON (MJ·day⁻¹)</th>
<th>CON vs. HIIR 95% CI*</th>
<th>CON vs. HIIR-ER 95% CI*</th>
<th>HIIR-ER vs. HIIR 95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>6.4 (1.4)</td>
<td>5.6 (1.5)</td>
<td>6.5 (1.4)</td>
<td>-0.16 to -0.03a</td>
<td>-1.0 to -0.7b</td>
<td>0.6 to 0.9c</td>
</tr>
<tr>
<td>Protein</td>
<td>54.4 (20.0)</td>
<td>47.1 (19.2)</td>
<td>54.1 (19.8)</td>
<td>-0.8 to 1.2</td>
<td>-8.6 to -4.6b</td>
<td>4.8 to 8.8c</td>
</tr>
<tr>
<td>CHO</td>
<td>218 (42)</td>
<td>196 (47)</td>
<td>222 (42)</td>
<td>-7 to -1a</td>
<td>-33 to -20b</td>
<td>16 to 29c</td>
</tr>
<tr>
<td>Fat</td>
<td>48.5 (15.4)</td>
<td>41.1 (14.5)</td>
<td>49.1 (15.8)</td>
<td>-1.0 to -0.4a</td>
<td>-9.5 to -6.2b</td>
<td>5.5 to 8.7c</td>
</tr>
<tr>
<td>% energy intake from protein</td>
<td>14 (4)</td>
<td>14 (4)</td>
<td>14 (4)</td>
<td>0.04 to 0.53a</td>
<td>-0.3 to 0.8</td>
<td>-0.3 to 0.4</td>
</tr>
<tr>
<td>% energy intake from CHO</td>
<td>58 (4)</td>
<td>59 (4)</td>
<td>58 (4)</td>
<td>-0.72 to 0.04</td>
<td>-0.4 to 1.8</td>
<td>-2.0 to 0.0c</td>
</tr>
<tr>
<td>% energy intake from fat</td>
<td>28 (5)</td>
<td>27 (4)</td>
<td>28 (5)</td>
<td>-0.2 to 0.3</td>
<td>-1.9 to 0.1</td>
<td>-0.04 to 1.97</td>
</tr>
</tbody>
</table>

Values are mean (SD) for n = 16. *95% confidence interval of the mean absolute difference between the experimental conditions.

CHO, carbohydrate.

a Significant difference between HIIR and CON (P < 0.05)

b Significant difference between HIIR-ER and CON (P < 0.05)

c Significant difference between HIIR and HIIR-ER (P < 0.05)
<table>
<thead>
<tr>
<th></th>
<th>HIIR</th>
<th>HIIR-ER</th>
<th>CON</th>
<th>CON vs. HIIR 95% CI*</th>
<th>CON vs. HIIR-ER 95% CI*</th>
<th>HIIR-ER vs. HIIR 95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily wear time (min)</td>
<td>852 (806-895)</td>
<td>819 (770-837)</td>
<td>800 (770-856)</td>
<td>-1 to 9%</td>
<td>-4 to 5%</td>
<td>-4 to 11%</td>
</tr>
<tr>
<td>Counts per minute</td>
<td>412 (363-484)</td>
<td>338 (298-397)</td>
<td>289 (262-336)</td>
<td>27 to 62%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2 to 37%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6 to 38%&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sedentary activity (min)</td>
<td>508 (479-553)</td>
<td>513 (435-550)</td>
<td>533 (495-551)</td>
<td>-13 to 4%</td>
<td>-12 to 6%</td>
<td>-12 to 10%</td>
</tr>
<tr>
<td>Light activity (min)</td>
<td>241 (200-319)</td>
<td>258 (182-283)</td>
<td>233 (188-278)</td>
<td>-2 to 26%</td>
<td>-7 to 12%</td>
<td>-5 to 25%</td>
</tr>
<tr>
<td>Moderate activity (min)</td>
<td>69 (56-77)</td>
<td>54 (40-62)</td>
<td>51 (43-59)</td>
<td>17 to 57%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-9 to 24%</td>
<td>3 to 57%&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vigorous activity (min)</td>
<td>14 (12-18)</td>
<td>9 (7-11)</td>
<td>1 (0-6)</td>
<td>209 to 1094%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110 to 640%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16 to 105%&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are median (interquartile range) for n = 16. Statistical analyses are based on natural log transformed data. *95% confidence interval for the ratio of geometric means.

<sup>a</sup> Significant difference between HIIR and CON (P < 0.05)

<sup>b</sup> Significant difference between HIIR-ER and CON (P < 0.05)

<sup>c</sup> Significant difference between HIIR and HIIR-ER (P < 0.05)
7.4.2 Free-living physical activity and sedentary time

On the pre-intervention day, no differences were seen in physical activity levels or sedentary time across the conditions ($P \geq 0.27$). Physical activity levels and sedentary time on the intervention day are displayed in Table 7.3. No significant differences were seen across the conditions for daily wear time ($P = 0.30$), sedentary time ($P = 0.47$) or time spent in light-intensity activities ($P = 0.15$). Average counts per minute (CPM) was higher than CON by 128 counts·min$^{-1}$ in HIIR ($ES = 1.49, P < 0.001$) and by 54 counts·min$^{-1}$ in HIIR-ER ($ES = 0.69, P = 0.03$); HIIR was 74 counts·min$^{-1}$ higher than HIIR-ER ($ES = 0.80, P = 0.01$). Time spent in moderate-intensity activities was higher in HIIR by 18 min and 15 min compared with CON ($ES = 1.06, P = 0.001$) and HIIR-ER ($ES = 0.85, P = 0.03$) respectively; CON and HIIR-ER were similar (3 min; $P = 0.39$). Time spent in vigorous-intensity activities was higher than CON by 12 min in HIIR ($ES = 1.59, P < 0.001$) and by 7 min in HIIR-ER ($ES = 1.21, P = 0.001$); HIIR was 5 min higher than HIIR-ER ($ES = 0.38, P = 0.01$). No differences were observed in free-living physical activity or sedentary time when accounting for the time spent resting or exercising in the laboratory on the intervention day ($P \geq 0.13$).

7.4.3 Responses to high-intensity interval running (HIIR)

The interval running session was performed at an average MAS of 11.5(1.1) km·h$^{-1}$ and was well tolerated by participants in HIIR and HIIR-ER. Two-way ANOVA revealed no differences between HIIR-ER and HIIR over running intervals 1 to 5 for heart rate, RPE or FS response ($P \geq 0.37$). During HIIR, there was a progressive increase from interval 1 to interval 10 for RPE (10(3) to 18(2) respectively; 95% CI 6 to 10, $ES = 2.82, P < 0.001$) and end interval heart rate (185(12) to 202(7) beats·min$^{-1}$ respectively; 95% CI 12 to 21 beats·min$^{-1}$, $ES = 1.36, P < 0.001$), corresponding to 91(4) and 99(2)% of peak heart rate respectively (95% CI 6 to 10%, $ES = 1.99, P < 0.001$). The FS response declined from interval 1 to interval 10 (3(2) to -2(3) respectively; 95% CI -6 to -3, $ES = 2.99, P < 0.001$). During HIIR-ER, there was a progressive increase from interval 1 to interval 5 for RPE (10(3) to 15(3) respectively; 95% CI 3 to 6, $ES = 1.50, P < 0.001$) and end interval heart rate (184(12) to 196(9) beats·min$^{-1}$ respectively; 95% CI 8 to 16 beats·min$^{-1}$, $ES = 0.99, P < 0.001$), corresponding to 90(4) and 96(2)% of peak heart rate respectively (95% CI 4 to 8%, $ES = 1.51, P < 0.001$), and a decline in the FS response (3(2) to -1(2) respectively; 95% CI -5 to -2, $ES = 1.57, P < 0.001$). The summed PACES score was similar between HIIR-ER and HIIR (57(9) vs. 56(10) respectively; 95% CI -6 to 3, $P = 0.55$).
**7.4.4 Resting whole-body energy expenditure (EE) and substrate oxidation**

Total resting EE over the 6.5 h postprandial period was similar across the conditions (HIIR 2.3(0.3) MJ, HIIR-ER 2.2(0.3) MJ, CON 2.3(0.3) MJ; \( P = 0.42 \)). The relative contribution of fat oxidation to total resting EE was greater than CON (44(17)%) in HIIR (53(17)%; 95% CI -4 to 22%, \( ES = 0.50, P = 0.14 \)) and HIIR-ER (51(13)%; 95% CI 0 to 14%, \( ES = 0.39, P = 0.05 \)); HIIR-ER and HIIR were similar (95% CI -11 to 15%, \( P = 0.74 \)) (Figure 7.2). Reciprocally, the relative contribution of carbohydrate oxidation to total resting EE was lower compared with CON (56(17)% in HIIR (47(17)%; 95% CI -22 to 4%, \( ES = 0.50, P = 0.14 \)) and HIIR-ER (49(13)%; 95% CI -14 to 0%, \( ES = 0.39, P = 0.05 \)); HIIR-ER and HIIR were not different (95% CI -15 to 11%, \( P = 0.74 \)) (Figure 7.2).

**Figure 7.2** Postprandial whole-body fat and carbohydrate oxidation expressed as a percentage of the total energy expenditure (EE) in the high-intensity interval running (HIIR), high-intensity interval running and energy-intake restriction (HIIR-ER) and control (CON) conditions (\( n = 15 \)). Fat and carbohydrate oxidation were estimated using stoichiometric equations, assuming that the contribution from protein was negligible (Chapter 3.3.3; Frayn, 1983). Values represent the total area under the substrate oxidation versus time curve divided by the duration of the postprandial period (6.5 h).
7.4.5 Plasma volume changes and fasting [TAG], [NEFA] and [glucose]

Average changes in plasma volume between the fasting and 6.5 h postprandial samples were not different across the three conditions (HIIR -0.3%, HIIR-ER 0.4%, CON -0.4%; \( P = 0.77 \)). Therefore, the raw plasma [TAG], [NEFA] and [glucose] were used in all statistical analyses without adjustment. The fasting plasma [TAG], [NEFA] and [glucose] for each condition are displayed in Table 7.4. One-way ANOVA revealed differences across the conditions in fasting plasma [TAG] \( (P = 0.01) \) and [NEFA] \( (P = 0.04) \), but not [glucose] \( (P = 0.41) \). Specifically, fasting plasma [TAG] was 16% and 8% lower than CON in HIIR \( (ES = 0.49, P = 0.01) \) and HIIR-ER \( (ES = 0.24, P = 0.14) \) respectively; HIIR was 8% lower than HIIR-ER \( (ES = 0.25, P = 0.04) \). Fasting plasma [NEFA] was 22% and 20% lower than CON in HIIR \( (ES = 0.65, P = 0.04) \) and HIIR-ER \( (ES = 0.58, P = 0.04) \) respectively; HIIR-ER and HIIR were not significantly different \(-3%; P = 0.78)\).

7.4.6 Plasma [TAG], [NEFA] and [glucose] in the postprandial period

Plasma TAG responses over the postprandial period for HIIR, HIIR-ER and CON are shown in Figure 7.3. Two-way ANOVA revealed differences in postprandial plasma [TAG] over time across conditions (main effect condition \( P = 0.01 \); main effect time \( P < 0.001 \); condition by time interaction \( P = 0.16 \)). Mean postprandial plasma [TAG] was 11% and 8% lower than CON in HIIR \(-17 to -4\%, \( ES = 0.27, P = 0.004 \)) and HIIR-ER \(-15 to -1\%, \( ES = 0.21, P = 0.03 \)) respectively; HIIR-ER and HIIR were similar \(-3%; -10 to 5\%, P = 0.48 \). The TAUC-TAG was 10% and 9% lower than CON in HIIR \( (ES = 0.30, P = 0.01) \) and HIIR-ER \( (ES = 0.28, P = 0.02) \) respectively; HIIR-ER and HIIR were similar \(-1%; P = 0.82 \) (Table 7.4). Specifically, TAUC-TAG was lower after HIIR than CON between 0 to 1 h by 16% \(-23 to -7\%, \( ES = 0.53, P = 0.002 \)) and 1 to 4.5 h by 11% \(-16 to -5\%, \( ES = 0.31, P = 0.003 \)); HIIR-ER was lower than CON between 0 to 1 h by 11% \(-17 to -5\%, \( ES = 0.37, P = 0.003 \)) and 1 to 4.5 h by 10% \(-17 to -3\%, \( ES = 0.30, P = 0.01 \)). No differences in TAUC-TAG over subsections of the total postprandial period were seen between HIIR-ER and HIIR \( (P \geq 0.13) \). No differences were seen in iAUC-TAG across the conditions \( (P = 0.53) \) (Table 7.4).
Table 7.4  Fasting and postprandial plasma triacylglycerol, non-esterified fatty acids (NEFA) and glucose concentrations in the high-intensity interval running (HIIR), high-intensity interval running and energy-intake restriction (HIIR-ER) and control (CON) conditions.

