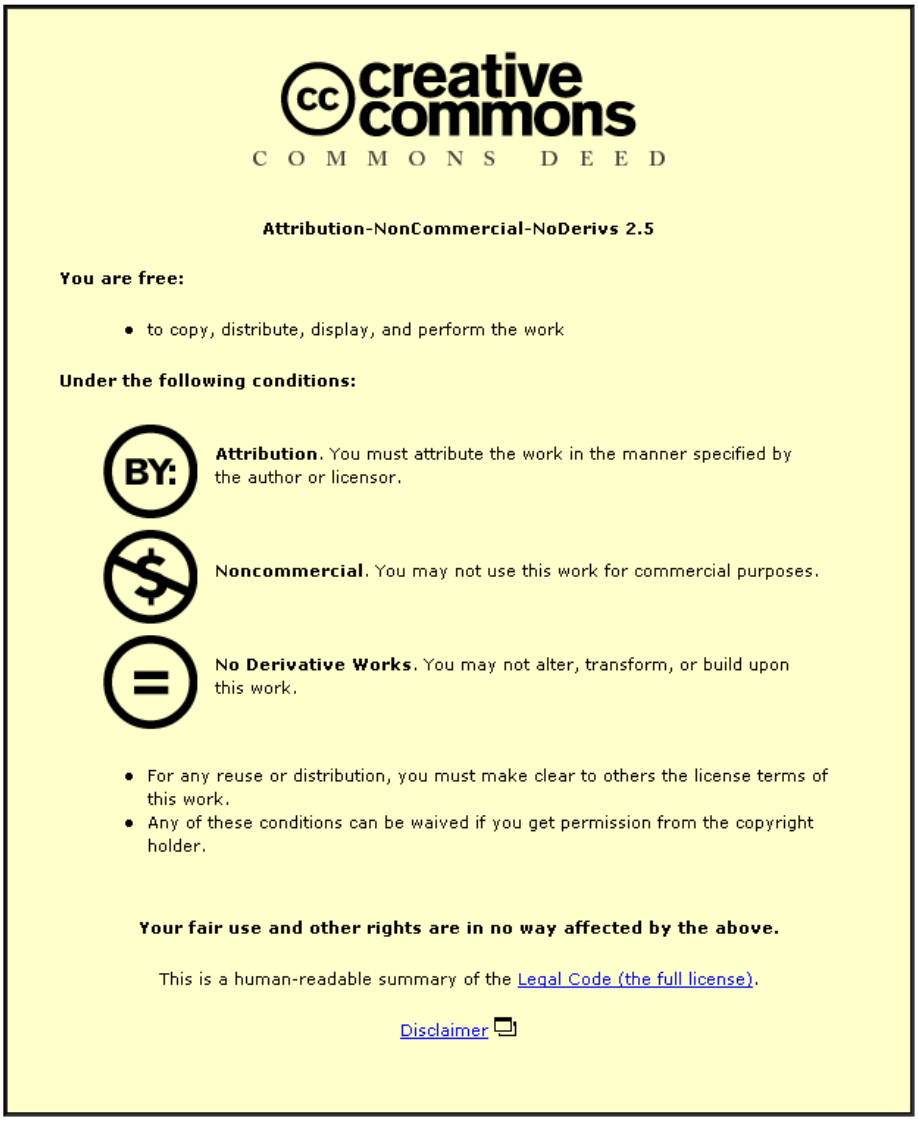

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EFFECT OF MANIPULATIONS IN EXERCISE AND BREAKFAST ON METABOLISM IN OVERWEIGHT AND NON-OVERWEIGHT CHILDREN AND ADOLESCENTS

Julia Zakrzewski

A Doctoral Thesis

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

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Abstract

Obesity and insulin resistance are serious health concerns in children and adolescents (young people). Interventions to increase the potential for fat oxidation and improve insulin sensitivity could have widespread clinical relevance. Although exercise is often advocated for health, the factors implicated in the relationship between exercise, fat oxidation and insulin resistance are not well understood in young people. This thesis has investigated the effect of manipulations in exercise and breakfast on metabolism in young people, focusing on fat oxidation and postprandial blood glucose control. The first experimental study, Chapter 4, compared two different exercise protocols for estimating the intensity corresponding to maximal fat oxidation (Fatmax) in non-overweight prepubertal children. A 3 min incremental protocol was recommended to provide an estimation of Fatmax using a wide range of intensities in this population. Using this protocol, Chapter 5 demonstrated that Fatmax was higher for treadmill compared with cycling exercise in pre- to early pubertal children. Furthermore, treadmill exercise resulted in higher rates of fat oxidation over a range of absolute and relative intensities and fat oxidation remained high over a wider range of intensities. Therefore, treadmill exercise (walking or slow running) is clearly preferential for promoting fat oxidation in this population. Subsequently, Chapter 6 examined the effect of mixed breakfast meals containing high (HGI) and low (LGI) glycaemic index carbohydrates on blood glucose, plasma insulin and fat oxidation in overweight and non-overweight girls. Breakfast GI did not affect fat oxidation during the postprandial rest period or subsequent exercise. However, the main finding of this study related to blood glucose; the higher blood glucose response following the HGI compared with LGI breakfast was more pronounced in the overweight girls. This suggested a reduced ability to cope with the metabolic demands of HGI breakfast consumption in overweight girls and highlighted that strategies to reduce insulin resistance in this population are required. Consequently, Chapter 7 investigated the effect of treadmill exercise at Fatmax performed 16 h prior to HGI breakfast consumption on blood glucose, plasma insulin and fat oxidation in overweight and non-overweight girls. Fatmax exercise reduced the postprandial insulin response in the non-overweight, but not the overweight, girls while blood glucose was unchanged in both groups. More encouragingly, fat oxidation was increased after exercise in both the overweight and non-overweight girls. Collectively, the four experimental studies within this thesis have demonstrated that treadmill exercise at Fatmax is an effective means of elevating fat oxidation both during and up to 16 h after exercise. When considering postprandial glucose and insulin responses to HGI breakfast consumption, LGI breakfasts should be recommended for overweight girls, whilst acute treadmill exercise at Fatmax can reduce postprandial insulin concentrations in non-overweight girls. Walking or slow running (Fatmax treadmill exercise) and LGI breakfast consumption may be best advocated in combination for promoting fat oxidation and improving postprandial blood glucose control in young people. These two simple lifestyle-related strategies may provide an effective, safe and attractive means for preventing and treating obesity, insulin resistance and related disorders.

Key words: exercise, metabolism, substrate oxidation, fat oxidation, glucose, insulin, glycaemic index, overweight, children, adolescents.

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List of Abbreviations

The following abbreviations were used to signify frequently used terms throughout the current thesis. All abbreviations were defined in the first instance they appear in the text.

ANOVA	Analysis of variance
beats·min^{-1}	Beats per minute
BM	Body mass
BMI	Body mass index
CE	Cycling exercise
CHO	Carbohydrate
CPT	Carnitine palmitoyltransferase
EMCL	Extramyocellular lipid
ES	Effect size
FFA	Free fatty acid
FFM	Fat free mass
HGI	High glycaemic index
HOMA-IR	Homeostasis model assessment for insulin resistance
HR	Heart rate
IMCL	Intramyocellular lipid
IMTG	Intramuscular triacylglycerol
LGI	Low glycaemic index
LIAB	Lactate increase above baseline
L·min^{-1}	Litres per minute
LoA	Limits of agreement
MFO	Maximal fat oxidation
MGI	Moderate glycaemic index
min	Minute
mL·kg·min^{-1}	Millilitres per kilogram per minute
NO	Non-overweight
OW	Overweight
RER	Respiratory exchange ratio
RPE	Rating of perceived exertion

revs·min^{-1}	Revolutions per minute
s	Seconds
SD	Standard deviation
TAUC	Total area under the curve
TM	Treadmill
V□CO$_2$	Carbon dioxide production
V□O$_2$	Oxygen uptake
V□O$_{2peak}$	Peak oxygen uptake
W	Watts
WC	Waist circumference

Chapter 1

Introduction

Overweight and obesity among children and adolescents (young people) is currently a major global health concern (Ebbeling et al., 2002; Health Survey for England, 2009; Wang et al., 2011). Recent figures from the Health Survey for England (2009) indicate that around one third of 2 to 15 year olds are classified as either overweight or obese (31% boys and 28% girls) and around half of these young people are obese (16% of boys and 15% of girls). Whilst there have been alarming increases over the last few decades in England, numbers have stabilised since 2005, but remain a major concern. Obesity is associated with a range of adverse metabolic and cardiovascular health outcomes in young people, including insulin resistance, type 2 diabetes mellitus, dyslipidaemia and hypertension (Burke, 2006; Chiarelli and Marcovecchio, 2008). It is also clear that childhood obesity tracks into adulthood (Gordon-Larsen et al., 2004) and can have adverse consequences on mortality and morbidity in later life, including increased risk of premature mortality, cardiometabolic morbidity (diabetes, hypertension, ischaemic heart disease and stroke), asthma and polycystic ovary syndrome (Reilly and Kelly, 2011). Adiposity during childhood, therefore, represents an early beginning of a potentially lifetime pathological process. In addition, the combined medical costs associated with the treatment of these preventable conditions are a significant economic burden and set to increase (Wang et al., 2011).

Insulin resistance represents a reduced ability to increase glucose uptake in response to a known quantity of exogenous or endogenous insulin and is the most common metabolic alteration related to obesity (Weiss and Kaufman, 2008). Population-based data has shown that insulin resistance is present in as many as 50% of obese adolescents (Lee et al., 2006). Evidence suggests that body fat contributes to the development of insulin resistance in young people; adiposity explains a large proportion of the variation in insulin resistance independent of age, sex and ethnicity (Lee et al., 2006) and insulin resistance increases in severity with the degree of adiposity (Weiss et al., 2004). Insulin resistance precedes the development of type 2 diabetes mellitus, with resultant high insulin levels and gradual development of impaired glucose tolerance (Weyer et al.,

1999), which are also common in obese children and adolescents (Caprio et al., 1995; Sinha et al., 2002a). Moreover, insulin resistance represents an important link between obesity and other metabolic and cardiovascular complications in young people (Cruz et al., 2004; Lee et al., 2006; Srinivasan et al., 2002; Weiss and Kaufman, 2008). The manifestation of insulin resistance in prepubertal children with a relatively short duration of adiposity is particularly concerning for young people, since the development of insulin resistance appears to be independent of obesity duration (Caprio et al., 1996).

The transition from adolescence to adulthood has been identified as a particularly high-risk period for weight gain and the later development of metabolic complications (Artz et al., 2005; Gordon-Larsen et al., 2004). Independent of obesity, the pubertal transition from Tanner stage 1 to 3 is associated with a 32% reduction in insulin sensitivity with concomitant increases in fasting glucose, insulin and the acute response to glucose, which recovers by Tanner stage 5 (Goran and Gower, 2001). Therefore, insulin resistance associated with obesity may be further exacerbated by the influence of puberty. The presence of these conditions in young people is thus particularly concerning and a major effort to alleviate overweight and obesity in young people could have widespread clinical relevance.