<table>
<thead>
<tr>
<th></th>
<th>HIIR</th>
<th>HIIR-ER</th>
<th>CON</th>
<th>CON vs. HIIR 95% CI*</th>
<th>CON vs. HIIR-ER 95% CI*</th>
<th>HIIR-ER vs. HIIR 95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Triacylglycerol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (mmol·L⁻¹)</td>
<td>0.75 (0.56-0.95)</td>
<td>0.82 (0.61-1.13)</td>
<td>0.87 (0.69-1.12)</td>
<td>-25 to -5%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-18 to 3%</td>
<td>-16 to -1%&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>TAUC (mmol·L⁻¹ 6.5 h)</td>
<td>7.78 (5.99-9.43)</td>
<td>6.99 (5.70-10.58)</td>
<td>7.64 (6.62-11.10)</td>
<td>-16 to -4%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-16 to -2%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-9 to 8%</td>
</tr>
<tr>
<td>iAUC (mmol·L⁻¹ 6.5 h)</td>
<td>3.14 (2.55-4.15)</td>
<td>2.39 (1.89-4.01)</td>
<td>3.24 (2.57-4.17)</td>
<td>-17 to 60%</td>
<td>-29 to 44%</td>
<td>-6 to 38%</td>
</tr>
<tr>
<td><strong>NEFA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (mmol·L⁻¹)</td>
<td>0.66 (0.56-0.78)</td>
<td>0.75 (0.61-0.85)</td>
<td>0.79 (0.62-1.22)</td>
<td>-39 to -1%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-35 to -1%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-22 to 21%</td>
</tr>
<tr>
<td>TAUC (mmol·L⁻¹ 6.5 h)</td>
<td>2.73 (2.23-3.26)</td>
<td>2.65 (2.20-3.33)</td>
<td>2.47 (2.28-2.98)</td>
<td>-9 to 18%</td>
<td>-3 to 17%</td>
<td>-10 to 6%</td>
</tr>
<tr>
<td>iAUC (mmol·L⁻¹ 6.5 h)</td>
<td>-1.30 (-2.19,-0.65)</td>
<td>-1.71 (-2.54,-0.73)</td>
<td>-2.41 (-5.08,-1.30)</td>
<td>6 to 132%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5 to 129%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-17 to 23%</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (mmol·L⁻¹)</td>
<td>5.72 (5.37-6.07)</td>
<td>5.68 (5.50-5.93)</td>
<td>5.74 (5.41-6.04)</td>
<td>-4 to 2%</td>
<td>-4 to 8%</td>
<td>-7 to 2%</td>
</tr>
<tr>
<td>TAUC (mmol·L⁻¹ 6.5 h)</td>
<td>44.4 (40.7-46.6)</td>
<td>43.2 (40.8-44.5)</td>
<td>42.4 (40.4-44.3)</td>
<td>2 to 6%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1 to 6%</td>
<td>-1 to 4%</td>
</tr>
<tr>
<td>iAUC (mmol·L⁻¹ 6.5 h)</td>
<td>10.07 (9.15-11.15)</td>
<td>9.10 (7.36-10.48)</td>
<td>7.54 (6.17-9.09)</td>
<td>5 to 37%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-41 to 28%</td>
<td>-6 to 104%</td>
</tr>
</tbody>
</table>

Values are median (interquartile range) for n = 16. Statistical analyses are based on natural log transformed data. *95% confidence interval for the ratio of geometric means.

TAUC, total area under the concentration versus time curve; iAUC, incremental area under the concentration versus time curve.
Chapter 7: Combined exercise, energy restriction and postprandial metabolism

\^ Significant difference between HIIR and CON \((P < 0.05)\)

\_ Significant difference between HIIR-ER and CON \((P < 0.05)\)

\textsuperscript{c} Significant difference between HIIR and HIIR-ER \((P < 0.05)\)

![Graph showing plasma triacylglycerol concentrations](image)

**Figure 7.3** Fasting (F) and postprandial plasma triacylglycerol concentrations ([TAG]) in the control (CON), high-intensity interval running and energy-intake restriction (HIIR-ER) and high-intensity interval running (HIIR) conditions \((n = 16)\). Values are mean (SD). Black rectangles denote consumption of breakfast and lunch meals at 08:00 and 12:00, respectively. Main effect condition \(P = 0.01\); main effect time \(P < 0.001\); condition by time interaction \(P = 0.16\).

Individual changes (delta) in TAUC-TAG for HIIR and HIIR-ER relative to CON are shown in Figure 7.4. The reductions in TAUC-TAG following HIIR and HIIR-ER were greater than changes in CON for ten (63%) and eleven (69%) girls respectively. Meaningful positive correlations were identified between the intervention-induced change in fasting plasma [TAG] and the change in TAUC-TAG relative to CON for HIIR \((r = 0.52, P = 0.04)\) and HIIR-ER \((r = 0.59, P = 0.02)\). The measured physical and physiological characteristics (Table 7.1), dietary intake (Table 7.2), free-living physical activity and sedentary time (Table 7.3), exercise responses (Section 7.4.3), resting whole-body EE and substrate oxidation (Section...
7.4.4, Figure 7.2) and fasting [NEFA] or [glucose] (Table 7.4) did not account for any of the inter-individual variability in delta TAUC-TAG for HIIR or HIIR-ER. The Pearson’s product moment correlation for the individual changes in TAUC-TAG between HIIR and HIIR-ER was small ($r = 0.31, P = 0.25$).

No differences were observed in postprandial plasma [NEFA] across the conditions over time (main effect condition $P = 0.63$; main effect time $P < 0.001$; condition by time interaction $P = 0.13$). No meaningful differences were evident for TAUC-NEFA across the conditions ($P = 0.45$) (Table 7.4). The iAUC-NEFA was 56% and 55% higher than CON in HIIR (ES = 0.67, $P = 0.03$) and HIIR-ER (ES = 0.65, $P = 0.03$) respectively; HIIR-ER and HIIR were not different (1%; $P = 0.91$) (Table 7.4).

Two-way ANOVA revealed a trend for differences in postprandial plasma [glucose] over time (main effect condition $P = 0.07$; main effect time $P < 0.001$; condition by time interaction $P = 0.33$). The TAUC-glucose was 4% higher in HIIR compared with CON (ES = 0.58, $P = 0.002$), but HIIR-ER was not different to HIIR (-1%; $P = 0.22$) or CON (2%; $P = 0.20$) (Table 7.4). The only significant difference in iAUC-glucose was a greater response in HIIR compared with CON (20%; ES = 0.80, $P = 0.01$) (Table 7.4).

### 7.5 Discussion

The primary finding from the present study is that acute manipulations of low volume HIIR and ER completed the day before standardised meals reduced postprandial plasma [TAG] and increased resting whole-body fat oxidation in healthy, 11 to 13 year old girls. The magnitude of this effect was marginally, although not meaningfully, greater following HIIR than HIIR-ER. The exercise and diet interventions were well tolerated by all participants and, therefore, may have practical metabolic health benefits in similar cohorts.

The exercise and dietary restriction induced reductions in fasting plasma [TAG] support the majority of previous exercise postprandial studies in young people (Chapters 5 and 6; Barrett et al., 2007; Lee et al., 2013; Tolfrey et al., 2008, 2012, 2014a). Although the lower fasting plasma [TAG] in HIIR and HIIR-ER are likely to influence the subsequent postprandial TAG response (Couch et al., 2000), substantial intra-individual variation is evident in childhood fasting [TAG] (Tolfrey et al., 1999), and fasting [TAG] are less predictive of CVD risk than postprandial [TAG] in women (Bansal et al., 2007).
Figure 7.4 Individual changes (delta) in the total area under the plasma triacylglycerol (TAG) concentration versus time curve (TAUC) between the high-intensity interval running (HIIR) and high-intensity interval running and energy-intake restriction (HIIR-ER) conditions compared with the control condition (CON): A) HIIR minus CON; B) HIIR-ER minus CON. Participant data are organised...
according to the size of the intervention-induced change in TAUC-TAG; thus, the order of the individual participants is not identical in A and B. A negative response indicates a reduction in TAUC-TAG in the intervention compared with CON.

Several adult studies have reported reductions in postprandial [TAG] following a single session of intermittent, high-intensity exercise (Ferreira et al., 2011; Freese et al., 2011; Gabriel et al., 2012, 2013; Tan et al., 2014; Trombold et al., 2013); however, this finding is not universal (Allen et al., 2014; Tan et al., 2013). The contrasting results in these studies may reflect the variety of high-intensity exercise protocols adopted which, coupled with differences in participant characteristics, exercise timing, meal content and blood sampling, is likely to promote heterogeneity in the individual responses (Allen et al., 2014; Tan et al., 2013). Nevertheless, we have demonstrated previously that a single session of HIIR promotes moderate reductions in postprandial plasma [TAG] in 11 to 12 year old boys (Chapter 4). The current study extends this novel finding to 11 to 13 year old girls, and supports the commonly reported reductions in postprandial [TAG] following acute moderate- to vigorous-intensity exercise in boys and girls (Table 2.2; Tolfrey et al., 2014b). An additional novel feature of the current study was the inclusion of a condition combining a lower volume of HIIR with a small reduction in energy intake (0.82(0.19) MJ, 195(46) kcal), which reduced postprandial plasma [TAG] to a similar extent as the full HIIR protocol (~10%; Table 7.4, Figure 7.3).

Acute energy-intake restriction alone has been shown to elicit a small reduction in postprandial [TAG] previously in healthy girls (-10%, ES = 0.32; Chapter 6) and pre-menopausal women (-12%; Maraki et al., 2010). Although an exercise-induced energy deficit appears a more potent stimulus to reduce postprandial [TAG] than an isoenergetic diet-induced energy deficit in girls (Chapter 6) and women (Gill and Hardman, 2000; Maraki et al., 2010), the combination of light walking and energy-intake restriction did match the reduction seen for exercise alone in sedentary, pre-menopausal women (Maraki et al., 2010). The similar reduction in postprandial plasma [TAG] following HIIR and HIIR-ER is promising, and highlights the potential for metabolic health benefits following time-efficient exercise combined with manageable dietary restriction in girls.

The mechanisms underpinning the acute exercise- and diet-induced reductions in postprandial plasma [TAG] in young people were not measured directly in the present study due to the invasive nature of the methods required to do this accurately. In adults, two primary pathways have been proposed involving the increased clearance of circulating TAG facilitated by
enhanced lipoprotein lipase (LPL) activity (Gill et al., 2003b; Kiens and Richter, 1998) and/or the secretion of fewer, TAG-richer very low-density lipoproteins (VLDL) that have a higher affinity for LPL (Magkos et al., 2006). A recent stable isotope enrichment study in obese women suggested that the TAG-lowering effect of acute exercise is mediated by a reduced abundance of endogenous fatty acids in plasma TAG and not the enhanced clearance of dietary fat (Davitt et al., 2013). The notion that endogenous, and not exogenous, TAG metabolism exerts a stronger influence on the postprandial TAG response is indirectly supported by the current study evidenced by the small differences in iAUC-TAG between the conditions, and the meaningful relationship seen between the intervention-induced changes in fasting plasma [TAG] and TAUC-TAG for HIIR (r = 0.52, P = 0.04) and HIIR-ER (r = 0.59, P = 0.02).

Elevated whole-body fat oxidation in the postprandial period after HIIR and HIIR-ER appears another novel finding in young people, and is consistent with the effect of acute moderate-intensity exercise in healthy boys (Chapter 5), along with exercise postprandial studies in adults employing acute high-intensity exercise protocols (Trombold et al., 2013; Whyte et al., 2013). The post-exercise shift in whole-body substrate utilisation towards fat oxidation reflects increases in exogenous and endogenous fat oxidation (Gill et al., 2001a), and has been linked to a number of regulatory mechanisms promoting the resynthesis of depleted skeletal muscle and/or hepatic glycogen stores (Kiens and Richter, 1998; Kimber et al., 2003). Circulating plasma fatty acids and triacylglycerol-rich lipoproteins (TRL) are potential lipid sources utilised for oxidation, which is in agreement with the lower postprandial plasma [TAG] after HIIR and HIIR-ER, likely mediated by enhanced LPL activity (Gill et al., 2003b; Kiens and Richter, 1998). However, the similar postprandial NEFA response between the three experimental conditions suggests that plasma fatty acids did not contribute to the greater whole-body fat oxidation in HIIR and HIIR-ER. Nevertheless, it is possible that differences in plasma [NEFA] were evident before the commencement of the postprandial period considering large increases in plasma free fatty acids have been shown in the early post-exercise recovery period (Kiens and Richter, 1998; Kimber et al., 2003). The lack of association between whole-body fat oxidation and indices of lipaemia in the current study supports a recent study in boys following moderate-intensity exercise (Chapter 5) but contrasts previous findings in adults (Burton et al., 2008; Trombold et al., 2013), suggesting that exercise- and diet-induced changes in postprandial plasma [TAG] and whole-body fat oxidation may occur independently in girls. Nevertheless, elevated
postprandial [TAG] are associated independently with CVD risk in women (Bansal et al., 2007), and low resting fat oxidation with an increased risk of weight gain (Ellis et al., 2010; Zurlo et al., 1990) and Type 2 diabetes mellitus (Blaak et al., 2001), highlighting the potential efficacy of acute high-intensity exercise and dietary restriction to improve metabolic health outcomes early in life.

Although the clinical significance of our findings cannot be established, the majority (93%) of the postprandial TAG samples were below the 2.3 mmol·L$^{-1}$ threshold considered a desirable concentration in young people (Kolovou et al., 2011a). The majority of girls fall short of the current physical activity guidelines for health (Hallal et al., 2012; Health Survey for England, 2012b; Verloigne et al., 2012), and time and enjoyment are reported frequently as barriers to exercise participation in adolescent girls (Butt et al., 2011; Kimm et al., 2006). Therefore, the potential for HIIR and HIIR-ER, with a total exercise time commitment of 24 and 14 min respectively (including warm-up and active recovery between intervals), to reduce postprandial plasma [TAG] and increase resting whole-body fat oxidation in girls is encouraging. The girls spent a greater amount of time engaged in vigorous-intensity activities in HIIR and HIIR-ER, and a greater amount of time in moderate-intensity activities in HIIR on the intervention day as a result of the prescribed exercise intervention. There were no differences between conditions after accounting for the time spent resting or exercising in the laboratory, suggesting that the implemented between condition control of free-living physical activity and sedentary time was effective. The high-intensity nature of the exercise adopted in the present study may better reflect the activity patterns of young people who spend a greater proportion of time engaged in high-intensity activities than adults (Hoos et al., 2004). Furthermore, it has been demonstrated that children associate moderate-intensity exercise interspersed with short high-intensity efforts with greater perceived enjoyment than completing continuous moderate-intensity exercise alone (Crisp et al., 2012). In the present study, the similarly high PACES score between HIIR and HIIR-ER suggests interval running performed at a high-intensity may be an attractive exercise model in girls independent of whether five or ten 1 min intervals are completed.

Previous high-intensity exercise postprandial studies highlight the substantial heterogeneity evident in postprandial TAG responses in young people (Chapter 4) and adults (Allen et al., 2014; Tan et al., 2013). We have shown previously in boys that exercising at a higher relative exercise intensity during HIIR is associated with a greater reduction in postprandial plasma [TAG] (Chapter 4); however, this relationship was not apparent in the current study with girls,
and the other measured variables in the study could not explain any of the heterogeneity present. A study with adults reported that exercise-induced changes in 3-OHB, a marker of hepatic fatty acid oxidation, was a strong predictor of the moderate-intensity exercise-induced reduction in fasting and postprandial [TAG] (Gill et al., 2007). Although it is possible that this marker may explain some of the heterogeneity in the present study, the 3-OHB assay used in the current study was unable to detect the concentration of 3-OHB in the majority of fasting and postprandial samples and, therefore, further investigation is required in young people.

The higher postprandial plasma [glucose] after HIIR compared with CON contrasts the majority of previous exercise postprandial studies in young people reporting no difference in postprandial [glucose] following acute exercise (Chapters 4, 5 and 6; Barrett et al., 2007; Lee et al., 2013; MacEneaney et al., 2009; Sedgwick et al., 2013, 2014; Tolfrey et al. 2008, 2014a), although one study reported a decrease in postprandial [glucose] following moderate-intensity exercise (Tolfrey et al., 2012). The reason for this discrepant finding is not known; however, it is unlikely that the higher postprandial [glucose] in HIIR is implicated in the TAG-lowering effect of HIIR considering glucose has not been linked to the potential mechanistic pathways discussed above.

In conclusion, acute manipulations of low volume HIIR and ER completed the day before standardised meals reduced postprandial plasma [TAG] and increased resting whole-body fat oxidation in healthy, 11 to 13 year old girls. The magnitude of this effect was marginally, though not meaningfully, greater following HIIR than HIIR-ER. Low volume, HIIR performed alone or in combination with a mild reduction in habitual energy intake may represent time-efficient and enjoyable strategies to improve metabolic health in girls, but further work is required to examine this chronically.
CHAPTER 8
Effect of acute high-intensity interval running on postprandial lipaemia: a comparison between boys and girls

8.1 Abstract

In two independent studies, we have shown that acute high-intensity interval running (HIIR) reduces postprandial triacylglycerol concentrations ([TAG]) in boys (Chapter 4) and girls (Chapter 7). However, it is not known whether differences in postprandial TAG metabolism exist between boys and girls in response to a single exercise session. Therefore, in the current chapter we combined and reanalysed data for boys (Chapter 4) and girls (Chapter 7) to compare the effect of acute HIIR on fasting and postprandial plasma [TAG] directly. Fifteen healthy boys (mean(SD): age 11.8(0.4) years; body mass 42.8(8.0) kg; peak oxygen uptake ($\dot{V}O_2$) 55(6) mL·kg$^{-1}$·min$^{-1}$) and sixteen healthy girls (age 12.1(0.7) years; body mass 45.1(7.6) kg; peak $\dot{V}O_2$ 43(6) mL·kg$^{-1}$·min$^{-1}$) completed two, 2-day conditions in a counterbalanced, crossover design. On day 1, participants rested (CON) or completed 10 × 1 min running intervals at 100% maximal aerobic speed, determined from an incremental peak $\dot{V}O_2$ test, with 1 min recovery between intervals (HIIR). On day 2, capillary blood samples were taken in the fasted state and at pre-determined intervals throughout the 6.5 h postprandial rest period. A standardised breakfast and lunch were consumed immediately and 4 h, respectively, after the fasting sample. Based on ratios of the geometric means (95% confidence intervals (CI) for ratios), fasting plasma [TAG] was 32% lower in boys than girls (-45 to -18%, effect size (ES) = 1.31, $P < 0.001$), and 12% lower after HIIR than CON (-19 to -4%, ES = 0.42, $P = 0.003$), but the magnitude of reduction following HIIR was similar in boys and girls (8% vs. 15% respectively; $P = 0.31$). The total area under the [TAG] versus time curve was 27% lower in boys than girls (-41 to -9%, ES = 1.02, $P = 0.01$), and 11% lower after HIIR than CON (-16 to -5%, ES = 0.37, $P = 0.001$); again, the magnitude of reduction following HIIR was similar in boys and girls (11% vs. 10% respectively; $P = 0.79$). This is the first study to show that 11 to 12 year old boys demonstrated lower fasting and postprandial plasma [TAG] than similarly aged girls, but the small-moderate reduction following a single bout of HIIR was similar between the sexes.
8.2 Introduction

The leading cause of death in the United Kingdom is cardiovascular disease (CVD), which accounted for 32% of all-cause mortality in 2010 in men and women (Townsend et al., 2012). Elevated postprandial triacylglycerol concentrations ([TAG]) are established as an independent risk factor for CVD in men and women (Bansal et al., 2007; Nordestgaard et al., 2007), and appear more discriminatory for CVD risk than traditional fasting concentrations (Bansal et al., 2007). The paediatric origins of atherosclerosis are well established (Froberg and Andersen, 2005; McGill et al., 2000a), and exaggerated fasting [TAG] in childhood predicts young adult CVD (Morrison et al., 2009, 2012). Considering people in Western society are exposed typically to the postprandial state for the majority of waking hours, low physical activity and poor diet may exacerbate the risk of future CVD with prolonged elevated [TAG] (Peddie et al., 2012). Therefore, lifestyle interventions that reduce postprandial [TAG] and delay precursors of atherosclerotic progression should begin early in life (Froberg and Andersen, 2005; McGill et al., 2000a).

Studies in adults have consistently demonstrated greater postprandial [TAG] in men than women (Couillard et al., 1999; Jackson et al., 2010; Kolovou et al., 2006; Koutsari et al., 2004). Although the mechanisms accounting for this sex difference have not been fully elucidated, greater skeletal muscle uptake and retention of plasma [TAG] (Horton et al., 2002), lower abdominal visceral adipose tissue lipolysis (Couillard et al., 1999), greater suppression of upper-body subcutaneous adipose tissue lipolysis (Jensen, 1995) and/or the protective effect of oestrogen (Westerveld, 1998) in women have been implicated. Nevertheless, Gill et al. (2002b) compared 38 men and 43 women and reported that the exercise-induced reduction in postprandial [TAG] was not different between men and women (23.5% vs. 19.8% respectively). A single session of moderate- to high-intensity exercise performed the day before a standardised meal reduces postprandial [TAG] in boys (Chapters 4 and 5; Barrett et al., 2007; Lee et al., 2013; MacEneaney et al., 2009; Sedgwick et al., 2013, 2014; Tolfrey et al., 2008, 2012) and girls (Chapters 6 and 7; Tolfrey et al., 2014a). However, it is not known whether the postprandial TAG response is different in boys and girls following a single session of exercise which represents an important gap in our understanding of this marker of future CVD risk in young people.

Therefore, the aim of this chapter was to compare directly the postprandial TAG response in healthy 11 to 13 year old boys and girls, and to examine the magnitude of change in
postprandial plasma [TAG] following a single bout of exercise. Data for boys and girls were combined and reanalysed from Chapters 4 and 7 respectively, and the sex differences in postprandial plasma [TAG] were examined following acute high-intensity interval running (HIIR) and rest control (CON) conditions.

8.3 Methods

8.3.1 Participants

Data for 15 boys and 16 girls were combined and reanalysed from Chapters 4 and 7 respectively. All participants indicated that they were generally physically active but not specifically accustomed to high-intensity running. Physical and physiological characteristics are presented in Table 8.1.

Table 8.1  Physical and physiological characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th>Girls</th>
<th>Girls vs. Boys 95% CI*</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.8  (0.4)</td>
<td>12.1  (0.7)</td>
<td>-0.7 to 0.1</td>
<td>0.49</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>42.8  (8.0)</td>
<td>45.1  (7.6)</td>
<td>-8.1 to 3.4</td>
<td>0.30</td>
</tr>
<tr>
<td>Stature (m)</td>
<td>1.53  (0.09)</td>
<td>1.55  (0.09)</td>
<td>-0.09 to 0.04</td>
<td>0.28</td>
</tr>
<tr>
<td>Body mass index (kg·m⁻²)</td>
<td>18.3  (2.8)</td>
<td>18.7  (2.1)</td>
<td>-2.1 to 1.5</td>
<td>0.14</td>
</tr>
<tr>
<td>Percent body fat (%)</td>
<td>13.5  (5.2)</td>
<td>21.4  (3.6)</td>
<td>-11.1 to -4.6</td>
<td>1.75</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>36.7  (5.4)</td>
<td>35.4  (5.5)</td>
<td>-2.7 to 5.3</td>
<td>0.24</td>
</tr>
<tr>
<td>Pubic hair development**</td>
<td>2  (1)</td>
<td>2  (3)</td>
<td>-2 to 1</td>
<td>0.03</td>
</tr>
<tr>
<td>Peak oxygen uptake (mL·kg⁻¹·min⁻¹)</td>
<td>55  (6)</td>
<td>43  (6)</td>
<td>8 to 17*</td>
<td>2.08</td>
</tr>
<tr>
<td>Maximal aerobic speed (km·h⁻¹)</td>
<td>12.5  (1.6)</td>
<td>11.5  (1.1)</td>
<td>-0.04 to 1.98</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Values are mean (SD) for $n = 15$ boys and $n = 16$ girls. *95% confidence interval of the mean absolute difference between the groups. **Self-assessment – median (interquartile range).

*a Significant difference between boys and girls ($P < 0.001$)
8.3.2 Preliminary exercise measurements

During the first visit to the laboratory, preliminary anthropometry was completed as described in Chapter 3.2. Following familiarisation with the treadmill, participants completed the speed-based incremental treadmill protocol to determine peak oxygen uptake (\(\dot{V}O_2\)) and maximal aerobic speed (MAS) detailed in Chapter 3.5.2.2.

8.3.3 Experimental design

Participants completed two, 2-day experimental conditions: rest control (CON) and high-intensity interval running (HIIR). The study design is presented schematically in Figure 8.1.

![Figure 8.1](image_url)  
**Figure 8.1**  
Diagram of the 2-day study protocol. TAG, triacylglycerol.  
+Evening meal replicated from first condition.

8.3.3.1 Standardisation of dietary intake

Participants weighed, recorded and replicated their habitual dietary intake on the pre-intervention and intervention day of all experimental conditions (Chapter 3.6.1). The macronutrient composition of the carbohydrate-rich cereal snack bar consumed at 19:45 on the intervention day of each condition was 1.2 g fat, 15.9 g carbohydrate and 1.0 g protein, which provided 335 kJ energy.

8.3.3.2 Day 1: Intervention day

During HIIR, the participants completed a 5 min warm-up at 60% MAS followed immediately by the acute high-intensity running intervals. The high-intensity running
Chapter 8: Sex differences in postprandial metabolism

comprised 10 × 1 min treadmill runs at 100% MAS, with 1 min active recovery between each interval. Participants dismounted the treadmill during the active recovery periods and were encouraged to pace around the lab to avoid venous pooling and feeling light headed. Heart rate was monitored continuously and the participants provided a rating of perceived exertion (RPE) in the last 10 s of each running interval as described previously (Chapter 3.4). During CON, participants rested in the laboratory for the duration of the visit.