Various inter-related factors have contributed to the large multi-national increase in numbers of overweight and obese young people (Ebbeling et al., 2002). Despite this, it is generally agreed that a complex interplay between excessive energy intake and insufficient energy expenditure is at the root of the problem. Therefore, lifestyle modification involving a combination of dietary and exercise strategies is advocated for weight management (Frieden et al., 2010). Exercise reduces body fat by creating a negative energy balance, attenuating the loss of lean body mass and reducing the accumulation of visceral adipose tissue (Owens et al., 1999). Although dietary interventions are often more successful for reducing body mass, exercise may confer additional health benefits (Ben Ounis et al., 2009). Independent of changes in body mass and composition, exercise training improves insulin sensitivity (Bell et al., 2007) and reduces cardiovascular risk factors (Watts et al., 2004) in young people. Moreover, fitness appears to be a stronger independent predictor of insulin resistance than fatness in young people (Allen et al., 2007; Jim Nez-Pav et al., 2011) and is protective against morbidity and all-cause mortality in adults (Fogelholm, 2010). This suggests that

adequate levels of exercise and physical activity may counteract the negative influence of body fat on health.

Collectively, the evidence suggests that efforts to reduce insulin resistance and other health markers in young people may be best focused on increasing physical activity rather than simply restricting energy intake to achieve weight loss. Moreover, it is widely recognised that obesity prevention provides a more effective and realistic solution than a cure (Frieden et al., 2010); thus, attention should be directed, not only to the obese, but also to overweight and non-overweight young people. It is crucial that these interventions are evidence-based, so continued research to enhance our understanding of exercise metabolism in young people is required.

Carbohydrate (CHO) and fat are the major substrates that contribute to energy expenditure during rest and exercise. Mounting evidence suggests that the development of strategies to maximise fat oxidation in particular could facilitate weight loss and reduce insulin resistance. High fat oxidation rates can protect against long term weight gain (DeLany et al., 2006; Seidell et al., 1992) and exercise training–induced changes in fat oxidation predicted fat loss (Barwell et al., 2009). The link between fat oxidation and insulin resistance is currently a topic of great interest; an imbalance between free fatty acid (FFA) availability and skeletal muscle FFA uptake, storage and oxidation may lead to the accumulation of harmful intracellular FA metabolites that disrupt insulin signalling (Holloway et al., 2009). Moreover, the amount of lipid stored in the muscle as triacylglycerol is associated with insulin resistance and is increased in obese young people (Sinha et al., 2002b; Weiss et al., 2005). Conversely, exercise training (Bruce et al., 2006; Dubé et al., 2011) and acute exercise (Schenk and Horowitz, 2007) increase fat oxidation, reduce the accumulation of intracellular fatty acid metabolites and increase insulin sensitivity. These data highlight that the mechanism of improved insulin sensitivity following exercise is perhaps the result of changes in muscle substrate utilisation. Indeed, reviews of the area have concluded that obese individuals would benefit from interventions that increase the potential for oxidising fat (Holloway et al., 2009; Kelley, 2002).

Several factors influence fat oxidation during exercise, including the exercise characteristics (intensity, mode, duration), individual participant characteristics (age,

sex, weight status) and pre-exercise diet. Among these factors, exercise intensity is of primary importance; fat oxidation increases from low to moderate intensities and then declines at higher intensities (Romijn et al., 1993). Much of the original work investigating fat oxidation during exercise can be criticised for estimating fat oxidation at only one or two intensities (Mácek et al., 1976; Rowland and Rimany, 1995). With this in mind, more recent work has estimated fat oxidation over a wide range of intensities using incremental exercise protocols and the relative exercise intensity that elicits the highest ('maximal') fat oxidation rate has been termed Fatmax (Achten et al., 2002). Fatmax generally occurs between 30 and 60% peak oxygen uptake ($V\square O_{2peak}$) in young people and may be influenced by puberty (Riddell et al., 2008), body composition (Zunquin et al., 2009b) and exercise training (Brandou et al., 2003). Importantly, exercise training at Fatmax improves body composition, fat oxidation and several metabolic health markers in obese young people (Ben Ounis et al., 2008; 2009). However, several weaknesses may be highlighted in many studies that have identified Fatmax in young people, which have used a variety of exercise protocols and methods that have not been systematically evaluated. Furthermore, conclusions from these studies are often based on findings from boys and cycling exercise. It is clear that this research is still in its infancy and the effect of factors such as exercise mode, sex and cardiorespiratory fitness on Fatmax remain to be investigated. In particular, exercise mode is clearly an important modifiable factor for increasing individual fat oxidation. Further examination of the factors influencing Fatmax in young people could help to optimise interventions aimed at improving fat oxidation and health-related outcomes in young people.

From a dietary perspective, the most effective way to enhance fat oxidation is to exercise in the fasted state (Horowitz et al., 1997). However, this would not be a practical or desirable option for many young people and humans are typically in the postprandial state. Furthermore, breakfast is often considered to be the 'most important meal of the day', providing a stable blood glucose concentration, which may subsequently influence appetite and energy reserves required for physical activity and cognitive tasks (Lien, 2007; Sandercock et al., 2010). Regarding health, there is convincing epidemiological evidence that it is important to not only encourage regular breakfast consumption (Barton et al., 2005; Timlin et al., 2008), but it is also critical to focus on breakfast composition (Deshmukh-Taskar et al., 2010; Djoussé and Gaziano,

2007). There has been great interest in the health benefits of breakfasts containing low glycaemic index (LGI) CHO for the prevention of obesity, diabetes and cardiovascular disease (Brand-Miller et al., 2009). The concept of glycaemic index (GI) was introduced as a method of classifying different CHO-rich foods according to their effect on postprandial glycaemia (Jenkins et al., 1981). The reduced postprandial glucose and insulin response to LGI compared with high GI (HGI) CHO consumption may have long term health implications for disease risk (Heine et al., 2004) and can promote satiety in young people, which has direct implications for weight management (Ludwig et al., 1999; Warren et al., 2003). Evidence in adults indicates that the attenuated glucose and insulin response to LGI breakfasts may also enhance postprandial fat oxidation during rest and subsequent exercise (Stevenson et al., 2009). This suggests that consuming a LGI breakfast may be a valuable compromise between promoting breakfast consumption and exercise in the fasted state to enhance fat oxidation in young people, but has yet to be investigated in this population. Furthermore, there is still a considerable degree of uncertainty regarding the effect of GI on fat oxidation even in the relatively well documented adult literature. The reduced glucose and insulin response, increased fat oxidation and prolonged satiety following LGI breakfast consumption may have clinical relevance for overweight and insulin resistant individuals (Holloway et al., 2009). Research of this nature may also be best focused adolescent girls, as physical activity levels are lower in girls compared with boys (Riddoch et al., 2007) and decline rapidly during adolescence (Armstrong and Welsman, 2006) and girls are less likely to eat breakfast daily (Timlin et al., 2008).

Similar to LGI breakfast consumption, an acute bout of exercise appears to be another effective strategy to reduce postprandial insulin concentrations and increase fat oxidation in adults, although glucose concentrations often remain unchanged (Burton et al., 2008; Kokalas et al., 2005). This suggests that long term training adaptations are not necessarily required to improve these metabolic health markers. Several studies in young people have shown improved insulin sensitivity and fat oxidation after exercise training (Bell et al., 2007; Ben Ounis et al., 2008; 2009; Nassis et al., 2005), but the acute effect of exercise remains to be investigated . Importantly, examining these acute responses may have direct relevance for those who do not participate in regular exercise training.

To summarise, the health advantages of enhancing fat oxidation through exercise and potential interactions with insulin resistance could have valuable implications for the management of obesity, insulin resistance and conditions in which fat oxidation is disturbed. However, much of this evidence is based on findings from adults. Despite well recognised differences in metabolism between children and adults (Boisseau and Delamarche, 2000), the number of experimental studies investigating exercise metabolism in young people is relatively small. This thesis bridged some of these gaps in the literature by enhancing our understanding of exercise, fat oxidation and insulin resistance in young people and has, ultimately, provided valuable evidence to inform lifestyle interventions aimed at improving these health markers.

1.1 Thesis structure and experimental aims

The overall theme of this thesis was to investigate the effect of manipulations in exercise and breakfast on metabolism in young people, focusing on fat oxidation, glucose and insulin. **Chapter 2** comprehensively and critically reviewed the literature that directly relates to the research line of enquiry, providing a basis and rationale for the experimental chapters. **Chapter 3** has then provided a brief overview of the general methods used throughout the experimental chapters that follow to reduce unnecessary replication. The first two experimental chapters focused on fat oxidation during exercise in children. **Chapter 4** examined the effect of exercise intensity on fat oxidation and compared two protocols for estimating Fatmax, as an examination of the literature revealed no consensus had been reached on the exercise protocol that should be used to estimate Fatmax in young people. The effect of exercise mode on Fatmax and fat oxidation was then examined in **Chapter 5** by comparing fat oxidation over a range of exercise intensities during treadmill and cycling exercise. Subsequently, **Chapter 6** addressed the effect of pre-exercise CHO consumption on fat oxidation, by specifically comparing the effect of HGI and LGI mixed breakfast meals on metabolism in overweight and non-overweight girls. Blood glucose and plasma insulin were determined primarily to detect potential differences between the HGI and LGI breakfasts in this study, but also highlighted that attention should be focused on improving the exaggerated glucose and insulin responses in overweight girls following HGI breakfast consumption. Consequently, **Chapter 7** examined the acute effect of Fatmax exercise on glucose, insulin and fat oxidation following HGI breakfast

consumption in overweight and non-overweight girls. Finally, the findings were discussed collectively in **Chapter 8**.

The specific aims of the experimental studies presented were:

Chapter 4: To compare Fatmax and maximal fat oxidation estimated using a 3 min incremental cycling protocol and a protocol consisting of several 10 min constant work rate exercise bouts in children.

Chapter 5: To compare Fatmax and fat oxidation over a range of intensities between treadmill and cycling exercise in children.

Chapter 6: To examine the effect of breakfast glycaemic index on glucose, insulin and fat oxidation during rest and subsequent exercise in overweight and non-overweight girls.

Chapter 7: To examine the acute effect of Fatmax exercise on glucose, insulin and fat oxidation in overweight and non-overweight girls.

Chapter 2

Review of Literature

This chapter reviews and critically examines the literature that has investigated the impact of exercise on metabolism in young people, focusing on fat oxidation, glucose, insulin. The first section provides an overview of the interplay between fat oxidation, obesity and insulin resistance. This is followed by a comprehensive discussion of the factors influencing fat oxidation during exercise in young people. Subsequently, the effect of breakfast consumption on metabolism is discussed, leading to a more in-depth review of the effect of GI on glucose, insulin and fat oxidation during rest and exercise. The final section of this chapter reviews the literature that has examined the acute effect of exercise on glucose, insulin and fat oxidation.

2.1 Fat oxidation, obesity and insulin resistance

2.1.1 Obesity

The partitioning of dietary fat between storage and oxidation may be important for weight management. Several lines of evidence suggest that low rates of fat oxidation can predispose individuals to weight gain. In adults, low rates of resting fat oxidation in the fasted state (Marra et al., 2004; Seidell et al., 1992) and over 24 hours (Zurlo et al., 1990) predicted long-term weight gain and also weight regain following weight loss (Froidevaux et al., 1993). Furthermore, there is evidence that obese (Kim et al., 2000) and formerly obese (Ranneries et al., 1998) adults have reduced rates of fat oxidation. While the impaired ability to oxidise fat during fasting conditions in obese adults does not improve following weight reduction (Berggren et al., 2008; Kelley et al., 1999), just 10 consecutive days of exercise training can increase fat oxidation in obese, formerly obese and non-obese adults (Berggren et al., 2008). Although evidence in young people is limited to just a few studies, longitudinal findings in 9 to 11 year old children showed that fat oxidation rates predicted fat gain two years later (DeLany et al., 2006). Nutritionally stunted children, a population at increased risk of overweight, also have impaired fasting and postprandial fat oxidation (Hoffman et al., 2000).

It is not clear whether young people who are already overweight or obese exhibit a reduced capacity for fat oxidation. During resting conditions, fat oxidation is elevated when expressed in absolute terms, similar when expressed per kg fat free mass (Maffeis et al., 1995; McMurray and Hosick, 2011; Zunquin et al., 2009b) and may be higher (Paz Cerezo et al., 2003) or similar (Butte et al., 2007) when expressed as a proportion of energy expenditure in overweight/obese compared with non-overweight young people. Obese young people, however, appear to have a reduced ability to oxidise fat during exercise (Lazzer et al., 2007; Zunquin et al., 2009b), which is discussed more extensively in section 2.5.3. A recent study concluded that, rather than increasing fat oxidation, it appears that overweight young people increase CHO oxidation to meet their increased energy requirements (McMurray and Hosick, 2011). Furthermore, their ability to be 'metabolically flexible' may be impaired (Aucouturier et al., 2011). Metabolic flexibility describes the ability to adapt substrate oxidation to availability and is perhaps best illustrated by the ability of skeletal muscle to switch between fat oxidation in the fasted state to CHO oxidation during insulin stimulated periods (Kelley and Mandarino, 2000). Signs of metabolic inflexibility in obese young people include a failure to stimulate glucose oxidation and suppress fat oxidation following insulin infusion (Caprio et al., 1995) and to increase insulin sensitivity during a high-CHO diet (Sunehag et al., 2005). Fasting hyperinsulinaemia (Odeleye et al., 1997) and the inability to adapt metabolically to changes in dietary macronutrient content (Eckel et al., 2006; Flatt, 1996) may predict further weight gain in these young people.

2.1.2 Insulin resistance

Mounting evidence has shown a strong association between the accumulation of fat within skeletal muscle and insulin resistance; this is currently a topic of great interest and has been reviewed by various authors (Eckardt et al., 2011; Holloway et al., 2009; Kelley et al., 2002a). Although much of this evidence is based on studies with adults due to the use of biopsy samples to measure intramuscular triacylglycerol (IMTG), intramyocellular lipid (IMCL) and extramyocellular lipid (EMCL), nuclear magnetic resonance spectroscopy techniques have been used in a few studies in young people. Sinha et al. (2002b) showed that both IMCL and EMCL content were elevated in obese compared with non-obese adolescents. Furthermore, IMCL content was correlated with waist circumference, body mass index (BMI) and fasting glucose:insulin ratio in healthy prepubertal boys (Ashley et al., 2002) and was closely linked to the

development of insulin resistance in obese prediabetic youth (Weiss et al., 2003). Interestingly, the relationship between IMCL and insulin resistance may be independent of adiposity; IMCL levels are elevated in obese insulin resistant adolescents (Weiss et al., 2005) and obese children with impaired glucose tolerance (Weiss et al., 2003) compared with their insulin sensitive/glucose tolerant obese counterparts. Furthermore, IMCL is elevated in lean insulin resistant offspring of type 2 diabetic adults (Jacob et al., 1999).

The observation that lean endurance-trained athletes have both high levels of IMTG and insulin sensitivity, often referred to as the athlete's paradox (Goodpaster et al., 2001), suggests the relationship between IMTG content and insulin resistance is not functional. Rather, IMTG provides a source for the generation of harmful fatty acid derived metabolites, including long-chain acyl CoA, diacylglycerol and ceramide, in skeletal muscle, which can disrupt the insulin-signalling pathway and induce insulin resistance (Eckardt et al., 2011; Itani et al., 2002). Thus, it appears that IMTG accumulation is perhaps a surrogate marker for other lipid species having a more direct effect on insulin action in obese and insulin resistant individuals, where it represents a dysfunction in fat metabolism (van Loon and Goodpaster, 2006). Conversely, greater IMTG storage in the trained state represents an adaptive response to endurance training, allowing a greater contribution of the IMTG pool as a substrate source during exercise (Bergman et al., 2010; Goodpaster et al., 2001; Schrauwen-Hinderling et al., 2003). Indeed, enhancements in insulin sensitivity following training occur with concomitant increased IMTG stores, reduced fatty acid metabolite accumulation and increased fat oxidation in adults (Bruce et al., 2006; Dubé et al., 2011).

The accumulation of IMTG and fatty acid metabolites may result from a combination of increased muscle FFA uptake and/or decreased fat oxidation in obese and insulin resistant individuals. Although the transport of FFA into the muscle is increased in obesity (Bonen et al., 2004), the capacity for fat oxidation appears to be paramount (Kelley et al., 1999). High resting fat oxidation rates may protect against insulin resistance in obese young people; fat oxidation was higher in obese insulin sensitive compared with obese insulin resistant adolescents, despite similar adiposity (Weiss et al., 2005). Furthermore, there is considerable evidence that metabolic inflexibility, which reflects a dysfunction in fat metabolism, is involved in the development of

insulin resistance in obese adults (Kelley and Mandarino, 2000) and some evidence in young people (Aucouturier et al., 2011). Indeed, obese insulin resistant compared with obese insulin sensitive adolescents have higher IMCL levels, reduced glucose disposal rate and a blunted suppression of fat oxidation during hyperinsulinaemia (Weiss et al., 2005).

Collectively, the evidence suggests a link between fat oxidation, insulin resistance and weight control in both young people and adults. Consequently, interventions to increase fat oxidation and thus improve the balance between FFA uptake and oxidation could have important health implications. Regular exercise may be a promising strategy to increase the ability for fat oxidation, reduce the accumulation of fatty acid metabolites and protect against insulin resistance (Bruce et al., 2006; Dubé et al., 2011).

2.2 Skeletal muscle fat metabolism

2.2.1 Regulation of skeletal muscle fat metabolism during exercise

Fat and CHO are the major energy substrates during exercise (Romijn et al., 1993). The regulation of skeletal muscle fat metabolism is complex and multifactorial, with different mechanisms dominating at different exercise intensities. The potential sites that control skeletal muscle fat metabolism and oxidation during exercise include adipose tissue lipolysis and FFA delivery to the muscle, FFA transport across the muscle membrane, IMTG lipolysis and FFA transport across the mitochondrial membranes. It should be noted that the mechanisms proposed to control fat oxidation during exercise are largely based on studies with adults due to the invasive nature of the techniques employed, including arterial cannulation (Costill et al., 1977; Romijn et al., 1993) and muscle biopsies (Kiens et al., 1997). Despite considerable progress during recent years, the factors controlling fat oxidation during exercise remain unclear and have not been investigated to any significant extent in young people.

Adipose tissue lipolysis and FFA delivery to the muscle: Long-chain FFAs from adipose tissue provide a major source of fat for the muscle during exercise. Hormone-sensitive lipase (HSL) is the rate-limiting enzyme involved in lipolysis; FFAs are then released from adipose tissue, bound to albumin in the blood and transported to the muscle. During exercise, elevated HSL activation and blood flow increases FFA delivery to the muscle and this increased FFA availability controls the up-regulation of

fat oxidation from rest to low and moderate intensity exercise (Costill et al., 1977; Romijn et al., 1993). During high intensity exercise, lactate accumulation may inhibit lipolysis (Boyd et al., 1974), although this has not always been confirmed (Trudeau et al., 1999). Nevertheless, elevating FFA availability during high intensity exercise does not increase fat oxidation to the same level seen at moderate intensity exercise (Romijn et al., 1995). This suggests that FFA availability only partially controls the down-regulation of fat oxidation during high intensity exercise, thus mechanisms within the skeletal muscle must also be involved.

FFA transport across the muscle membrane: The uptake of FFAs into the muscle likely involves both passive diffusion and protein-mediated transport. Over the last few years, knowledge of the regulation of cellular FFA uptake has risen dramatically. There is now evidence that the majority of FFAs enter the muscle via plasma membrane-associated proteins, most notably fatty acid translocase (FAT)/CD36, plasma membrane-bound fatty acid binding protein (FABPpm) and, possibly, fatty acid transport protein (FATP) (Spriet, 2002). More specifically, FAT/CD36 (Bonen et al., 2000) and FABPpm (Han et al., 2007) are acutely translocated from an intracellular pool to the plasma membrane during muscular contractions, thus increasing FFA transport across the plasma membrane and into the muscle cell. This suggests these transporter proteins are particularly relevant when considering exercise.

IMTG lipolysis: A second major source of fat during exercise is the release of FFAs from IMTG (Watt et al., 2002). Although the mechanisms regulating IMTG lipolysis are largely unknown, HSL has been identified in skeletal muscle (Langfort et al., 1999; 2000) and is activated during moderate intensity exercise, partly due to stimulation by adrenaline (Kjaer et al., 2000). Muscle HSL activity, therefore, appears to be another important regulator of exercise fat oxidation.

FFA transport across the mitochondrial membranes: Fatty acid binding proteins chaperone FFAs in the cytoplasm to the surface of the outer mitochondria membrane. The carnitine palmitoyltransferase (CPT) complex, consisting of CPT-1, acylcarnitine translocase and CPT-2, regulates the transport of FFAs into the mitochondria. CPT-1, located on the outer mitochondrial membrane, catalyses the transfer of long chain fatty acyl groups from coenzyme (CoA) to carnitine. The generated acylcarnitine then

permeates the inner membrane, via acylcarnitine/carnitine translocase. CPT-2, located on the inner mitochondrial membrane, catalyses the transfer of the acyl group from carnitine to CoA. The re-formed acyl-CoA enters the ß-oxidation pathway, is further metabolised in the tricarboxylic acid (TCA) pathway and, finally, adenosine triphosphate (ATP) is produced in the electron transport chain in the process of oxidative phosphorylation (McGarry and Brown, 1997).

CPT-1 is considered to be the rate-limiting enzyme involved in FFA transport into the mitochondria and numerous regulators of CPT-1 activity have been proposed. Malonyl-CoA reversibly inhibits CPT-1 activity (Berthon et al., 1998; Starritt et al., 2000) and evidence in rodent muscle suggests a role for malonyl-CoA in the suppression of fat oxidation at high exercise intensities (Rasmussen et al., 1997). Malonyl-CoA is formed from acetyl-CoA, a reaction catalysed by acetyl-CoA carboxylase. Increased acetyl-CoA and acetylcarnitine concentrations during high intensity exercise (Odland et al., 1998) may thus increase malonyl-CoA (Saddik et al., 1993) and reduce fat oxidation. However, studies in humans have failed to provide evidence that malonyl-CoA is an important regulator of exercise fat metabolism (Odland et al., 1996; 1998). Despite this lack of evidence relating to exercise intensity, the sensitivity of CPT-1 to malonyl-CoA is increased in trained compared with untrained skeletal muscle (Starritt et al., 2000). Further, CPT-1 may bind to malonyl-CoA more efficiently at low pH levels (Mills et al., 1984), which may contribute to the down-regulation of fat oxidation at high exercise intensities when hydrogen ion accumulation reduces muscle pH. Moreover, a direct effect of reduced muscle pH on fat oxidation has been proposed; even small decreases in pH reduced CPT-1 activity in humans (Starritt et al., 2000). Reduced free carnitine availability may also be limiting to the CPT-1 reaction during high intensity exercise when glycolytic flux and acetyl-CoA formation are high, and thus contribute to reductions in fat oxidation (Stephens et al., 2007, van Loon et al., 2001). In addition to CPT-1, more recent evidence has shown that fatty acid transporter proteins are present on the mitochondrial membrane and are involved in the transport of FFA into the mitochondria, as well as the muscle. There is evidence that FAT/CD36 facilitates FFA mitochondrial transport, whereas the role of FABPpm appears to be related to transporting reducing equivalents into the mitochondrial matrix (Holloway et al., 2006; 2007).