8.3.3.3 Day 2: Postprandial day

Capillary blood samples were taken at pre-determined intervals throughout the 6.5 h postprandial rest period prior to, and following the consumption of standardised breakfast and lunch meals (Chapter 3.6.4). The breakfast meal provided 1.5 g fat (60.7% of meal total energy), 1.8 g carbohydrate (32.6%), 0.4 g protein (6.7%) and 93 kJ energy per kilogram body mass. The lunch meal provided 1.2 g fat (51.9%), 1.9 g carbohydrate (36.5%), 0.6 g protein (11.6%) and 89 kJ energy per kilogram body mass.

8.3.4 Analytical methods

Capillary blood samples were analysed for plasma [TAG] and glucose concentration ([glucose]) (Chapter 3.7.2). The within-batch coefficient of variation for plasma [TAG] and [glucose] were 1.0 and 0.4% respectively for boys (Chapter 4.3.4) and 1.6 and 0.8% respectively for girls (Chapter 7.3.4).

8.3.5 Statistical analyses

Data were analysed using the statistical methods presented in Chapter 3.8. Student’s independent t-tests were used to compare physical and physiological characteristics (Table 8.1) and HIIR responses (Table 8.3) between boys and girls. Physical maturity was compared between groups using a non-parametric Mann Whitney-U test and presented as median (interquartile range). The 95% confidence interval (CI) was derived using a bootstrapping method and the effect size (ES) was calculated from the z-score (Field, 2009) (Table 8.1). Concentrations of plasma TAG and glucose were natural log transformed prior to analysis. These data are presented as median (interquartile range) and analysis is based on the ratios of the geometric means and 95% CI for ratios. Energy and macronutrient intakes, estimated changes in plasma volume, fasting plasma [TAG] and [glucose] and total (TAUC) and incremental (iAUC) area under the concentration versus time curve were compared using separate 2 × 2 (condition by group) mixed measures analysis of variance (ANOVA) repeated
for condition. Differences in plasma [TAG] and [glucose] over the total 6.5 h postprandial period were examined using separate $2 \times 2 \times 7$ (condition by group by time) mixed measures ANOVA repeated for condition and time. Temporal changes in TAUC-TAG between the experimental conditions were examined over sub-sections of the total postprandial period (0 to 1 h, 1 to 4.5 h and 4.5 to 6.5 h) using separate $2 \times 2$ (condition by group) mixed measures ANOVA repeated for condition. All ANOVA analyses were adjusted appropriately for the period effect (Senn, 2002). The ES for between group comparisons were calculated using the boys’ and girls’ pooled SD as follows:

$$ES = \frac{Mean_v - Mean_i}{\sqrt{Boys \ SD_v^2 + Girls \ SD_i^2/2}}$$ (Cumming and Finch, 2001)

### 8.4 Results

#### 8.4.1 Participant characteristics

Girls were marginally older than boys (ES = 0.49, $P = 0.19$), and percent body fat was lower in boys compared with girls (ES = 1.75, $P < 0.001$) (Table 8.1). Peak VO$_2$ was greater in boys than girls (ES = 2.08, $P < 0.001$), with a trend for MAS to be higher in boys than girls (ES = 0.70, $P = 0.06$) (Table 8.1). The other measured physical and physiological variables were similar between boys and girls ($P \geq 0.41$) (Table 8.1).

#### 8.4.2 Dietary intake

Energy and macronutrient intakes were similar on the pre-intervention day across conditions and groups (main effect condition $P \geq 0.11$; main effect group $P \geq 0.08$; condition by group interaction $P \geq 0.38$). Energy and macronutrient intakes during the intervention day are displayed in Table 8.2. No differences were seen in energy and macronutrient intakes between CON and HIIR ($P \geq 0.74$). Energy intake (ES = 1.02, $P = 0.004$), and absolute intakes of protein (ES = 0.83, $P = 0.03$) and fat (ES = 0.97, $P = 0.005$) were significantly greater in boys compared with girls. Carbohydrate intake tended to be greater in boys than girls (ES = 0.67, $P = 0.06$). No differences in the contribution of protein, carbohydrate and fat to total energy intake were seen between CON and HIIR ($P \geq 0.26$); however, the contribution of carbohydrate to total energy intake was lower (ES = 0.91, $P = 0.02$) and the contribution of fat to total energy intake was higher (ES = 0.72, $P = 0.05$) in boys than girls.
Table 8.2  Energy and macronutrient intakes during the intervention day in the control (CON) and high-intensity interval running (HIIR) conditions in boys and girls.

<table>
<thead>
<tr>
<th></th>
<th>Boys (n = 15)</th>
<th>Girls (n = 16)</th>
<th>Girls vs. Boys 95% CI*</th>
<th>CON vs. HIIR 95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>HIIR</td>
<td>CON</td>
<td>HIIR</td>
</tr>
<tr>
<td>Energy (MJ·day⁻¹)</td>
<td>8.0 (1.7)</td>
<td>8.1 (1.7)</td>
<td>6.5 (1.4)</td>
<td>6.4 (1.4)</td>
</tr>
<tr>
<td>Protein (g·day⁻¹)</td>
<td>69.7 (19.1)</td>
<td>70.9 (19.3)</td>
<td>54.1 (19.8)</td>
<td>54.4 (20.0)</td>
</tr>
<tr>
<td>CHO (g·day⁻¹)</td>
<td>249 (42)</td>
<td>248 (64)</td>
<td>222 (42)</td>
<td>218 (42)</td>
</tr>
<tr>
<td>Fat (g·day⁻¹)</td>
<td>68.4 (25.8)</td>
<td>70.5 (24.6)</td>
<td>49.1 (15.8)</td>
<td>48.5 (15.4)</td>
</tr>
<tr>
<td>% energy intake from protein</td>
<td>14.7 (2.7)</td>
<td>15.2 (3.8)</td>
<td>13.9 (3.6)</td>
<td>14.1 (3.7)</td>
</tr>
<tr>
<td>% energy intake from CHO</td>
<td>53.7 (6.6)</td>
<td>52.0 (8.6)</td>
<td>57.9 (3.9)</td>
<td>57.6 (3.7)</td>
</tr>
<tr>
<td>% energy intake from fat</td>
<td>31.6 (6.3)</td>
<td>32.8 (8.3)</td>
<td>28.2 (4.6)</td>
<td>28.3 (4.6)</td>
</tr>
</tbody>
</table>

Values are mean (SD) for n = 15 boys and n = 16 girls. *95% confidence interval of the mean absolute difference between the groups or experimental conditions.

CHO, carbohydrate.

a Significant difference between boys and girls (P < 0.05)
8.4.3 Responses to high-intensity interval running (HIIR)

Temporal changes in HIIR responses between running interval 1 and 10 are presented in Chapter 4.4.2 for boys and Chapter 7.4.3 for girls. Differences in average heart rate and RPE responses to HIIR between boys and girls are displayed in Table 8.3. Average start interval heart rate was lower in boys compared with girls when expressed in beats-min\(^{-1}\) (ES = 1.73, \(P < 0.001\)) and as a percentage of peak heart rate (ES = 1.65, \(P < 0.001\)). Average end interval heart rate was lower in boys compared with girls (ES = 0.75, \(P = 0.05\)), but was not significantly different when expressed as a percentage of peak heart rate (\(P = 0.25\)). Average RPE over the 10 running intervals was greater in boys than girls (ES = 0.85, \(P = 0.03\)).

**Table 8.3** Average responses to the high-intensity interval running (HIIR) session.

<table>
<thead>
<tr>
<th></th>
<th>Boys ((n = 15))</th>
<th>Girls ((n = 16))</th>
<th>Girls vs. Boys 95% CI*</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak heart rate ((\text{beats} \cdot \text{min}^{-1})^{**})</td>
<td>200 (6)</td>
<td>203 (7)</td>
<td>-9 to 1</td>
<td>0.57</td>
</tr>
<tr>
<td>Start interval heart rate ((\text{beats} \cdot \text{min}^{-1}))</td>
<td>144 (10)</td>
<td>164 (12)</td>
<td>-28 to -11(^a)</td>
<td>1.73</td>
</tr>
<tr>
<td>Percent peak heart rate (%)</td>
<td>72 (5)</td>
<td>80 (5)</td>
<td>-12 to -5(^a)</td>
<td>1.65</td>
</tr>
<tr>
<td>End interval heart rate ((\text{beats} \cdot \text{min}^{-1}))</td>
<td>190 (7)</td>
<td>196 (8)</td>
<td>-11.3 to -0.1(^a)</td>
<td>0.75</td>
</tr>
<tr>
<td>Percent peak heart rate (%)</td>
<td>95 (3)</td>
<td>96 (2)</td>
<td>-3 to 1</td>
<td>0.43</td>
</tr>
<tr>
<td>Rating of perceived exertion</td>
<td>16 (1)</td>
<td>14 (2)</td>
<td>0.2 to 2.6(^a)</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Values are mean (SD) for \(n = 15\) boys and \(n = 16\) girls. *95% confidence interval of the mean absolute difference between the groups. **Peak heart rate was determined during the speed-based incremental treadmill protocol (Section 8.3.2).

\(^a\) Significant difference between boys and girls \((P < 0.05)\)

8.4.4 Plasma volume changes and fasting plasma [TAG] and [glucose]

Average changes in plasma volume between the fasting and 6.5 h postprandial samples were not different across the conditions or groups (main effect condition \(P = 0.88\); main effect group \(P = 0.44\); condition by group interaction \(P = 0.95\)). Therefore, the raw plasma [TAG] and [glucose] were used in all statistical analyses without adjustment. The fasting plasma [TAG] and [glucose] for each condition are displayed in Table 8.4. Fasting plasma [TAG]
was 32% lower in boys than girls (ES = 1.31, \( P < 0.001 \)) and 12% lower after HIIR than CON (ES = 0.42, \( P = 0.003 \)), but the magnitude of reduction following HIIR was similar in boys and girls (8% vs. 15% respectively; condition by group interaction \( P = 0.31 \)). No differences were seen in fasting plasma [glucose] across conditions or groups (main effect condition \( P = 0.35 \); main effect group \( P = 0.14 \); condition by group interaction \( P = 0.68 \)).

### 8.4.5 Plasma [TAG] and [glucose] in the postprandial period

Plasma TAG responses over the postprandial period for CON and HIIR are shown in Figure 8.2. Two-way ANOVA revealed differences in postprandial plasma [TAG] over time across conditions and groups (main effect condition \( P < 0.001 \); main effect group \( P = 0.01 \); condition by group interaction \( P = 0.83 \)). The TAUC-TAG was 27% lower in boys than girls (ES = 1.02, \( P = 0.01 \)) and 11% lower after HIIR than CON (ES = 0.37, \( P = 0.001 \)), but the magnitude of reduction following HIIR was similar in boys and girls (11% vs. 10% respectively; condition by group interaction \( P = 0.79 \)) (Table 8.4). The TAUC-TAG was lower in boys than girls between 0 to 1 h by 26% (-39 to -10%, ES = 1.03, \( P = 0.004 \)), 1 to 4.5 h by 32% (-46 to -14%, ES = 1.15, \( P = 0.002 \)) and 4.5 to 6.5 h by 18% (-33 to 0%, ES = 0.68, \( P = 0.05 \)). The TAUC-TAG was lower in HIIR than CON between 0 to 1 h by 11% (-16 to -6%, ES = 0.40, \( P < 0.001 \)), 1 to 4.5 h by 11% (-17 to -5%, ES = 0.36, \( P = 0.001 \)) and 4.5 to 6.5 h by 9% (-15 to -3%, ES = 0.33, \( P = 0.01 \)). No differences were seen in iAUC-TAG across conditions or groups (main effect condition \( P = 0.81 \); main effect group \( P = 0.43 \); condition by group interaction \( P = 0.17 \)) (Table 8.4).
### Table 8.4
Fasting and postprandial plasma triacylglycerol and glucose concentrations in the control (CON) and high-intensity interval running (HIIR) conditions in boys and girls.

<table>
<thead>
<tr>
<th></th>
<th>Boys ($n = 15$)</th>
<th>Girls ($n = 16$)</th>
<th>Girls vs. Boys</th>
<th>CON vs. HIIR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>HIIR</td>
<td>CON</td>
<td>HIIR</td>
</tr>
<tr>
<td><strong>Triacylglycerol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (mmol·L$^{-1}$)</td>
<td>0.53 (0.50-0.66)</td>
<td>0.51 (0.45-0.55)</td>
<td>0.87 (0.69-1.12)</td>
<td>0.75 (0.56-0.95)</td>
</tr>
<tr>
<td>TAUC (mmol·L$^{-1}$ 6.5 h)</td>
<td>6.25 (4.87-8.35)</td>
<td>5.92 (4.54-6.90)</td>
<td>7.64 (6.62-11.10)</td>
<td>7.78 (5.99-9.43)</td>
</tr>
<tr>
<td>iAUC (mmol·L$^{-1}$ 6.5 h)</td>
<td>2.85 (2.22-4.04)</td>
<td>2.78 (1.76-3.32)</td>
<td>3.24 (2.57-4.17)</td>
<td>3.14 (2.55-4.15)</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (mmol·L$^{-1}$)</td>
<td>5.44 (5.26-5.79)</td>
<td>5.37 (5.07-5.46)</td>
<td>5.74 (5.41-6.04)</td>
<td>5.72 (5.37-6.07)</td>
</tr>
<tr>
<td>TAUC (mmol·L$^{-1}$ 6.5 h)</td>
<td>43.1 (40.8-43.8)</td>
<td>42.3 (41.2-44.3)</td>
<td>42.4 (40.4-44.3)</td>
<td>44.4 (40.7-46.6)</td>
</tr>
<tr>
<td>iAUC (mmol·L$^{-1}$ 6.5 h)</td>
<td>9.18 (7.60-12.35)</td>
<td>10.55 (8.56-12.45)</td>
<td>7.54 (6.17-9.09)</td>
<td>10.07 (9.15-11.15)</td>
</tr>
</tbody>
</table>

Values are median (interquartile range) for $n = 15$ boys and $n = 16$ girls. Statistical analyses are based on natural log transformed data. *95% confidence interval for the ratio of geometric means.

TAUC, total area under the concentration versus time curve; iAUC, incremental area under the concentration versus time curve.

$^a$ Significant difference between boys and girls ($P < 0.05$)

$^b$ Significant difference between HIIR and CON ($P < 0.05$)
Figure 8.2  Fasting (F) and postprandial plasma triacylglycerol concentrations ([TAG]) in the control (CON) and high-intensity interval running (HIIR) conditions for \( n = 16 \) girls and \( n = 15 \) boys. Values are mean (SD). Black rectangles denote consumption of breakfast and lunch meals at 08:00 and 12:00, respectively. Main effect condition \( P < 0.001 \); main effect group \( P = 0.01 \); condition by group interaction \( P = 0.83 \).

Individual changes (delta) in TAUC-TAG between HIIR and CON for boys and girls are shown in Figure 8.3. The reductions in TAUC-TAG following HIIR were greater than changes in CON for ten (67%) boys and ten (63%) girls. Furthermore, 7 out of the top 11 participants that experienced a HIIR-induced reduction in TAUC-TAG were girls. End interval percent peak heart rate demonstrated a meaningful relationship with delta TAUC-TAG \( (r = -0.44, P = 0.01) \). In addition, the intervention-induced change in fasting plasma [TAG] was positively correlated with the change in TAUC-TAG relative to CON \( (r = 0.50, P = 0.005) \).
Figure 8.3  Individual changes (delta) in the total area under the plasma triacylglycerol (TAG) concentration versus time curve (TAUC) in the high-intensity interval running (HIIR) condition compared with the control condition (CON) in boys (□) and girls (■). A negative response indicates a reduction in TAUC-TAG in HIIR compared with CON.
Two-way ANOVA revealed no differences in postprandial plasma \([\text{glucose}]\) across conditions or groups (main effect condition \(P = 0.35\); main effect group \(P = 0.31\)), but a tendency for a condition by group interaction emerged \((P = 0.05)\). The TAUC-glucose was similar across conditions and groups (main effect condition \(P = 0.16\); main effect group \(P = 0.42\)), but a trend for a different magnitude of change following HIIR was seen between boys and girls (0% vs. 3% respectively; condition by group interaction \(P = 0.07\)) (Table 8.4). No differences were observed in iAUC-glucose across conditions or groups (main effect condition \(P = 0.90\); main effect group \(P = 0.71\); condition by group interaction \(P = 0.23\)) (Table 8.4).

8.5 Discussion

The primary finding in the present study was that fasting and postprandial plasma \([\text{TAG}]\) were lower in 11 to 12 year old boys compared with similarly aged girls, but the magnitude of reduction following a single session of HIIR was similar (fasting \([\text{TAG}]\): 8% vs. 15% and postprandial \([\text{TAG}]\): 11% vs. 10%, respectively). To the author’s knowledge, this is the first study to directly compare the differences in fasting and postprandial plasma \([\text{TAG}]\) following acute exercise in boys and girls.

The HIIR-induced reduction in fasting plasma \([\text{TAG}]\) supports the majority of previous exercise postprandial studies in young people (Barrett et al., 2007; Lee et al., 2013; Tolfrey et al., 2008, 2012, 2014a), but the considerably lower fasting plasma \([\text{TAG}]\) in boys compared with girls appears a novel finding in young people. The greater fasting plasma \([\text{TAG}]\) in girls compared with boys is likely to contribute to the heightened postprandial TAG response (Couch et al., 2000). However, substantial intra-individual variation is evident in childhood fasting \([\text{TAG}]\) (Tolfrey et al., 1999), and impaired clearance of postprandial \([\text{TAG}]\) is associated independently with an increased risk of CVD (Bansal et al., 2007; Nordestgaard et al., 2007) suggesting that postprandial \([\text{TAG}]\) may be more informative of metabolic health in young people.

The lower postprandial lipaemic response in boys compared with girls contrasts previous findings in adults, which have demonstrated consistently that premenopausal women experience lower postprandial \([\text{TAG}]\) than men (Couillard et al., 1999; Jackson et al., 2010; Kolovou et al., 2006; Koutsari et al., 2004). The temporal time-zone analysis revealed that the boys experienced a lower postprandial TAG response throughout the entire 6.5 h
postprandial period compared with girls. The mechanistic pathways underlying the lower postprandial TAG in boys cannot be determined directly from the present study, but it seems reasonable to speculate that differences in biological maturation, body composition, cardiorespiratory fitness and/or dietary intake may be implicated. Insulin resistance is an independent predictor of the postprandial TAG response in healthy adults (Boquist et al., 2000; Jeppesen et al., 1995). A transient state of insulin resistance occurs during puberty in boys and girls, with the greatest insulin resistance experienced at pubertal stage 3, and girls appearing more insulin resistant than boys at all stages of sexual maturation (Moran et al., 1999). While an estimate of insulin resistance is not available in the present study, it is possible that greater insulin resistance in girls may be implicated in the exaggerated postprandial plasma TAG response compared with similarly aged boys. Although the median rating of pubic hair development was not different between boys and girls, a range of self-assessed biological maturity ratings were identified (pubic hair development stage 1: n = 2/4; stage 2: n = 11/6; stage 3: n = 2/1; stage 4: n = 0/3; stage 5: n = 0/2 for boys/girls respectively). As discussed in Chapter 3.2, a limitation of secondary sex characteristics is that discrete stages are used to characterise a continuous biological process and, therefore, this measure may not be accurate enough to detect small differences in biological maturation in the present study. Girls enter and end puberty approximately two years before boys and pubertal events do not occur in the same sequence between the sexes (Baxter-Jones et al., 2005). Consequently, it is difficult to align girls and boys for pubertal status when making between-sex comparisons (Sherar et al., 2004). Despite the limitations associated with self-report data and stages of sexual maturation (Chapter 3.2; Baxter-Jones et al., 2005), validity studies suggest self-assessment of secondary sex characteristics provides an accurate and reliable surrogate measure of biological maturation (Matsudo and Matsudo, 1994; Schlossberger et al., 1992). Nevertheless, a previous study demonstrated a significant association between sexual maturation and fasting [TAG] independent of age in 10 to 13 year old boys and girls (Bertrais et al., 2000); therefore, it seems plausible that biological maturation may influence postprandial lipaemia in boys and girls.

Percent body fat was significantly lower in boys compared with girls and has been identified as a strong determinant of insulin resistance in children previously (Arslanian and Suprasongsin, 1996). Furthermore, measures of adiposity are associated with CVD risk factors including fasting [TAG] in young people (Lamb et al., 2011; Owens et al., 1998; Steinberger et al., 2005), although the reported sex differences in body fat distribution in
young people may be more discriminatory in explaining the sex dimorphism in postprandial [TAG] seen in the present study (Huang et al., 2001; Owens et al., 1998). In this regard, a recent postprandial exercise study demonstrated that visceral adipose tissue was the strongest predictor of the postprandial TAG response in overweight white adolescents; however, the limited sample size precluded a direct comparison between boys and girls (Lee et al., 2013). Alternatively, it is possible that the greater peak VO\(_2\) in boys (Table 8.1) confers a protective effect on postprandial lipaemia. The implications of this finding are unclear and may be confined to early adolescence considering cardiorespiratory fitness was not a predictor of the postprandial lipaemic response in adults (Gill et al., 2002b). However, it is worth noting that the study by Gill et al. (2002b) represents a retrospective analysis of 38 men and 43 women taking part in seven independent exercise postprandial studies, and it is possible that the different protocols adopted to determine maximum VO\(_2\) in the men and women could influence this relationship (treadmill test with gas analysis \((n = 62)\), cycling test with gas analysis \((n = 8)\), extrapolated from sub-maximal tests \((n = 11)\)). Finally, although dietary intake was substantially different on the intervention day between boys and girls (Table 8.2), energy and macronutrient intakes were all greater in boys than girls and the relative proportion of carbohydrate, a potent stimulator of plasma [TAG], was lower in boys; therefore, this component is unlikely to be an influencing factor based on the available evidence. While a number of potential explanations have been highlighted, further research is required to clearly elucidate the precise mechanisms accounting for the different postprandial TAG profiles in boys and girls.

The reduction in postprandial plasma [TAG] following HIIR supports several studies in adults adopting acute intermittent, high-intensity exercise interventions (Ferreira et al., 2011; Freese et al., 2011; Gabriel et al., 2012, 2013; Tan et al., 2014; Trombold et al., 2013); however, this finding is not universal (Allen et al., 2014; Tan et al., 2013). In addition, this is the first study to demonstrate that the HIIR-induced reduction in postprandial plasma [TAG] was similar in boys and girls (11% vs. 10% respectively; Table 8.4, Figure 8.2), suggesting that 11 to 13 year old boys and girls may acquire similar metabolic health benefits in postprandial TAG metabolism following a single bout of HIIR. Similarly, in the retrospective analysis by Gill et al. (2002b), the moderate-intensity exercise-induced reduction in postprandial [TAG] was similar in men and women (23.5% vs. 19.8% respectively). Other studies have likewise demonstrated that the magnitude of reduction in postprandial [TAG] was similar in men and women following a single session of moderate-intensity exercise.
(Hardman and Aldred, 1995; Tsetsonis and Hardman, 1996) and high-intensity exercise (Freese et al., 2011). The mechanisms responsible for the HIIR-induced reduction in postprandial plasma [TAG] were not measured directly in the present study due to the invasive nature of the techniques required. In adults, increased clearance of circulating TAG facilitated by enhanced lipoprotein lipase (LPL) activity (Gill et al., 2003b; Kiens and Richter, 1998) and/or the secretion of fewer, TAG-richer very low-density lipoproteins (VLDL) that have a higher affinity for LPL (Magkos et al., 2006) have been implicated. Indeed, the meaningful relationship seen between the HIIR-induced change in fasting plasma [TAG] and TAUC-TAG ($r = 0.50, P = 0.005$), and the small differences in iAUC-TAG between the conditions may point to changes in endogenous and not exogenous TAG pathways (Davitt et al., 2013).

Although the clinical significance of our findings cannot be elucidated, the majority of the postprandial TAG samples (97%) were below the 2.3 mmol·L$^{-1}$ threshold proposed as a desirable concentration in children and adolescents (Kolovou et al., 2011a). Postprandial [TAG] is established as an independent risk factor for future CVD (Bansal et al., 2007; Nordestgaard et al., 2007), and CVD risk factors including physical inactivity track into adulthood (Eisenmann et al., 2004; Telama et al., 2005). The majority of young people fail to meet the current physical activity guidelines of 60 min of moderate- to vigorous-intensity daily physical activity (Hallal et al., 2012; Health Survey for England, 2012b; Verloigne et al., 2012); therefore, the potential for a single session of HIIR to reduce an important marker of future atherogenic risk in boys and girls is encouraging. The inter-individual variability in postprandial plasma [TAG] following HIIR (Figure 8.3) is similar to the heterogeneous response reported in previous moderate-intensity exercise postprandial studies in young people (Chapters 5 and 6; Tolfrey et al., 2012, 2014a) and adults (Gill et al., 2002b, 2007). A similar proportion of boys and girls experienced a HIIR-induced reduction in postprandial plasma [TAG] (~65%; Figure 8.3). Furthermore, a greater proportion of the top 11 participants demonstrating a reduction in postprandial lipaemia were girls (64%; Figure 8.3), but the reason for this finding is unclear. Average end interval percent peak heart rate demonstrated a significant correlation with delta TAUC-TAG suggesting that exercising at a higher relative intensity augments the reduction in postprandial plasma [TAG]. Consequently, small increases in effort could afford greater potential gains in long-term health providing the exercise stimulus can be applied regularly without risking injury or burn-out in the young participants.
The present study adopted a retrospective study design whereby data was pooled from two independent studies in boys (Chapter 4) and girls (Chapter 7) separated by two years. However, the data from the two studies are comparable considering the studies were conducted in the same laboratory setting and adopted identical standardisation of dietary intake, exercise protocols and postprandial measurements. The postprandial meals were similar for all participants, with only a slight variation in the energy and macronutrient composition (< 7 kJ energy and < 0.2 g fat, carbohydrate and protein per kilogram body mass). A limitation of this study is that the retrospective study design precluding any matching of possible physical or physiological determinants or correlates of TAG and glucose metabolism between the two groups. Furthermore, the absence of objective quantification of free-living physical activity and sedentary time in boys (Chapter 4) represents a second possible limitation. The boys and girls were asked to subjectively minimise and record their physical activities 48 h before the postprandial day and then replicate this during the same period before the second condition. Although this procedure was verified verbally and by comparing the diaries, the absence of an objective measure to quantify free-living physical activity and sedentary time in boys precludes a direct comparison of differences that may exist between conditions and/or groups which may influence the postprandial measures. However, despite these limitations, the analysis has provided a meaningful and important insight into postprandial metabolism in young people which is not available currently in the literature.