2.2.2 Assessment of fat oxidation: indirect calorimetry

The non-invasive nature of indirect calorimetry is particularly attractive for the estimation of fat oxidation in young people. Indirect calorimetry relies on the assumption that oxygen consumption ($V\square O_2$) and carbon dioxide production ($V\square CO_2$) reflect gas exchange at the tissue level and that a physiological steady state has been reached (Frayn, 1983). However, $V\square CO_2$ increases due to the buffering of H^+, resulting in non-oxidative $V\square CO_2$ production during high intensity exercise (Wasserman, 1984) and the respiratory exchange ratio (RER) may reflect the state of respiration rather than gas exchange at the muscle during hyperventilation and hypoventilation. Indirect calorimetry also assumes that other metabolic processes involving oxygen consumption and carbon dioxide production are negligible, such as gluconeogenesis, lipogenesis and ketogenesis (Frayn, 1983). Stable isotope tracer techniques represent another promising method of non-invasively estimating substrate oxidation in young people. In more recent years, this method has been used in conjunction with indirect calorimetry to estimate endogenous and exogenous substrate oxidation rates by measuring labelled CO_2 in exhaled air (Riddell et al., 2000; Timmons et al., 2003; 2007a).

Despite the issues associated with indirect calorimetry, under well-controlled steady state conditions the RER compares well with gas exchange at the tissue level (respiratory quotient, RQ) (Jansson and Kaijser, 1987) and can be used to estimate substrate oxidation. Romijn et al. (1992) found that indirect calorimetry was a valid measurement of substrate oxidation up to 80 to 85% $V\square O_{2peak}$ when compared with a breath $^{13}C/^{12}C$ ratio method in five well-trained male cyclists, although more recent findings indicate that indirect calorimetry and stable isotope techniques started to deviate from 75% $V\square O_{2peak}$ (Rowlands and Jeukendrup, 2004). Unfortunately, a similar exercise intensity does not appear to have been identified in young people. However, as fat oxidation tends to peak at low to moderate exercise intensities in children and adolescents (Brandou et al., 2003; Riddell et al., 2008), it is unlikely that fat oxidation will be estimated at intensities exceeding 75% $V\square O_{2peak}$ in studies examining exercise fat oxidation, and particularly Fatmax, in this population.

2.3 Exercise intensity and fat oxidation: the concept of Fatmax

Exercise intensity is one of the primary factors influencing substrate oxidation during exercise. As exercise intensity increases, there is a progressive increase in the relative

contribution of CHO and decrease in the relative contribution of fat oxidation to energy expenditure (Brooks and Mercier, 1994). In absolute terms, CHO oxidation increases proportionally with exercise intensity, whereas fat oxidation increases from low to moderate intensities and then declines at higher intensities (Achten et al., 2002; Romijn et al., 1993). The exercise intensity that corresponds to maximal fat oxidation (MFO) has been termed Fatmax (Achten et al., 2002) and is typically expressed as % V□O_{2peak}, as displayed in Figure 2.1.

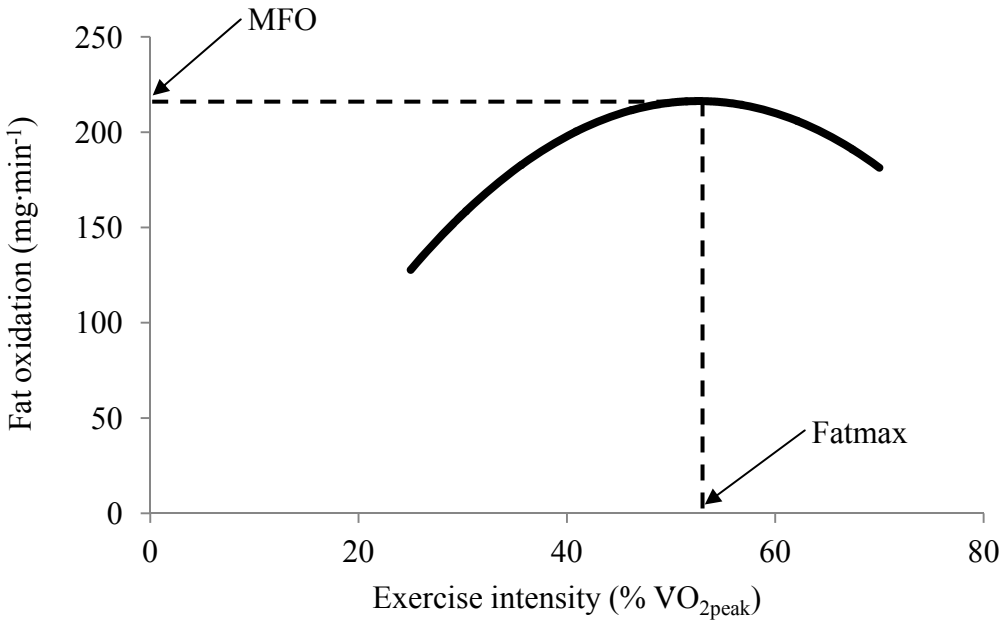


Figure 2.1 Fatmax and maximal fat oxidation (MFO)

Fat oxidation during exercise has traditionally been assessed using a small number of prolonged steady state exercise bouts to ensure the valid use of indirect calorimetry in adults (Friedlander et al., 1998; Romijn et al., 1993) and young people (Mácek et al., 1976; Rowland and Rimany, 1995). Using this method, comparative studies have determined the influence of age (Martinez and Haymes, 1992; Rowland and Rimany, 1995), exercise mode (Mácek et al., 1976) and training status (Duncan and Howley, 1998) on fat oxidation in young people. However, the use of a small number of exercise intensities used to estimate fat oxidation does not allow for a detailed examination of the effect of exercise intensity on fat oxidation and thus a precise estimation of Fatmax. Moreover, findings from comparative studies using a limited range of intensities may be confounded for failing to account for possible interactions with intensity.

Consequently, Achten et al. (2002) developed and validated a protocol to assess fat oxidation over a wide range of exercise intensities in trained men. Fat oxidation during an incremental exercise test with 3 min stages was compared with isolated exercise bouts performed at similar intensities. This comparison was required since there are two potential issues with a 3 min incremental protocol: (1) whether a physiological steady state is attained before the onset of the sampling period; and (2) whether there is a residual effect from stage to stage as the increments progress that influence subsequent fat oxidation estimations. Using the data from the 3 min incremental protocol, the relationship between fat oxidation and exercise intensity was determined and Fatmax was estimated subsequently for each individual. It was concluded that an incremental exercise test on a cycle ergometer starting at 95 W with 35 W increments every 3 min to exhaustion can be used for the determination of Fatmax and MFO in trained men, where Fatmax occurred at 64% $V\dot{O}_{2peak}$. In addition to the identification of Fatmax, the 3 min incremental exercise test was used to estimate the range of intensities where fat oxidation was within 10% of maximum, which was termed the 10% Fatmax zone (Achten et al., 2002). This spanned a relatively large range of intensities between 55 and 72% $V\dot{O}_{2peak}$. However, a potential flaw of this study was that fat oxidation was estimated from average $V\dot{O}_2$ and $V\dot{C}O_2$ values from the final 2 min of each 3 min exercise stage, whereas a steady state is normally reached in 2 to 3 min in adults (Xu and Rhodes, 1999). It is, therefore, possible that not all participants reached a steady state after the first minute of each stage, questioning the validity of indirect calorimetry (Frayn, 1983). It should also be highlighted that comparisons of fat oxidation between the two protocols were only made at the group level and examination of within-participant random variability between the measures would have been more insightful (Bland and Altman, 1986; Ludbrook, 1997).

The 3 min incremental protocol is desirable for practical reasons and allows the estimation of fat oxidation across a wide range of exercise intensities compared with the use of prolonged isolated exercise bouts. Consequently, this protocol has been adapted for other studies with adults (Venables et al., 2005) and children (Riddell et al., 2008). However, the conclusion that the 3 min protocol provides a valid estimation of Fatmax reached by Achten et al. (2002) was challenged more recently (Meyer et al., 2007; 2009). Meyer et al. (2007) reported that Fatmax could not be determined using five 1 hour exercise bouts at different intensities below the individual anaerobic threshold

(IAT), as there was no significant difference in fat oxidation between exercise intensities for the group. However, fat oxidation did begin to decline at the highest exercise intensity and the inclusion of intensities above IAT may be required to elicit a significant decline in fat oxidation. Other potential limitations of this study should also be highlighted; daily fluctuations in RER may have influenced comparisons in fat oxidation between the 1 hour exercise bouts performed on 5 different days (Bagger et al., 2003), participants were not fasted and CHO ingestion has a profound effect on Fatmax and fat oxidation (Achten and Jeukendrup, 2003b), the group rather than individual level analysis and small sample size (10 male athletes) may have limited the ability to detect significant differences in fat oxidation and, finally, one of the men was not able to complete the highest intensity for 1 h due to exhaustion, suggesting the protocol may be inappropriate for other groups, such as children and individuals with low fitness levels.

In a follow-up study, this group also questioned the reliability of Fatmax estimated using a 6 min incremental exercise protocol, reporting very large intra-individual variability in Fatmax, expressed as V□O_2 ($L \cdot min^{-1}$) (Meyer et al., 2009). The combination of only five exercise stages and a subjective visual interpretation of the data may have precluded a precise estimation of Fatmax and contributed to a large proportion of the observed intra-individual variability. When using a 3 min incremental cycle test to estimate Fatmax, the coefficient of variation (CV) was 9.6% (Achten and Jeukendrup, 2003a) and a moderate test–retest reliability (r=0.60) was reported (Glass et al., 1999). However, using a bivariate relationship to quantify reliability does not account for systematic bias in the paired measurements and may depend on the range of values within the sample (Bates et al., 1996; Bland and Altman, 1995).