In conclusion, fasting and postprandial plasma [TAG] were lower in healthy 11 to 12 year old boys compared with similarly aged girls, but the magnitude of reduction following acute HIIR was similar between the sexes (fasting [TAG]: 8% vs. 15% and postprandial [TAG]: 11% vs. 10%, respectively). Further work is required to systematically investigate the mechanisms responsible for the higher TAG profile in girls compared with boys. Nevertheless, boys and girls can experience similar metabolic health benefits in fasting and postprandial TAG metabolism following acute HIIR regardless of the initial concentration of TAG.
CHAPTER 9
General discussion

9.1 Introduction

The associated morbidity and mortality rates from cardiovascular disease (CVD) represent a continuing public health burden worldwide and prevention by targeting modifiable risk factors is high on the public health agenda. Elevated postprandial triacylglycerol concentrations ([TAG]) are implicated in the development and progression of atherosclerosis, a disease process that has its origins in childhood and is central to the pathology of CVD. Considering most waking hours are postprandial, a combination of low physical activity and an atherogenic diet may exacerbate future CVD risk with prolonged elevated [TAG]. Therefore, preventative lifestyle interventions that delay precursors of atherosclerotic disease should be paediatric centred. However, research investigating the effect of manipulations in exercise energy expenditure (EE) and dietary energy intake on postprandial [TAG] in young people is relatively sparse. Therefore, the primary focus of this thesis was to extend our understanding by examining postprandial TAG metabolism following novel acute exercise protocols and dietary energy intake manipulations in 11 to 13 year old boys and girls. The aim of this chapter is to consolidate and discuss the collective findings that have emerged from these studies. Table 9.1 provides a summary of the study protocols and variables measured during each experimental chapter.
Table 9.1  Summary of the studies presented in the experimental chapters of this thesis.

<table>
<thead>
<tr>
<th>Chapter</th>
<th>n</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Conditions</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>15</td>
<td>M</td>
<td>11.8</td>
<td>1. CON: rest</td>
<td>TAG, glucose, insulin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2. HIIR: 10 × 1 min TM @ 100% MAS</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>M</td>
<td>12.3</td>
<td>1. CON: rest</td>
<td>TAG, glucose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2. EX-DEF: 56 min TM @ 60% peak (\dot{V}O_2)</td>
<td>Resting energy expenditure and</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3. EX-REP: 56 min TM @ 60% peak (\dot{V}O_2) with energy replacement</td>
<td>substrate oxidation</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>F</td>
<td>12.1</td>
<td>1. CON: rest</td>
<td>TAG, glucose, insulin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2. EX: 73 min TM @ 60% peak (\dot{V}O_2) (energy deficit 1.55 MJ)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3. ER: Energy intake reduced by 1.51 MJ</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>F</td>
<td>12.1</td>
<td>1. CON: rest</td>
<td>TAG, NEFA, glucose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2. HIIR: 10 × 1 min TM @ 100% MAS</td>
<td>Resting energy expenditure and</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>3. HIIR-ER: 5 × 1 min TM @ 100% MAS, energy intake reduced by 0.82 MJ</td>
<td>substrate oxidation</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>M</td>
<td>11.8</td>
<td>1. CON: rest</td>
<td>TAG, glucose</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>F</td>
<td>12.1</td>
<td>2. HIIR: 10 × 1 min TM @ 100% MAS</td>
<td></td>
</tr>
</tbody>
</table>

M, male; CON, rest control; HIIR, high-intensity interval running; TM, treadmill; MAS, maximal aerobic speed; TAG, triacylglycerol; EX-DEF, exercise with energy deficit; \(\dot{V}O_2\), oxygen uptake; EX-REP, exercise with energy replacement; F, female; EX, moderate-intensity exercise; ER, energy-intake restriction; HIIR-ER, high-intensity interval running and energy-intake restriction; NEFA, non-esterified fatty acids.
9.2 Exercise protocols and postprandial metabolism

One aim of the studies presented in this thesis was to examine the efficacy of traditional, moderate-intensity exercise and novel, high-intensity exercise protocols on postprandial plasma [TAG] and resting whole-body fat oxidation in boys and girls. Accumulating evidence in boys and girls demonstrates the postprandial TAG-lowering effect of acute moderate- to vigorous-intensity exercise interventions (Table 2.2; Tolfrey et al., 2014b), and the work presented in Chapters 5 and 6 extends this growing evidence base in young people. The potential for acute moderate-intensity exercise performed in accordance with current physical activity guidelines to reduce postprandial plasma [TAG] advocates the promotion of exercise to improve metabolic health early in life. Chapters 4 and 7 represent the first experimental insight into the postprandial responses to intermittent, high-intensity interval running (HIIR) in young people. The results revealed that 10 × 1 min running intervals performed at 100% maximal aerobic speed (MAS) with 1 min recovery between intervals reduced postprandial plasma [TAG] in boys and girls, supporting the majority of studies in adults (Ferreira et al., 2011; Freese et al., 2011; Gabriel et al., 2012, 2013; Tan et al., 2014; Trombold et al., 2013). Importantly, the exercise session was well tolerated by all participants. Therefore, this pattern of exercise may be better suited to the stop-start nature of young people’s activity habits and represent an attractive and efficacious alternative to traditional moderate-intensity exercise that enables young people to acquire metabolic health benefits.

Chapter 8 presented a retrospective comparison of the postprandial lipaemic response to HIIR in boys (Chapter 4) and girls (Chapter 7) and, although the postprandial TAG response was significantly lower in boys than girls, the HIIR-induced reduction was similar (11% vs. 10% respectively). The sexual dimorphism in postprandial [TAG] contrasts the commonly reported exaggerated response in men compared with women (Couillard et al., 1999; Jackson et al., 2010; Kolovou et al., 2006; Koutsari et al., 2004). The lower postprandial plasma [TAG] in boys may reflect differences in biological maturation and the associated transient state of insulin resistance that occurs during puberty with girls appearing more insulin resistant than boys throughout this period (Moran et al., 1999). Furthermore, differences in body composition and cardiorespiratory fitness, possibly mediated by biological maturation, in addition to habitual dietary intake and free-living physical activity and sedentary time are also plausible mechanistic pathways. These are the first postulations attempting to explain the sexual dimorphism in postprandial plasma [TAG] between boys and girls and, therefore,
future research is required to examine this systematically. Nevertheless, the potential for boys and girls to experience similar HIIR-induced reductions in postprandial plasma [TAG] is promising, and the high levels of perceived enjoyment (Chapter 7) combined with a lower investment of time may help to promote greater long-term adherence to exercise.

In agreement with postprandial exercise studies in adults (Burton et al., 2008; Davitt et al., 2013; Trombold et al., 2013, 2014), elevated resting whole-body fat oxidation was observed in the postprandial period following acute moderate-intensity exercise with energy deficit in boys (Chapter 5) and HIIR in girls (Chapter 7). However, the apparent dissociation between whole-body fat oxidation and indices of lipaemia in these studies was somewhat surprising considering TAG is a likely lipid source for oxidation and these parameters have been linked mechanistically (Chapter 2.6.2). This finding indicates that exercise-induced changes in postprandial plasma [TAG] and whole-body fat oxidation occur independently in boys and girls. Nevertheless, it is encouraging that a single session of exercise induces a state of negative fat balance the following day, and further supports the acquisition of metabolic health benefits following acute exercise in young people.

9.3 Energy deficit and postprandial metabolism

The mitigating effect of acute exercise on postprandial [TAG] may be attributable to the associated energy deficit or skeletal muscle contraction. This concept has received considerably less attention even in the adult literature, and the findings presented in Chapters 5, 6 and 7 represent the first time this important avenue of research has been explored in relation to postprandial lipaemia in young people. Chapter 5 demonstrated that immediate replacement of the exercise EE negates the exercise-evoked reduction in postprandial plasma [TAG] in boys, supporting previous evidence in adults (Burton et al., 2008; Freese et al., 2011; Harrison et al., 2009; Trombold et al., 2014). Furthermore, an exercise-induced energy deficit was required to promote a meaningful increase in resting whole-body fat oxidation. This supports the contention that the body relies upon lipids as a fuel source to facilitate the resynthesis of depleted skeletal muscle and/or hepatic glycogen stores, whereas the provision of exogenous carbohydrate in the post-exercise meal presumably contributes to the accelerated restoration of glycogen homeostasis (Casey et al., 2000; Wallis et al., 2008). These findings have important practical implications for those wanting to experience the full benefit of exercise, and emphasises the importance of maintaining a negative energy balance.
to maximise the acute physiological effect of exercise on postprandial lipaemia and whole-body fat oxidation.

The importance of the energy deficit was investigated further in Chapters 6 and 7 through comparisons of exercise and carefully controlled reductions in habitual energy intake. Chapter 6 demonstrated a trend for a larger reduction in postprandial plasma [TAG] following an exercise-induced energy deficit compared with an isoenergetic diet-induced energy deficit in girls, supporting the studies conducted in pre- and post-menopausal women (Gill and Hardman, 2000; Maraki et al., 2010). This suggests the physiological origin of the energy deficit may mediate the magnitude of change in postprandial plasma [TAG]. In Chapter 7, the reduction in postprandial plasma [TAG] and increase in whole-body fat oxidation observed for HIIR was matched when a lower volume of high-intensity exercise was superimposed with mild, energy-intake restriction to augment the energy deficit. Consequently, combining exercise and energy-intake restriction may represent an attractive model to acquire metabolic health benefits in young people by reducing the total exercise commitment and promoting enjoyment. This is particularly encouraging considering time and enjoyment are the most frequently cited barriers to exercise participation in adolescents (Butt et al., 2011; Kimm et al., 2006).

9.4 Heterogeneity in postprandial lipaemia

A common theme highlighted throughout this thesis was the substantial inter-individual variability evident in the postprandial TAG response following the various exercise and diet interventions (Chapters 4 to 8). The degree of heterogeneity in postprandial lipaemia has been reported in a small number of exercise postprandial studies in adults (Allen et al., 2014; Arjunan et al., 2013; Gill et al., 2002b, 2007; Tan et al., 2013) and young people (Tolfrey et al., 2012, 2014a); however, the vast majority of studies focus on main effects and group differences and do not present individual responses. Potential factors responsible for the heterogeneity in postprandial lipaemia in this thesis are explored in each experimental chapter (Chapters 4 to 8). The most consistent finding to emerge was the positive correlation observed between the intervention-induced change in fasting plasma [TAG] and change in the total area under the [TAG] versus time curve (TAUC) relative to the control condition (Chapters 5 to 8), although this relationship was not seen in Chapter 4. Nevertheless, this association is perhaps not surprising considering elevated fasting [TAG] has been associated with postprandial [TAG] in children previously (Couch et al., 2000).
A recent analysis of pooled exercise intervention studies in men and women (n = 1,687) reported that 10.4% of participants experienced an adverse response in fasting [TAG] following exercise training (Bouchard et al., 2012). This study supports the presence of ‘responders’ and ‘non-responders’ to acute lifestyle interventions highlighted throughout this thesis. Consequently, universal exercise and diet prescription may be inappropriate (Bouchard et al., 2012; Buford et al., 2013), and efforts should be intensified to identify the intervention-induced predictors of postprandial lipaemia in young people so that individuals at risk of an adverse response can be identified and offered alternative approaches that optimise metabolic health outcomes.

9.5 Practical implications

The studies in this thesis have advanced the evidence base in young people regarding the efficacy of novel exercise and dietary manipulations on postprandial metabolism. The research findings presented may have practical implications in terms of guiding future experimental research, promoting healthy lifestyle behaviours in young people and informing public health policy. The suggested recommendations discussed in this section are based on the research findings in healthy, recreationally active boys and girls; therefore, further work is required to determine the applicability of the recommendations to more diverse paediatric populations (e.g., overweight/obese, insulin resistant, diabetic).