2.4 Protocols to estimate Fatmax in young people

Since the work by Achten et al. (2002), a variety of protocols have been used to estimate Fatmax in young people. The majority of studies have used incremental exercise tests, with stages of 3 (Riddell et al., 2008), 3.5 (Zunquin et al., 2009a; 2009b), 4 (Aucouturier et al., 2009; Lazzer et al., 2010), 5 (Lazzer et al., 2007) and 6 (Brandou et al., 2006) min. However, adapting a protocol validated using trained adult male cyclists (Achten et al., 2002) may not be appropriate for other populations, including young people. Although 3 min stages were suggested to be too short to estimate Fatmax

in sedentary adults, an average underestimation of 2 W is unlikely to have any meaningful practical implications (Bordenave et al., 2007) and others have considered a mean step-increment of 26 W to be 'small' when estimating Fatmax (Meyer et al., 2009). To the author's knowledge, no studies have assessed potential differences in Fatmax between the different protocols employed in young people. Nevertheless, $V\dot{O}_2$ kinetics research has provided evidence that children reach a steady state faster than adults (Fawkner et al., 2002), suggesting that indirect calorimetry is valid earlier after exercise onset and supporting the use of shorter stages in this population. Furthermore, prior moderate or heavy intensity exercise does not affect the $V\dot{O}_2$ kinetic response to subsequent moderate exercise in adults (Burnley et al., 2000; Gerbino et al., 1996), whilst prior heavy intensity exercise may even speed $V\dot{O}_2$ kinetics during heavy intensity exercise (Gerbino et al., 1996). This implies that early exercise stages will not affect the time taken to reach a steady state during an incremental exercise test, although these studies used two square wave transitions separated by a 6 min rest period rather than an incremental protocol. Studies in children do not appear to have examined the potential residual effect of earlier exercise stages on fat oxidation during later exercise stages. In adults, prior bouts of exercise may increase fat oxidation (Goto et al., 2007) and adipose tissue lipolysis (Stich et al., 2000) during subsequent exercise when compared with a single bout of prolonged exercise. Also, warm-up influences the metabolic response during a subsequent exercise bout, possibly due to increased blood flow and oxygen delivery to active muscles (Robergs et al., 1991), increased muscle temperature (Starkie et al., 1999) and lower blood and muscle lactate accumulation (Gray et al., 2002).

Alternatively, other studies in young people have continued to use a more traditional approach of isolated exercise bouts lasting 6 min (Stephens et al., 2006) and 8 to 10 min (Maffeis et al., 2005) with standardised recovery periods to assess fat oxidation. The use of longer duration exercise bouts with rest periods increases the likelihood of attaining a steady state and may reduce the potential residual effect of previous exercise stages on fat oxidation. However, the use of this type of protocol has only allowed for the assessment of fat oxidation at three (Maffeis et al., 2005) and five (Stephens et al., 2006) different exercise intensities. Although an understanding of the relationship between exercise intensity and fat oxidation may be possible, the small number of different exercise intensities precludes a precise estimation of Fatmax.

A general issue with the protocols used to estimate fat oxidation is that during very prolonged exercise fat oxidation rates increase with time and are, therefore, unlikely to attain a 'true' steady state in both children (Delamarche et al., 1992; Timmons et al., 2003) and adults (Romijn et al., 1992). Therefore, it should be noted that fat oxidation values estimated from protocols using short stages will likely underestimate those during prolonged exercise.

2.4.1 Determination of $V\dot{O}_{2peak}$

The accurate determination of $V\dot{O}_{2peak}$ is essential for a meaningful expression of Fatmax, a parameter invariably expressed relative to this measurement. Several studies have used progressive exercise tests lasting 8 to 12 min to determine $V\dot{O}_{2peak}$ when assessing Fatmax in young people (Aucouturier et al., 2009; Maffeis et al., 2005; Stephens et al., 2006), whereas others have determined fat oxidation and $V\dot{O}_{2peak}$ during the same test lasting around 30 min in the fasted (Riddell et al., 2008) and non-fasted (Zunquin et al., 2009a; 2009b) state. It is possible that the longer stage and overall exercise duration in the latter studies resulted in blunted $V\dot{O}_{2peak}$ values, although evidence in this area is unclear (Bentley et al., 2007). In addition, examination of maximal effort criteria suggests that $V\dot{O}_{2peak}$ may not have been reached consistently in some studies (Aucouturier et al., 2009; Riddell et al., 2008; Stephens et al., 2006).

Many of the studies that have estimated Fatmax in obese young people have not measured $V\dot{O}_{2peak}$ directly, but have provided values based on the predictive equations of Wasserman et al. (1987) (Brandou et al., 2003; 2005; 2006) and the $V\dot{O}_2$-heart rate relationship up to theoretical maximum heart rate (HR_{max}) and American College of Sports Medicine prediction equations (ACSM, 1995) (Lazzer et al., 2007; 2008). The theoretical maximal aerobic power (W_{max}th) has also been calculated using what Brandou et al. (2003; 2005; 2006) described as the Tanner equation, although it has not been possible to locate the original reference for this equation. However, Aucouturier et al. (2009) reported that the predictive equations of Wasserman et al. (1987) and the ACSM prediction equations overestimated $V\dot{O}_{2peak}$ compared with $V\dot{O}_{2peak}$ values obtained using a progressive incremental exercise test in obese young people. Consequently, 95% limits of agreement demonstrated that Fatmax was underestimated when expressed as % $V\dot{O}_{2peak}$ determined using the Wasserman et al. (1987) equations

(% V□O_{2peak}W) and the ACSM prediction equations (% V□O_{2peak}ACSM). Agreement between methods was also poor when comparing Fatmax expressed relative to measured (% W_{max}m) and theoretical (% W_{max}th) maximal aerobic power. Despite these concerns, it should be noted that the direct measurement of V□O_{2peak} may not be feasible in certain populations and within study comparisons in Fatmax may not be influenced when measuring V□O_{2peak} indirectly providing the error is consistent.

2.4.2 Identification of Fatmax

Fatmax is typically identified using a graph of fat oxidation vs. exercise intensity and curve-fitting techniques (Achten et al., 2002). The start of the sampling period for V□O_2 and V□CO_2 used to estimate fat oxidation at each exercise intensity should be considered to assess whether the values used to estimate fat oxidation reflect a physiological steady state. Studies in young people have used respiratory values from 2.5 to 3 min (Riddell et al., 2008), 3 to 3.5 min (Zunquin et al., 2009a; 2009b), 3 to 4 min (Aucouturier et al., 2009), 3 to 6 min (Brandou et al., 2005; 2006), 4 to 5 min (Lazzer et al., 2007), 5 to 6 min (Stephens et al., 2006) and 5 to 10 min (Maffeis et al., 2005) of exercise. Typically, it has been assumed that a steady state has been reached by the beginning of these sampling periods (Riddell et al., 2008) and only rarely have measurements been taken to confirm the attainment of a steady state in RER (Stephens et al., 2006).

Although a clear advantage of an incremental test is the estimation of fat oxidation over a large number of intensities, studies have often not stated the number of exercise intensities used to derive the curve for the estimation of Fatmax (Riddell et al., 2008; Zunquin et al., 2009a; 2009b). Some studies using incremental exercise tests have estimated Fatmax using only five intensities (Brandou et al., 2003; 2006; Lazzer et al., 2007), which is no more than when using isolated exercise bouts (Stephens et al., 2006). Furthermore, exercise intensities have often been restricted to up to 60% V□O_{2peak} (Brandou et al., 2003; Zunquin et al., 2009a; 2009b), although Fatmax may have occurred out of this range (>60% V□O_{2peak}) in some individuals due to high inter-individual variability in Fatmax values (Riddell et al., 2008). In addition, only one study using curve-fitting techniques has reported r^2 values (Riddell et al., 2008) to provide an indication of the goodness of the fit.

In studies using isolated exercise bouts, the highest rate of fat oxidation and corresponding exercise intensity (Fatmax) have been identified visually rather than using curve-fitting procedures (Maffeis et al., 2005; Stephens et al., 2006). This provides an indication of where the protocol specific peak fat oxidation and Fatmax occurred, rather than an interpolated estimation that may proffer greater precision. Furthermore, fat oxidation values for each intensity were pooled and the highest fat oxidation rate was determined from the averaged values, thus these studies did not account for individual differences in Fatmax. Riddell et al. (2008) used both polynomial curve-fitting techniques and a simple visual method to calculate Fatmax and MFO on an individual basis. However, as the difference between the modelling and visual method was not examined systematically, the level of agreement between these two methods is not available.

It is clear that the techniques used to construct individual curves of fat oxidation against exercise intensity are not standardised across studies. Using individual curves is common and provides a more consistent measure of Fatmax for data that do not align to a perfect curve compared with using point estimates (measured values), although they may underestimate the measured MFO in adults (Chenevière et al., 2009). Alternatively, measured Fatmax values provide a direct indication and may be preferred for this reason. Therefore, it may be prudent to report both modelled and measured values.

2.5 Factors influencing Fatmax and fat oxidation during exercise in young people

Fat oxidation during exercise can influenced by several factors relating to the exercise characteristics (intensity, mode, duration), participant (sex, puberty, training) and pre-exercise diet (Achten and Jeukendrup, 2003b; Boisseau and Delamarche, 2000; McMurray and Hosick, 2011; Venables et al., 2005). The following section reviews potential factors affecting exercise fat oxidation in young people, including puberty, sex, obesity, exercise mode and exercise training, by drawing on studies that have assessed fat oxidation over a range of intensities.

2.5.1 Puberty

It was first demonstrated that children have lower RER values during exercise compared with adults 70 years ago (Robinson, 1938). Lower RER values have been

observed consistently in children compared with adults during submaximal exercise performed at similar absolute (Montoye, 1982; Robinson, 1938) and relative (Foricher et al., 2003; Martinez and Haymes, 1992) exercise intensities, indicating higher rates of fat oxidation in children. Younger children also have higher fat oxidation rates compared with older children during exercise at the same relative intensity (Timmons et al., 2007a; 2007b), suggesting puberty may modulate these age-related changes in fat oxidation. Only recently has the effect of puberty on fat oxidation during exercise been assessed over a wide range of intensities (Brandou et al., 2006; Stephens et al., 2006; Riddell et al., 2008; Zunquin et al., 2009a).

In the only longitudinal study available, the effect of pubertal status on Fatmax was examined in five boys (Riddell et al., 2008). With a baseline age of 11 to 12 years, Fatmax was estimated annually over a period of 3.5 years and compared independently to nine adult males, who were only assessed on one occasion. Fatmax was higher in the boys at each pubertal stage compared with the men and decreased with increasing pubertal status over the 3.5 years. Similarly, MFO ($mg \cdot kg$ lean body $mass^{-1} \cdot min^{-1}$) was comparable at Tanner stage 1 and 2, but had decreased to values akin to the men by Tanner 4. Fatmax was assessed using a 3 min incremental protocol and polynomial curve fitting-procedures; fat oxidation was estimated using values from the final 30 s of each stage, increasing the likelihood that a steady state was achieved when compared to the 2 min sampling period used by Achten et al. (2002). Although it was stated that a physiological steady state was reached during each 3 min stage, it is not clear how this was verified. The longitudinal design is a major strength of this study, permitting a more causative interpretation of the findings than cross sectional studies. Furthermore, this appears to be the only study to report the strength of the polynomial models used to define Fatmax; the r^2 values were 0.75 (Tanner 1), 0.85 (Tanner 2/3), 0.79 (Tanner 4) and 0.81 (men), suggesting relatively strong goodness of fit. Despite these strengths, the small sample size limits the wider applicability of the findings, which should be replicated on a larger scale with both boys and girls.

In support of Riddell et al. (2008), cross-sectional studies examining the influence of puberty on Fatmax using incremental exercise protocols have demonstrated that Fatmax and MFO (expressed per kg FFM) declines as pubertal status increases in obese children (Brandou et al., 2006; Zunquin et al., 2009a). In another study, the influence of

puberty on fat oxidation was assessed using fasted 5 to 6 min exercise bouts at 30, 40, 50, 60 and 70% V□O_{2peak} interspersed with 5 to 10 min rest periods in early-, mid- and late-pubertal boys and young men (Stephens et al., 2006). Fat oxidation was higher in prepubertal compared with pubertal boys. Peak fat oxidation was observed at 40% V□O_{2peak} in the early and mid-pubertal boys and 30% V□O_{2peak} in the late-pubertal boys, again implying that Fatmax decreases with puberty, although a precise estimation of Fatmax was precluded by the use of visual analysis to identify Fatmax rather than using customary individual curve-fitting procedures. However, it is noteworthy that participants performed a second V□O_{2peak} test if maximal effort was not achieved initially. It was concluded that puberty reduces fat oxidation during exercise, with the development of an adult-like metabolic profile occurring between mid- to late-puberty and being complete by the end of puberty (Stephens et al., 2006). Very recent data indicate that the higher fat oxidation observed in prepubertal compared with pubertal boys may not apply to girls; no difference in fat oxidation (per kg FFM) during exercise was found between prepubertal and pubertal girls (McMurray and Hosick, 2011). This suggests a need for more studies including girls.

Collectively, the available research suggests that advances in puberty reduce both Fatmax and the ability of FFM to oxidise fat during exercise in boys, a finding that has not been replicated in girls. However, the practical implications of 3 (Brandou et al., 2006), 4 (Zunquin et al., 2009a) and 9 (Riddell et al., 2008) % V□O_{2peak} reductions in Fatmax between prepubertal and postpubertal young people are more difficult to interpret and may have limited practical significance. A much larger reduction in Fatmax (25% V□O_{2peak}) seems to occur when comparing the extremes of prepubertal boys and adult males (Riddell et al., 2008). These findings highlight the need to consider pubertal status when investigating exercise fat oxidation in young people. This may be achieved by providing an assessment of secondary sexual characteristics using Tanner scales (Tanner, 1962). Although this measure of pubertal status is widely used in paediatric exercise science (e.g., Brandou et al., 2006; Riddell et al., 2008; Stephens et al., 2006; Zunquin et al., 2009a), potential limitations should be highlighted. Firstly, individual variation and between-sex variation in the timing and tempo of pubertal events have long been recognised (Marshall and Tanner, 1969; 1970). Indeed, the length of time taken to progress through a stage depends on the individual and what is actually being measured is the interval between two stages. Problems in aligning

individuals may occur when an individual in the early phase of a stage of pubic hair development is rated the same as an individual in the late phase of the same stage and, as different pubertal events occur at different times, a girl at stage 3 of breast development is not necessarily at stage 3 for pubic hair development. Furthermore, girls enter and end puberty approximately 2 years before boys and pubertal events do not occur in the same sequence between the sexes. Therefore, it is difficult to align girls and boys for pubertal status when making between-sex comparisons (Sherar et al., 2004). The categorisation of Tanner stages can be performed by a trained physician (e.g., Brandou et al., 2006; Stephens et al., 2006; Zunquin et al., 2009a) or self reported by the participant (e.g., Riddell et al., 2008). Direct visual observation may be appropriate for clinical settings, but many young people (and their parents) feel uncomfortable with this method. In order to address these concerns, self-assessment techniques were developed and there is evidence that this method is accurate and reliable (Matsudo and Matsudo, 1994; Schlossberger et al., 1992; Williams et al., 1988). However, there are still concerns that young people may overestimate early stages and underestimate later stages of pubertal development (Cameron, 2002).

Mechanisms controlling the higher Fatmax and fat oxidation in children are unclear. It has been proposed that high fat oxidation in children may be a default mechanism due to an underdeveloped glycolytic system; muscle biopsies have provided evidence of a higher proportion of type I muscle fibres in children compared with untrained adults (Bell et al., 1980; Fournier et al., 1982) and reduced activity of the enzymes phosphofructokinase (PFK) (Eriksson, 1972) and lactate dehydrogenase (LDH) (Kaczor et al., 2005) in children compared with adults. However, these results are limited and have not always been confirmed (Berg et al., 1986; Haralambie, 1982). During exercise, blood lactate concentration, an intermediate of CHO oxidation, is also lower in children than adults (Mácek et al., 1976; Mahon et al., 1997) and is inversely related to fat oxidation (Achten and Jeukendrup, 2004), perhaps due to an inhibition of adipose tissue lipolysis (Boyd et al., 1974). Moreover, the lactate increase above baseline (LIAB) coincides with the intensity at which fat oxidation begins to decline in children (Tolfrey et al., 2010) and adults (Achten and Jeukendrup, 2004) and the increase in blood lactate with intensity is more pronounced in men than boys (Mahon et al., 1997). This suggests increased lactate concentration between childhood and adulthood may explain reductions in both fat oxidation rates and Fatmax with puberty. Children also appear to

rely less on endogenous CHO; muscle biopsy data have shown reduced muscle glycogen content in children (Erikson et al., 1971; 1973) and, using stable isotope techniques, reduced endogenous CHO oxidation during exercise was reported in children (Timmons et al. 2003; 2007a; 2007b). However, exogenous CHO oxidation was higher in boys than men (Timmons et al., 2003) and decreased with puberty (Timmons et al., 2007a). No difference in exogenous CHO oxidation was reported in 12 and 14 year old girls, although this may have been due to the small difference in puberty between the two groups (Tanner 3 and 4) (Timmons et al., 2007b). This work suggests that children do not have an underdeveloped glycolytic flux and it is more likely that glycogen stores limit CHO oxidation. It is possible that higher IMTG stores observed in prepubertal children may contribute to an increased ability to oxidise fat, although such data obtained from muscle biopsy in children are extremely limited and may depend on training status (Bell et al., 1980). Increased FFA availability and uptake during exercise in children compared with adults has also been reported (Delamarche et al., 1992), but not always confirmed (Boisseau and Delamarche, 2000; Martinez and Haymes, 1992). Although no age-related changes in CPT activity or major differences in enzyme activities of fat metabolism were observed in children compared with adults (Haralambie, 1982; Kaczor et al., 2005), the CPT/2-oxoglutarate dehydrogenase ratio of enzyme activities in skeletal muscle may be higher in children (Kaczor et al., 2005), suggesting a preferential oxidation of fat over CHO.

2.5.2 Sex

The effect of sex on fat oxidation may be negligible before puberty, as between-sex differences in fat oxidation in adults appear to be due to sex hormones (Campbell and Febbraio, 2001; Hamadeh et al., 2005; Timmons et al., 2007a). Despite the documented effect of oestrogen supplementation increasing fat oxidation (Hamadeh et al., 2005), the effect of menstrual cycle phase on substrate oxidation remains unclear (Oosthuyse and Bosch, 2010). During exercise, testosterone concentration was inversely correlated with endogenous fat and exogenous CHO oxidation, but positively correlated with endogenous CHO oxidation, in boys (Timmons et al., 2007a). In girls, oestrogen concentration was inversely correlated with total fat and positively correlated with endogenous CHO oxidation (Timmons et al., 2007b). Thus, increases in sex hormones during the pubertal period may affect fat metabolism, resulting in differences in fat oxidation rates between the sexes.

The vast majority of studies assessing Fatmax in young people have only included boys (Table 2.1). It appears that only one study has compared Fatmax in boys and girls, specifically a group of obese and non-obese pubertal and postpubertal young people (Lazzer et al., 2007). It was found that sex did not influence Fatmax in the obese or non-obese groups. In agreement, no effect of sex on Fatmax was reported in overweight adults (Bogdanis et al., 2008), although Fatmax was higher in healthy women compared with men (Chenevière et al., 2011). Lazzer et al. (2007) also reported absolute MFO and fat oxidation at 30, 40, 50, and 60% V□O_{2peak} was higher in obese and non-obese boys compared to girls. However, the higher FFM and predicted V□O_{2peak} in the boys may explain these higher fat oxidation rates, rather than an independent sex effect. Indeed, a recent study reported no difference in FFM relative fat oxidation during three exercise intensities between girls and boys (McMurray and Hosick, 2011) and the higher absolute MFO in overweight men compared with women was diminished when scaled to FFM (Bogdanis et al., 2008). When men and women were matched for age, BMI and V□O_{2peak}, MFO and fat oxidation between 35 and 85% V□O_{2peak} (expressed per kg FFM) was greater in the women (Chenevière et al., 2011). In agreement, studies with adults have shown that women rely more on fat compared with men when comparing single exercise intensities (Devries et al., 2007; Tarnopolsky et al., 2007). Further research is required to examine the independent effect of sex on fat oxidation in young people, where participants are carefully matched for pubertal status, fitness and body composition.

2.5.3 Obesity

It is of great interest to estimate Fatmax in obese young people, since low fat oxidation may predispose obesity and insulin resistance (see section 2.1). Many Fatmax studies with young people have included obese individuals and have shown that Fatmax tends to occur around 40 to 55% V□O_{2peak} in this group (Table 2.1). When comparing obese and non-obese pubertal boys directly, Fatmax and MFO expressed per kg FFM were lower in the obese boys (Zunquin et al., 2009b). In addition, fat oxidation was lower in the obese boys during exercise at 40, 50 and 60% V□O_{2peak}, with no difference at 20 and 30% V□O_{2peak}. When obese and non-obese adolescent girls and boys were matched for V□O_{2peak} and physical activity, Lazzer et al. (2007) reported only slightly lower Fatmax values in the obese groups and no difference in absolute fat oxidation rates over

five different exercise intensities. However, absolute fat oxidation tended to be higher in the obese adolescents for exercise intensities lower than 40% V□O_{2peak} and lower for exercise intensities above 40% V□O_{2peak}, which supports the results of Zunquin et al. (2009b). These findings suggest that obesity may reduce relative (Zunquin et al., 2009b) and absolute (Lazzer et al., 2007) fat oxidation during moderate intensity exercise (40 to 60% V□O_{2peak}). Importantly, matching obese and non-obese adolescents for fitness may reduce between-group differences in Fatmax and fat oxidation (Lazzer et al., 2007), suggesting increased cardiorespiratory fitness can improve these metabolic markers. Indeed, Fatmax and MFO are higher in trained compared with untrained adults (Nordby et al., 2006). However, predicted measures of V□O_{2peak} used in the study by Lazzer et al. (2007) may not reflect measured V□O_{2peak} (Aucouturier et al., 2009) and assessments of physical activity based on a questionnaire (Lazzer et al., 2004) rather than more objective measures, such as accelerometry and doubly labelled water, should be interpreted with caution (Arvidsson et al., 2005; Rush et al., 2008). Indeed, the sporadic nature of children's physical activity (Bailey et al., 1995) makes these activities difficult to recall, quantify and categorise and the lower cognitive functioning of children compared with adults reduces their ability to accurately recall the intensity, frequency and duration of activities (Sallis, 1991). Consequently, these findings require confirmation.