Promoting a physically active lifestyle from a young age may stimulate transient benefits in postprandial lipid metabolism and have long-term implications regarding CVD risk. However, there is no consensus currently on the optimum exercise mode, intensity or duration of exercise required to maximise these benefits. In line with the current international physical activity guidelines in children and adolescents (Department of Health, Physical Activity, Health Improvement and Protection, 2011; Janssen and LeBlanc, 2010), approximately 60 min of brisk walking or slow running (6.0 to 9.1 km·h⁻¹) appears a sufficient stimulus to reduce postprandial plasma [TAG] and increase resting whole-body fat oxidation (Chapters 5 and 6). These responses manifest when exercise is prescribed at a relative intensity of 60% peak \( \text{VO}_2 \), an average heart rate of 79% of the age-predicted maximum and an average rating of perceived exertion (RPE) of 11 (‘fairly light’ on the scale). However, many children and adolescents, especially girls, fail to meet the current physical activity guidelines (Hallal et al., 2012; Health Survey for England, 2012b; Verloigne et al., 2012), and evidence suggests that engagement in physical activity declines with age (Health Survey for England, 2012b).
Consequently, as a novel alternative, a reduction in postprandial plasma [TAG] and elevation in resting whole-body fat oxidation can be achieved by exercising at a high intensity involving 10 min of running performed in 1 min intervals with 1 min active recovery between intervals (27 min in total including warm-up, recovery and cool-down) (Chapters 4, 7 and 8). The 1 min running intervals are performed at 100% MAS (i.e., 100% peak \( \dot{V}O_2 \)), an average heart rate of 96% of the age-predicted maximum and an average RPE rating of ‘hard’ (corresponding to 15 on the scale). The protocol was well tolerated by the boys and girls in this thesis, and in adult sedentary and patient populations previously (Hood et al., 2011; Little et al., 2011), and may be better suited to the typical activity patterns of young people (Hoos et al., 2004). Furthermore, the high levels of perceived enjoyment following HIIR (Chapter 7) could have important implications for exercise adherence. Performing a combination of moderate- and high-intensity exercise regimes on a regular basis may be more attractive to young people by fostering exercise variety whilst maintaining the health benefits in postprandial lipid metabolism. From a practical perspective, these two exercise protocols could be incorporated into the daily school routine (e.g., active commuting to school, playground activities, physical education lessons, extra-curricular activities), free-play activities and organised sports clubs.

A physically active lifestyle is associated with a multitude of cardiovascular health benefits in young people (Daniels et al., 2011; Janssen and LeBlanc, 2010), and the work presented in this thesis highlights the benefits of acute exercise in relation to postprandial lipid metabolism. However, judicious use of post-exercise energy intake is recommended to maximise the positive health gains of exercise (Chapter 5). Specifically, compensatory increases in energy intake during the post-exercise period are not advised in young people to ensure the exercise-induced reduction in postprandial plasma [TAG] and elevation in whole-body fat oxidation is maximised. Evidence of compensatory increases in appetite and energy intake following acute exercise is not well supported in adults (Caudwell et al., 2013) or young people (Thivel et al., 2013). However, partial compensation may occur in response to chronic exercise (Caudwell et al., 2013), and the large individual variability observed in physiological responses highlights the importance of considering exercise EE and energy intake in combination (Caudwell et al., 2009; Thivel et al., 2014).

The degree of energy-intake restriction imposed in Chapter 6 (1.5 MJ) was less potent than an isoenergetic bout of exercise in reducing postprandial plasma [TAG]. Although reductions in dietary energy intake may be an attractive alternative in young people who find it difficult to
accumulate sufficient physical activity, this level of restriction may be too challenging to implement safely and effectively in practice. Exercise prescription in combination with a smaller, carefully managed reduction in energy intake may be better advocated to improve postprandial lipid metabolism in young people, and supports a recent review emphasising the importance of lifestyle modification through exercise and diet for health (Frieden et al., 2010). A combination of exercise (e.g., HIIR) and energy-intake restriction may represent a more practical and appealing model to young people, and contributes to providing an array of lifestyle options that may reduce postprandial plasma [TAG] and increase resting whole-body fat oxidation. Mild energy-intake restriction (e.g., 816 kJ; Chapter 7) could be achieved through a reduction in portion size (e.g., reduction of ~60 g jacket potato (348 kJ), ~3 g butter (92 kJ), ~44 g baked beans (152 kJ), ~13 g cheese (224 kJ)) or the omission of snack type foods (e.g., different combinations of 1 scoop ice cream (50 ml; 130 kJ), 1 cookie (11 g; 220 kJ), 1 packet jelly sweets (20 g; 292 kJ), 1 muffin (545 kJ), 1 packet of crisps (25 g; 549 kJ), 1 slice of sponge cake (830 kJ)). It is essential that any restriction in energy intake is carefully monitored and informed by exercise and diet practitioners to ensure that daily energy intake remains sufficient to meet the growing, developing and maturing body’s energy demands.

9.6 Limitations and future directions

There are some notable limitations of the studies presented in this thesis which have been identified and discussed within each experimental chapter. A limitation common to all studies is the recruitment of a fairly homogenous cohort of participants in terms of their physical and physiological characteristics (i.e., healthy, recreationally active), which limits the ability to extrapolate the findings to wider populations. Thus, future studies are required to characterise the postprandial lipaemic and whole-body fat oxidation responses to exercise and diet manipulations in individuals already demonstrating CVD risk factors such as fasting hypertriglyceridaemia, obesity, insulin resistance and Type 2 diabetes mellitus. Furthermore, building on the findings presented in Chapter 8, the influence of sex and maturation in modifying postprandial lipaemia following exercise (and diet) interventions warrants additional attention with a large cohort of boys and girls spanning the full spectrum of maturity ratings.

There is also scope to further investigate the heterogeneity observed in the postprandial TAG responses, perhaps with a larger sample size and by increasing the number of measured outcome variables, to identify potential factors that discriminate ‘responders’ and ‘non-
responders’. This would enable the identification of individuals at risk of an adverse postprandial lipaemic response to an exercise or diet intervention and provide a framework for tailored exercise and diet prescription (Bouchard et al., 2012; Buford et al., 2013). In addition, the experimental studies in this thesis quantified the postprandial responses to a single exercise or diet intervention; therefore, it is not known how the findings observed in postprandial plasma [TAG] and whole-body fat oxidation in response to an acute intervention translate over the long-term. This research is vital to determine the chronic benefits of regular exercise and diet manipulations in relation to these important markers of CVD risk.

The studies within this thesis were conducted in a controlled and standardised laboratory setting, which is not representative of a young person’s typical daily routine. Given that young people spend a large proportion of their time in a school environment, translating the established laboratory-based exercise postprandial lipaemia findings into a working school environment warrants future attention and would determine the applicability of the laboratory-based findings in the ‘real-world’. It would be possible to capitalise on existing school-based physical activities and extra-curricular sports to induce an exercise EE, and to utilise more representative meals (e.g., school meals, pre-prepared packed meals) rather than the traditionally high-fat meals adopted in research to date.

Finally, studies providing a mechanistic insight would also be timely, although it is appreciated that the invasive nature of many techniques precludes their application in studies with young people. Nevertheless, employing indirect and relatively non-invasive techniques may help to develop our understanding of the mechanisms responsible for the exercise-, and to a lesser extent diet-induced reductions in postprandial lipaemia in young people (e.g., ultrasound for blood flow, 3-hydroxybutyrate (3-OHB) for hepatic fatty acid oxidation, isotope-ratio mass spectrometry for gas analysis).

9.7 Conclusion

The work contained in this thesis highlights the efficacy of novel acute exercise and diet interventions to reduce postprandial plasma [TAG] and elevate whole-body fat oxidation in healthy, 11 to 13 year old boys and girls. This work has contributed to the existing evidence base in young people, and demonstrates the potential for lifestyle interventions to promote a healthier cardiovascular risk profile in early adolescence at a time when many adverse health behaviours become established. It is hoped that the work presented in this thesis will be used
to direct and develop future research in this important avenue of paediatric exercise and health physiology, especially considering the importance of promoting the long-term health and well-being of young people.
CHAPTER 10

References


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Chapter 10: References


Health Screen Questionnaire for Study Volunteers

As a volunteer participating in a research study, it is important that your child is currently in good health and has had no significant medical problems in the past. This is (i) to ensure your child’s continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

If your child has a blood-borne virus, or think that they may have one, please do not take part in this research.

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 your general practitioner ............... Yes [ ] No [ ]
   (c) on a hospital waiting list .......................... Yes [ ] No [ ]

2. **In the past two years**, has your child had any illness which required them to:
   (a) consult your 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) Problems with bones or joints .................... Yes [ ] No [ ]
   (j) Disturbance of balance / co-ordination .......... Yes [ ] No [ ]
   (k) Numbness in hands or feet ....................... Yes [ ] No [ ]
   (l) Disturbance of vision .............................. Yes [ ] No [ ]
   (m) Ear / hearing problems ........................... Yes [ ] No [ ]
   (n) Thyroid problems .................................. Yes [ ] No [ ]
   (o) Kidney or liver problems ......................... Yes [ ] No [ ]
   (p) Allergy to nuts ..................................... Yes [ ] No [ ]
4. **Has any**, otherwise healthy, member of your family under the age of 35 died suddenly during or soon after exercise? Yes ☐ No ☐

If YES to any question above, please describe briefly (e.g., to confirm problem was/is short-lived, insignificant or well controlled.)

............................................................................................................................
............................................................................................................................
............................................................................................................................
............................................................................................................................

5. **Allergy information**
   (a) is your child allergic to any food products? Yes ☐ No ☐
   (b) is your child allergic to any medicines? Yes ☐ No ☐
   (c) is your child allergic to plasters? Yes ☐ No ☐

If YES to any of the above, please provide additional information on the allergy

............................................................................................................................
............................................................................................................................
............................................................................................................................
............................................................................................................................

6. **Additional questions for female participants**
   (a) are your daughter’s periods normal/regular? Yes ☐ No ☐
   (b) is your daughter on “the pill”? Yes ☐ No ☐
   (c) could your daughter be pregnant? Yes ☐ No ☐
   (d) is your daughter taking hormone replacement therapy (HRT)? Yes ☐ No ☐

7. Please provide contact details of a suitable person for us to contact in the event of any incident or emergency.

   Name: ......................................................................................................................

   Telephone Number: ...............................................................................................  
   Work ☐ Home ☐ Mobile ☐

   Relationship to participant: ....................................................................................

8. Is your child currently involved in any other research studies at the University or elsewhere? Yes ☐ No ☐

   If yes, please provide details of the study ..................................................................
   ............................................................................................................................
   ............................................................................................................................
   .............................................................................................................................
STUDY TITLE

INFORMED CONSENT FORM

(to be completed after Parent Information Sheet has been read)

The purpose and details of this study have been explained to me. I understand that this study is designed to further scientific knowledge and that all procedures have been approved by the Loughborough University Ethical Approvals (Human Participants) Sub-Committee.

I have read and understood the information sheet and this consent form.

I have had an opportunity to ask questions about my child’s participation.

I understand that my child is under no obligation to take part in the study.

I understand that my child has the right to withdraw from this study at any stage for any reason, and that I will not be required to explain their reasons for withdrawing.

I understand that all the information my child provides will be treated in strict confidence and will be kept anonymous and confidential to the researchers unless (under the statutory obligations of the agencies which the researchers are working with), it is judged that confidentiality will have to be breached for the safety of my child or others.

I agree for my child to participate in this study.

Your name

Your signature

Signature of investigator

Date
Young Person’s Willingness to Participate Form

Study Title

Please read the statements below and indicate whether you are willing to participate in this study

Willingness to Participate Statement:

- I have read the information about the tests and measurements. All of the tests and measurements have also been explained to me. I have had the opportunity to ask questions and I understand what I am being asked to do if I volunteer to take part in this study. I know that I can say that I do not wish to continue with the tests or measurements at any time and I do not have to give a reason.

- I agree to take part in the tests and measurements (please tick the box):

Full name: ______________________________ (print)
Signed: ______________________________
Date: ______________________________
Witnessed by: ______________________________
1. GENITAL DEVELOPMENT – TESTES, SCROTUM AND PENIS (MATURITY RATINGS)

The pictures on this page show the different stages of development of the testes, scrotum and penis. A boy passes through each of the five stages shown by these pictures. Please look at each of the pictures and read the sentences alongside the pictures. Then choose the picture closest to your stage of development and circle the corresponding number (1-5) on the enclosed confidential form. In choosing the right picture, look only at the stage of development, not at pubic hair.

**Stage 1**
The testes, scrotum and penis are about the same size and shape as they were when you were a child.

**Stage 2**
The testes and scrotum are a little larger. The skin of the scrotum has changed. The scrotum, the sack holding the testes, has lowered a bit. The penis is only a little larger.

**Stage 3**
The penis has grown mainly in length. The testes and scrotum have grown and dropped lower than in stage 2.

**Stage 4**
The penis has grown even larger. It is wider. The head of the penis (the glans) is bigger. The scrotum is darker and bigger than before.

**Stage 5**
The penis, scrotum and testes are the size and shape of that of an adult male.

It is very important that you try to be as accurate as you possibly can when completing this self-assessment. We are not interested in who is the most or the least physically mature in our study. With a range of students from school years 7 and 8, it is perfectly normal to find that values differ between individuals; in fact, we would be very surprised to find that everyone is the same. You can be 100% confident that whatever values you write on your response form will never be available to anyone not involved in the study. Also, all of your responses are coded so we will not know what values you have provided specifically.
2. PUBIC HAIR DEVELOPMENT (MATURITY RATINGS)

The pictures on this page show different amounts of male pubic hair. A boy passes through each of the five stages shown by these drawings. Please look at each of the pictures and read the sentences alongside the pictures. Then choose the picture closest to your stage of development and circle the corresponding number (1-5) on the enclosed confidential form. In choosing the right picture, look only at the pubic hair and not at the size of the testes, scrotum and penis.

Stage 1
There is no pubic hair at all.

Stage 2
There is a little soft, long, lightly coloured hair. Most of the hair is at the base of the penis. This hair may be straight or a little curly.

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

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

Stage 5
The hair has spread out to touch the thighs. The hair is now like that of an adult male. It covers the same area as that of an adult male.

It is very important that you try to be as accurate as you possibly can when completing this self-assessment. We are not interested in who is the most or the least physically mature in our study. With a range of students from school years 7 and 8, it is perfectly normal to find that values differ between individuals; in fact, we would be very surprised to find that everyone is the same. You can be 100% confident that whatever values you write on your response form will never be available to anyone not involved in the study. Also, all of your responses are coded so that we will not know what values you have provided specifically.
1. BREAST DEVELOPMENT (MATURITY RATINGS)

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

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

Stage 2
This is the breast bud stage. In this stage, the nipple is raised more than in stage 1. The breast is a small round. The areola (coloured skin around the nipple) is larger than in stage 1.

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

Stage 4
The areola and the nipple make up a round that sticks up above the shape of the breast. This stage may not happen at all for some girls, some girls will develop from stage 3 to stage 5 with no stage 4.

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

It is very important that you try to be as accurate as you possibly can when completing this self-assessment. We are not interested in who is the most or the least physically mature in our study. With a range of students from school years 7 and 8, it is perfectly normal to find that values differ between individuals; in fact, we would be very surprised to find that everyone is the same. You can be 100% confident that whatever values you write on your response form will never be available to anyone not involved in the study. Also, all of your responses are coded so that we will not know what values you have provided specifically.
2. PUBIC HAIR DEVELOPMENT (MATURITY RATINGS)

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

Stage 1
(No picture)

Stage 1
There is no pubic hair at all.

Stage 2
There is a little soft, long, lightly coloured hair. This hair may be straight or a little curly.

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

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

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

It is very important that you try to be as accurate as you possibly can when completing this self-assessment. We are not interested in who is the most or the least physically mature in our study. With a range of students from school years 7 and 8, it is perfectly normal to find that values differ between individuals; in fact, we would be very surprised to find that everyone is the same. You can be 100% confident that whatever values you write on your response form will never be available to anyone not involved in the study. Also, all of your responses are coded so that we will not know what values you have provided specifically.
Appendix 4: Stages of sexual maturation

PHYSICAL MATURATION SHEET

Please circle the number where the written description and picture is most like you. Please remember that none of the people that you have met at the University will be able to link this information directly to you.

1. Genital / Breast development
   1  2  3  4  5

2. Pubic hair development
   1  2  3  4  5

Thank you for answering the questions. Please bring this sheet back to the University in the sealed envelope on your next visit. Your responses are completely confidential and would have no meaning to anyone not involved in the study because of the code we have used.
Appendix 5: Rating of perceived exertion scale

Rating of Perceived Exertion (RPE)

6

7 — Very, very light

8

9 — Very light

10

11 — Fairly light

12

13 — Somewhat hard

14

15 — Hard

16

17 — Very hard

18

19 — Very, very hard

20 — Maximum
Appendix 6: Child-specific effort perception scales

Children’s Effort Rating Table (CERT)

1 Very, very easy
2 Very easy
3 Easy
4 Just feeling a strain
5 Starting to get hard
6 Getting quite hard
7 Hard
8 Very hard
9 Very, very hard
10 So hard I’m going to stop


Cart and Load Effort Rating (CALER) scale

Appendix 6: Child-specific effort perception scales

Pictorial Children’s Effort Rating Table (PCERT)


OMNI scale

DIET RECORD: VISIT 2 & 3

This form is for you to fill in your normal food and drink intake during the two days leading up to visit 3, the day when you come to the University during school time, play on the Wii and let us take some small blood samples. We have provided you with some scales so that you can weigh what food and drink you are having.

Before your 5th and 7th visit to the University we will ask you to copy all the food and drink you had before your 3rd visit. Therefore, it is important that you record everything you eat and drink. There is an example of how to fill in the diet record overleaf.

1. Please fill in the tables on the attached pages. This task asks about what you eat and drink on the two days leading up to visit 3.

2. Record the amount of food and drink you have in grams as you weigh each item. Also, make sure you record the weight of anything you don't eat or drink. Refer to the weighing instructions included with the scales for information on how to weigh your food and drink, and any leftovers.

3. The cooking method may be boiled (added salt), fried, grilled, roasted (type of fat), toasted, barbecued, microwaved, steamed, baked, poached.

4. Include sandwich spreads (butter, jam, honey etc.) and the type of bread (wholemeal medium slice, white thick slice etc.).

5. Include the brand of the food and drink (e.g. Tesco value, Tesco Finest, Kellogg’s, Warburtons, Heinz etc.).

6. Include all snacks between meals – e.g. crisps, chocolate, fruit, sweets, fizzy drinks.

Please bring your completed form with you on your 3rd visit to the University.
## EXAMPLE DIET RECORD

<table>
<thead>
<tr>
<th>TIME</th>
<th>FOOD AND DRINK</th>
<th>AMOUNT (g)</th>
<th>AMOUNT LEFTOVER (g)</th>
<th>COOKING METHOD</th>
<th>BRAND</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:00</td>
<td>Frosties</td>
<td>47 g</td>
<td>2 g</td>
<td></td>
<td>Kellogg's</td>
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<tr>
<td></td>
<td>Skimmed milk</td>
<td>150 g</td>
<td>30 g</td>
<td></td>
<td>Tesco organic</td>
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<tr>
<td></td>
<td>Orange juice</td>
<td>206 g</td>
<td>0 g</td>
<td></td>
<td>Tesco</td>
</tr>
<tr>
<td>10:30</td>
<td>Apple</td>
<td>214 g</td>
<td>73 g (core)</td>
<td></td>
<td>Royal Gala</td>
</tr>
<tr>
<td>13:00</td>
<td>Thick wholemeal bread</td>
<td>2 slices (88 g)</td>
<td>0 g</td>
<td></td>
<td>Tesco</td>
</tr>
<tr>
<td></td>
<td>Butter</td>
<td>5 g</td>
<td>0 g</td>
<td></td>
<td>Anchor</td>
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<tr>
<td></td>
<td>Cooked sandwich ham</td>
<td>2 slices (25 g)</td>
<td>0 g</td>
<td></td>
<td>Tesco</td>
</tr>
<tr>
<td></td>
<td>Strawberry fruit corner</td>
<td>150 g</td>
<td>0 g</td>
<td></td>
<td>Muller</td>
</tr>
<tr>
<td></td>
<td>Ready salted crisps</td>
<td>24 g</td>
<td>0 g</td>
<td></td>
<td>Tesco (multipack)</td>
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<tr>
<td></td>
<td>Coca cola</td>
<td>330 g</td>
<td>0 g</td>
<td></td>
<td>Coca Cola</td>
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<tr>
<td>16:00</td>
<td>Orange squash</td>
<td>43 g</td>
<td>53 g (total)</td>
<td></td>
<td>Robinsons</td>
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<td></td>
<td>Water</td>
<td>256 g</td>
<td>53 g (total)</td>
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<tr>
<td>18:00</td>
<td>Chicken</td>
<td>124 g</td>
<td>14 g</td>
<td>Grilled skinless</td>
<td>Tesco</td>
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<tr>
<td></td>
<td>Peas</td>
<td>43 g</td>
<td>7 g</td>
<td>Boiled</td>
<td>Birdseye frozen</td>
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<tr>
<td></td>
<td>Carrots</td>
<td>78 g</td>
<td>0 g</td>
<td>Boiled</td>
<td>Tesco loose</td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td>168 g</td>
<td>0 g</td>
<td>Jacket (no skin)</td>
<td>Tesco loose</td>
</tr>
<tr>
<td></td>
<td>Chocolate chip digestive</td>
<td>64 g (4 biscuits)</td>
<td>0 g</td>
<td></td>
<td>Tesco</td>
</tr>
<tr>
<td>20:30</td>
<td>Water</td>
<td>233 g</td>
<td>68 g</td>
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<td>Tap water</td>
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As you know, we are keen for you to **minimise the amount of physical activity** that you do in the two days leading up to visit 3, the day when you come to the University during school time. We have given you an activity monitor to wear during these 2 days. This form is for you to record all of the physical activities completed during the two day period before visit 3 (even if it does not amount to very much).

We are also keen for you to replicate (copy) any physical activities that you completed over these two days before your 5\(^{th}\) and 7\(^{th}\) visit to the University.

1. Please fill in the tables on the attached pages. This task asks about what physical activities you complete every 30 minutes in the two days leading up to visit 3.

2. Please fill the activity diary in as the day progresses rather than attempting to remember everything that you did at the end of the day (or worse still trying to remember a few days later!).

3. Write a basic description of the physical activity (e.g., walking to school or walking to first lesson) in the first column of the activity record. When you write this, please remember that you will be copying the activity two weeks later – so the description needs to be clear to you.

4. Then decide whether the activity that you did was light, moderate, hard or very hard in intensity (see examples overleaf) and put an ‘X’ in the correct column on the table (see the example table at the bottom of the page overleaf if you are unsure). As we have asked you to minimise your physical activity on these two days, we are hoping that hard and very hard activities do not appear on your record. However, if you feel that they are unavoidable please include them on your record and then be prepared to replicate them leading up to your 5\(^{th}\) and 7\(^{th}\) visit to the University.

5. If you do more than one physical activity in any 30 min period, try to fit both descriptions into the space provided in the tables.

*Please bring your completed form with you on your 3\(^{rd}\) visit to the University.*
EXAMPLES OF PHYSICAL ACTIVITY INTENSITY

**Light** – Activities that involve slow, little or no movement and breathing remains normal e.g. watching TV, reading, listening to music, getting dressed, playing on the computer, eating.

![Light Example](image1)

**Moderate** – Activities that involve more movement and cause you to breathe a little heavier than when you are resting e.g. brisk walking, slow cycling, golf, swimming.

![Moderate Example](image2)

**Hard** – Activities that cause you to breathe faster and sweat e.g. jogging, football, dance.

![Hard Example](image3)

**Very Hard** – Activities that involve very quick movement and cause you to breathe very fast e.g. sprinting, fast cycling, aerobics.

![Very Hard Example](image4)

<table>
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<th>Time</th>
<th>Activity</th>
<th>L</th>
<th>M</th>
<th>H</th>
<th>VH</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.00 - 7.30</td>
<td>Eating breakfast and cleaning teeth</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>7.30 - 8.00</td>
<td>Walking to school</td>
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<td></td>
<td>X</td>
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Date: .......... Day: .................. Name: ........................................ Code: ..........

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<td>3.00 - 3.30</td>
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# Appendix 8: Physical activity record

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Appendix 9: Visual analogue scale

Visual Analogue Scale

Code:..................Date:..................Time:..................

Read the questions below and place a mark on the line to show your answer:

How hungry do you feel?

I am not hungry at all .................................................. I have never been more hungry

How satisfied do you feel?

I am completely empty .................................................. I cannot eat or drink anything else

How full do you feel?

Not full at all .................................................. Totally full

How much do you think you can eat?

Nothing at all .................................................. A lot

How nauseous (sick) do you feel?

Not nauseous at all .................................................. Very nauseous

How much did you like the milkshake?

Not at all .................................................. A lot

THANK YOU! 😊
Feeling Scale

+5 — very good
+4
+3 — good
+2
+1 — fairly good
0 — neutral
-1 — fairly bad
-2
-3 — bad
-4
-5 — very bad
Participant code: ……………

Physical Activity Enjoyment Scale (PACES)

After thinking carefully about it, please circle one number for each question.

When I was exercising……

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<th>Disagree a lot</th>
<th>Agree a lot</th>
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<td>I enjoyed it</td>
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<tr>
<td>I felt bored</td>
<td>1   2  3  4  5</td>
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<tr>
<td>I disliked it</td>
<td>1   2  3  4  5</td>
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<tr>
<td>I found it pleasurable</td>
<td>1   2  3  4  5</td>
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<tr>
<td>It was no fun at all</td>
<td>1   2  3  4  5</td>
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<tr>
<td>It gave me energy</td>
<td>1   2  3  4  5</td>
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<td>It made me depressed</td>
<td>1   2  3  4  5</td>
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<td>It was very unpleasant</td>
<td>1   2  3  4  5</td>
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<td>My body felt good</td>
<td>1   2  3  4  5</td>
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<td>I got something out of it</td>
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<td>It was very exciting</td>
<td>1   2  3  4  5</td>
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<td>It frustrated me</td>
<td>1   2  3  4  5</td>
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<td>It was not at all interesting</td>
<td>1   2  3  4  5</td>
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<tr>
<td>It gave me strong feelings of success</td>
<td>1   2  3  4  5</td>
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<tr>
<td>It felt good</td>
<td>1   2  3  4  5</td>
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<tr>
<td>It felt as though I would rather be doing something else</td>
<td>1   2  3  4  5</td>
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THANK YOU! 😊