Few studies have examined the relationship between exercise intensity and fat oxidation during treadmill exercise. Maffeis et al. (2005) studied fat oxidation at 4, 5 and 6 $km \cdot h^{-1}$ in prepubertal boys heterogeneous for adiposity using 8 to 10 min exercise bouts. This may be a compromise between prolonged bouts which would not be appropriate for children (Meyer et al., 2007) and incremental protocols (Achten et al., 2002). However, a precise estimation of Fatmax was not possible from only three walking speeds. Peak fat oxidation occurred at the slowest speed and corresponded to an exercise intensity of 45% V□O_{2peak}, which is similar to the 42% V□O_{2peak} treadmill Fatmax later reported in obese boys (Lazzer et al., 2010). However, the possibility that higher fat oxidation may have occurred at even slower speeds should not be discounted, as data below the peak fat oxidation were not available. Moreover, fat oxidation did not change significantly with increasing walking speed, suggesting that larger increases in exercise intensity are required to elicit changes in fat oxidation in obese boys. This could also support the contention that fat oxidation may be relatively stable at exercise intensities below the

lactate threshold (Meyer et al., 2007). The lack of difference in fat oxidation between tertiles of obesity (Maffeis et al., 2005) may suggest that larger differences in adiposity are required to detect differences in fat oxidation. However, these findings were based on absolute rates of fat oxidation; the similar fat oxidation coupled with higher FFM in the severely obese group indicates that they oxidised less fat relative to FFM. Moreover, CHO oxidation was higher in the heavier children and a closer examination of the results suggested a tendency for fat oxidation to decrease with increasing severity of obesity; the non-significant differences may have resulted from a lack of statistical power (eight participants per tertile group). In support, a very recent study that estimated substrate oxidation at 4, 5.6 and 8 $km \cdot h^{-1}$ reported greater CHO oxidation with similar fat oxidation in overweight compared with non-overweight girls and boys (McMurray and Hosick, 2011). Similar to the study of Maffeis et al. (2005), fat oxidation peaked at the slowest speed and corresponded to approximately 30 to 40% $V\dot{O}_{2peak}$ in the overweight and non-overweight children. The lack of difference between groups (McMurray and Hosick, 2011) is in contrast to reports of reduced Fatmax values in obese young people (Zunquin et al., 2009b), although the inclusion of only three intensities did not allow a precise estimation of Fatmax.

The lower Fatmax observed in obese young people suggests that this population must exercise at a slightly lower intensity to elicit MFO and that fat oxidation begins to decline at a lower exercise intensity, which may limit fat oxidation to a narrower range of exercise intensities. However, it is not clear whether this difference in Fatmax is particularly meaningful and there is limited evidence to support this finding (Lazzer et al., 2007; Zunquin et al., 2009b). It is also difficult to compare the findings of studies that have included participants with different characteristics, such as sex and pubertal status, and different exercise protocols performed in the fasted (Lazzer et al., 2007) and non-fasted (Maffeis et al., 2005; Zunquin et al., 2009b) states. Adult data, however, support these findings, with reports that Fatmax and fat oxidation (absolute and FFM relative) over a range of intensities were reduced in obese compared with non-obese adults (Pérez-Martin et al., 2001), although lower rates of fat oxidation have not always been confirmed in studies using fewer intensities (Goodpaster et al., 2002; Steffan et al., 1999). Therefore, these findings require clarification in both young people and adults.

The mechanisms contributing to lower rates of fat oxidation in obese individuals are not well documented in young people, but have been examined more extensively in adults. It should be noted that these mechanisms are often based on resting measures and may not directly relate to exercise. Hormonal responses in obese young people have the potential to inhibit fat oxidation, including reduced growth hormone concentration, greater leptin and insulin concentrations and a blunted adrenaline response to exercise (Eliakim et al., 2006; McMurray and Hackney, 2005). Obesity also blunts the influence of oestrogen on fat metabolism (McMurray and Hackney, 2005). A lower percentage of type I muscle fibres, which primarily oxidise fat, has also been proposed to limit fat oxidation in obese individuals (Karjalainen et al., 2006; Kriketos et al., 1997). Lipolysis may decrease with obesity in adults (Blaak, 2003) and there is some evidence indicating reduced lipolysis and FFA availability in obese young people; under resting conditions obese children had a blunted rise in plasma FFA and glycerol during adrenaline infusion (Bougnères et al., 1997) and obese adolescent girls had an impaired responsiveness of ß2-adrenergic receptors to promote lipolysis in adipose tissue (Enoksson et al., 2000). Others have suggested that reductions in fat oxidation may not be explained by reduced FFA delivery, as obesity is associated with increased plasma FFA concentrations in young people (Heptulla et al., 2001) and adults (Boden, 2003) and increased plasma membrane FFA transport in adults (Bonen et al., 2004). Therefore, a reduction in the capacity of mitochondria to oxidise fat has been proposed as a plausible mechanism (Kelley et al., 2002b; Ritov et al., 2005). Reduced FFA transport into the mitochondria may limit fat oxidation in the obese. This has been attributed to reduced CPT-1 activity (Kim et al., 2000; Simoneau et al., 1999), which may be related to higher malonyl-CoA concentrations (Bandyopadhyay et al., 2006), and the redistribution of FAT/CD36 to the plasma membrane making less available for the mitochondria (Bonen et al., 2004). Moreover, obesity has been associated with an inability of FAT/CD36 to translocate to the plasma membrane in response to muscle contraction in rodents (Han et al., 2007). Increased FABP content was associated with increased weight loss and fat oxidation, suggesting a potential role in the low fat oxidation rates in obese individuals (Blaak et al., 2001). Although both mitochondrial dysfunction and content may be implicated in obesity-induced reductions in fat oxidation, mitochondrial content appears to be more important (Holloway et al., 2009). Obese adults have markedly reduced mitochondrial size, even when corrected for fibre type distribution (Kelley et al., 2002b) and reduced activity of the key enzymes citrate

synthase and β-hydroxy acyl dehydrogenase, which are common markers of mitochondrial volume and fat oxidation (Kim et al., 2000; Holloway et al., 2007; Simoneau et al., 1999). Furthermore, low levels of enzyme activities of the TCA cycle, the electron transport chain and β-oxidation have been observed in obese insulin resistant adults who concomitantly exhibit high resting RER values (Kelley et al., 1999; Simoneau et al., 1999).

2.5.4 Exercise mode

Studies comparing fat oxidation between exercise modes (treadmill and cycling exercise) in young people have traditionally used a small number of exercise intensities corresponding to the exercise mode-specific $V\dot{O}_{2peak}$ (Mácek et al., 1976). However, $V\dot{O}_{2peak}$ is typically 7 to 10% higher for treadmill compared with cycling exercise in untrained young people and adults (Mácek et al., 1976; Millet et al., 2009), thus comparing relative exercise intensities often results in a higher absolute $V\dot{O}_2$ for treadmill exercise, which may explain differences in fat oxidation between exercise modes. The comparison of fat oxidation over a wide range of both relative (% mode-specific $V\dot{O}_{2peak}$) and absolute (absolute $V\dot{O}_{2peak}$ values) exercise intensities may overcome these issues and is perhaps the most appropriate procedure for examining the effect of exercise mode on fat oxidation.

Lafortuna et al. (2010) estimated fat oxidation over a range of seven intensities during treadmill and cycling exercise in obese adolescent boys. Treadmill exercise promoted higher fat oxidation and lower RER values compared with cycling at comparable absolute and relative intensities (Lafortuna et al., 2010). Although similar data in young people do not appear to be available, the higher fat oxidation during treadmill compared with cycling exercise over a range of intensities is emerging as a consistent finding in adults (Achten et al., 2003; Capostagno and Bosch, 2010; Glass et al., 1999). However, a recent study reported no difference in MFO between treadmill and cycling exercise in moderately trained men and women (Chenevière et al., 2010).

To the author's knowledge, research directly comparing Fatmax between treadmill and cycling exercise in young people is not available. Although Fatmax was not estimated in the study of Lafortuna et al. (2010), graphical representations of fat oxidation versus exercise intensity suggest that peak fat oxidation occurred at a slightly higher intensity

during treadmill exercise in the obese adolescent boys. Similarly, Fatmax was higher for treadmill compared with cycling exercise in moderately trained adults (Chenevière et al., 2010). In contrast, other studies with adults have shown no difference in Fatmax between treadmill and cycling exercise in moderately trained men (Achten et al., 2003) or untrained men and women (Glass et al., 1999). Differences in the treadmill exercise protocols used in these adult studies may explain such discrepancies, where treadmill intensity has been increased via changes in gradient (Achten et al., 2003) or speed (Chenevière et al., 2010).

The higher fat oxidation during treadmill exercise coincided with lower blood lactate concentrations compared with cycling exercise in the obese boys (Lafortuna et al., 2010), indicating blood lactate accumulation may be a possible mechanism limiting fat oxidation during cycling in young people. In line with this, higher blood lactate and lower arterial pH and bicarbonate concentrations have been reported during cycling compared with treadmill exercise at comparable metabolic rates in adults (Miles et al., 1980). Hydrogen ion accumulation in the sarcoplasm may inhibit CPT-1 activity, thus reducing fat oxidation (Starritt et al., 2000). The reduced muscle mass used during cycling compared with running performed at the same relative intensity has a long standing history (Hermansen and Saltin, 1969). This would result in a higher energy expenditure relative to active muscle mass and the recruitment of more type II muscle fibres during cycling, inducing a higher contribution of CHO and lower contribution of fat to energy expenditure. Furthermore, the release of catecholamines is proportional to exercising muscle mass (Lewis et al., 1983). As catecholamines are potent stimulators of lipolysis (Martin, 1996), the larger muscle mass during treadmill exercise may elicit a larger catecholamine response, contributing to increased FFA mobilisation and oxidation. Also, the eccentric muscle action in running may delay peripheral fatigue and reduce the recruitment of type II muscle fibres during running compared with cycling for the same relative exercise intensity (Carter et al., 2000).

2.5.5 Exercise training

A potential important practical application of Fatmax relates to exercise prescription to enhance fat oxidation, a training adaptation that may have particular relevance for obesity and insulin resistance (see section 2.1). It is well documented that exercise training improves insulin sensitivity in young people (Bell et al., 2007; Shaibi et al.,

2008). In adults, enhancements in insulin sensitivity following training occur with concomitant increased IMTG stores, reduced FA metabolite accumulation and increased fat oxidation (Bruce et al., 2006; Dubé et al., 2008; 2011), suggesting the increased fat oxidation may mediate improvements in insulin sensitivity through reductions the accumulation of harmful FA metabolites. Moreover, increased IMTG content is a very early response to training, preceding improvements in insulin sensitivity (Schrauwen-Hinderling et al., 2003). Although both low (Schrauwen et al., 2002) and high (Perry et al., 2008) intensity training can increase the capacity to oxidise fat, it is plausible that training at individual Fatmax can optimise these effects. Indeed, the primary benefit of training at this intensity is the promotion of high fat oxidation rates. Furthermore, it is possible that shifting Fatmax to a higher intensity may also favour fat oxidation by reducing the range of higher exercise intensities at which fat oxidation is low. The maintenance of high fat oxidation rates at high exercise intensities may also delay glycogen depletion and fatigue (Noakes, 2000). However, the practical significance of shifting Fatmax has not been investigated systematically.

Training at Fatmax has been shown to both increase (Brandou et al., 2003) and have no effect (Lazzer et al., 2008) on Fatmax in obese young people. When increases in Fatmax were demonstrated, obese adolescents completed a combined programme involving dietary manipulation, nutrition classes and 45 min cycling at Fatmax every day for two weeks and, subsequently, a sub-group continued exercise training for a further 6 weeks (Brandou et al., 2003). Increased Fatmax (32 to 45% W_{max}th) and absolute fat oxidation at 30, 40 and 50% W_{max}th were only observed following the full eight week programme. However, part of this 'improvement' may be due to random variation in the measurement of Fatmax (Meyer et al., 2009) and control of diet or exercise in the days prior to testing was not mentioned. Interestingly, Fatmax was unchanged following a multidisciplinary weight-control programme involving two hours per week of exercise over a longer period of eight months in obese children, although, perhaps more importantly, MFO increased by 21% (Lazzer et al., 2008). Increases in $V\square O_{2peak}$ following the programme may have contributed to the lack of change in Fatmax, whilst MFO improved (Lazzer et al., 2008). However, as these programmes involved a combination of exercise and dietary management, it is not possible to ascertain whether training at Fatmax would exert an independent effect (Brandou et al., 2003; Lazzer et al., 2008).

More recent work has investigated the effect of diet, training at Fatmax and combined diet and training at Fatmax (diet + training) on metabolism in obese young people (Ben Ounis et al., 2008; 2009; Elloumi et al., 2009). These studies have shown increases in the ability to oxidise fat during exercise following two month training and diet + training interventions, but not following dietary intervention alone (Ben Ounis et al., 2008; 2009; Elloumi et al., 2009). Moreover, training and diet + training, but not diet alone, improved several health markers, including insulin resistance, adiponectin, resistin and HDL cholesterol, whereas dietary intervention induced more favourable changes in body composition (Ben Ounis et al., 2008; Elloumi et al., 2009). It was clear, however, that the diet + training groups improved all of the measured body composition and health markers (Ben Ounis et al., 2008; 2009; Elloumi et al., 2009). These findings suggest that training at Fatmax can have an independent effect on improving fat oxidation and various health markers in adolescents. However, combining Fatmax training with dietary intervention may result in more pronounced improvements in a larger number of health markers.

Although these studies have shown health benefits of training at Fatmax, it is possible that training at a different exercise intensity is equally or more effective. Brandou et al. (2005) studied the influence of low intensity (LI) and high intensity (HI) exercise and a hypoenergetic diet on substrate oxidation during exercise in obese boys. Training consisted of cycling at Fatmax (average 51% W_{max}th) for the LI group and Fatmax +40% of Fatmax (average 61% W_{max}th) for the HI group, with energy expenditure being constant between groups, twice a week for two months. Although the absolute power output was significantly different between LI and HI, the difference in exercise intensity expressed as % W_{max}th and HR did not reach significance, thus the reported difference in exercise training between the LI and HI groups may not have been sufficient to induce a differential effect between the groups. Furthermore, the term 'high intensity' may be misleading, usually referring to exercise intensities higher than the 61% W_{max}th or 156 beats·min^{-1} reported during HI training (Perry et al., 2008; van Aggel-Leijssen et al., 2002). After training, substrate oxidation was unchanged in the LI group, whereas the HI group oxidised less fat and more CHO during exercise at 20% and 30% W_{max}th. It was concluded that LI training maintains the ability to oxidise fat

during exercise, whilst HI training results in a shift towards CHO oxidation. These findings conflict with the existing literature that has generally reported higher fat oxidation rates following moderate intensity exercise training (Lazzer et al., 2008; van Aggel-Leijssen et al., 2002) and that high intensity training typically increases (Burgomaster et al., 2008; Talanian et al., 2007) or may not affect (van Aggel-Leijssen et al., 2002) exercise fat oxidation. Indeed, the increased CHO oxidation following HI training (Brandou et al., 2005) is somewhat surprising since training induces an array of skeletal muscle adaptations that promote fat oxidation whilst reducing CHO oxidation (Eriksson et al., 1973; Fournier et al., 1982; Kiens and Lithell, 1989; Tarnopolsky et al., 2007). Moreover, in a well-controlled crossover study involving obese men who were not subject to energy restriction, fat oxidation increased by 44% after training at Fatmax, but not after isoenergetic interval training at intensities 20% above and below Fatmax. These improvements were evident despite no reported changes in body mass, body fat or $V\square O_{2peak}$ (Venables and Jeukendrup, 2008). It was postulated that the failure to increase fat oxidation in the study by Brandou et al. (2005) may have been due to possible reductions in fat oxidation following the hypoenergetic diet (Franssila-Kallunki et al., 1992; Schutz et al., 1992) that began two weeks before exercise training started.

When examining the acute effects of Fatmax and HI exercise, total fat oxidation was higher during Fatmax (42% predicted $V\square O_{2peak}$ for 45 min) compared with HI (67% predicted $V\square O_{2peak}$ for 30 min) treadmill exercise in obese adolescent boys, despite a similar energy expenditure between exercise intensities (Lazzer et al., 2010). Although exercise intensity did not affect fat oxidation 60 min post-exercise, cumulative fat oxidation (exercise plus recovery) remained higher in the Fatmax group. Importantly, the groups were matched for body fat and $V\square O_{2peak}$. However, a crossover design may have been more appropriate to control for unknown factors that may result in individual differences in fat oxidation. The higher fat oxidation during Fatmax compared with HI exercise suggests that exercise training at Fatmax may promote fat oxidation when compared to higher intensities and supports the notion that larger differences in exercise intensity (around 25% $V\square O_{2peak}$) than those used by Brandou et al. (2005) (around 10% $V\square O_{2peak}$) may be required to elicit differences in fat oxidation when comparing Fatmax and HI training. However, potential benefits of training at Fatmax compared with other exercise intensities clearly require further study in young people.

Adaptations within skeletal muscle may be partly responsible for the metabolic adaptations to training in young people. Changes in oxidative enzyme activity following endurance training with little modification in glycolytic activity has been reported in boys (Eriksson et al., 1973; Fournier et al., 1982) and endurance, but not sprint training, increased the surface area of oxidative type I and more oxidative fast twitch type II fibres in adolescent boys (Fournier et al., 1982). Likewise, increased mitochondrial area/volume (Tarnopolsky et al., 2007), oxidative enzyme activity (Berthon et al., 1998; Kiens et al., 1997; Tarnopolsky et al., 2007) and a shift toward more oxidative fibre type composition (Dubé et al., 2008) may contribute to exercise-induced increases in fat oxidation in adults. The adult-based literature investigating additional mechanisms to explain the increased fat oxidation following endurance may be insightful when considering potential mechanisms in young people. Exercise-induced increases in fat oxidation do not appear to be due to increased plasma FFA availability; in fact training may reduce peripheral lipolysis and plasma FFA rate of appearance (Martin et al., 1993; Phillips et al., 1996). There is strong evidence that the elevated use of fat in the trained state results from increased IMTG storage, lipolysis and, ultimately, oxidation (Dubé et al., 2008; Kiens and Lithell, 1989; Phillips et al., 1996; Schrauwen-Hinderling et al., 2003; van Loon and Goodpaster, 2006). Exercise training increases total protein expression of FAT/CD36 (Tunstall et al., 2002) and FABPpm (Kiens et al., 1997) in adults, which is likely to improve FFA transport across the muscle membrane, while the higher CPT-1activity in trained compared to untrained adults (Berthon et al., 1998) and increased CPT-1 mRNA following training (Tunstall et al., 2002) may improve FFA transport across the mitochondrial membrane. Furthermore, exercise training alters the localisation of FAT/CD36 and increases its association with CPT-1, which may augment fat oxidation (Schenk and Horowitz, 2006).

2.5.6 Conclusions

Research examining Fatmax in young people is growing, but still appears to be in its infancy. The available literature indicates that Fatmax generally occurs between 30 and 60% $V\square O_{2peak}$ in young people (Table 2.1). Fatmax and exercise fat oxidation may decrease with puberty (Riddell et al., 2008) and obesity (Zunquin et al., 2009b), and increase following exercise training (Brandou et al., 2003). However, inconsistencies in the methods employed to estimate Fatmax limit inter-study comparisons. Future studies

estimating Fatmax in young people should address issues relating to the exercise protocol used to determine fat oxidation and V□O_{2peak}, the appropriate use of indirect calorimetry, the control of diet and physical activity and the selected participant population (puberty, sex, age, training status). Research is required to verify whether the incremental protocol recommended by Achten et al. (2002), as used widely in adult and paediatric studies, is suitable for determining Fatmax specifically in young people. Reaching consensus on the exercise protocol used to estimate Fatmax in this population would provide more objective exercise prescription for maximising fat oxidation during exercise. Well-controlled studies examining the factors influencing Fatmax and fat oxidation, including exercise mode, sex, cardiorespiratory fitness and free living physical activity would help to further individualise exercise prescription for young people. Despite evidence in adults that treadmill compared with cycling exercise is preferential for enhancing fat oxidation, studies in young people have typically used cycling exercise and the effect of exercise mode on Fatmax has not been investigated. Additionally, conclusions from these studies are largely based on boys; it may be argued that girls should be targeted since physical activity levels are lower in this population (Riddoch et al., 2007). The practical function of the Fatmax concept also requires further work in young people, including the potential effect of exercise prescription at Fatmax on health biomarkers and body composition when compared to other more traditional exercise prescriptions.

Table 2.1 Summary of studies that have estimated Fatmax in young people

Author	*n*	Weight status	Pubertal status	Sex	Age (y)	Fatmax
Brandou et al. (2003)	7	OB	Mixed stages	M+F	13.7	32% W_{max}th (pre-training) 45% W_{max}th (post-training)
Brandou et al. (2005)	7	OB	Mixed stages	M	11.8	51% W_{max}th
Maffeis et al. (2005)	24	OB	Prepubertal	M	10	45% V□O_{2peak}
Brandou et al. (2006)	7	OB	Prepubertal	M	10.6	~50% W_{max}th
	8	OB	Postpubertal	M	13.5	~47% W_{max}th
Stephens et al. (2006)	9	NO	EP	M	10.3	40% V□O_{2peak}
	12	NO	MP	M	12.3	40% V□O_{2peak}
	11	NO	LP	M	15.0	30% V□O_{2peak}
Lazzer et al. (2007)	15	OB	Pubertal and postpubertal	M	15.9	40% V□O_{2peak}
	15	OB		F	15.6	38% V□O_{2peak}
	15	NO		M	15.0	45% V□O_{2peak}
	15	NO		F	15.0	42% V□O_{2peak}
Lazzer et al. (2008)	19	OB	Mixed	M + F	8-12	48% V□O_{2peak} (pre- and post-training)
Riddell et al. (2008)	5	NO	T1	M	12.0	56% V□O_{2peak}
	5	NO	T2/3	M	13.2	55% V□O_{2peak}
	5	NO	T4	M	14.7	45% V□O_{2peak}
	9	NO	Postpubertal	M	23.8	31% V□O_{2peak}
Aucouturier et al. (2009)	20	OB	Not stated	M+F	13.0	53 %V□O_{2peak} 47% V□O_{2peak}ACSM 38% V□O_{2peak}W 38% V□O_2W_{max} 36% V□O_2W_{max}th
Zunquin et al. (2009a)	16	OB	Prepubertal	M	9.7	49% V□O_{2peak}
	16	OB	Pubertal	M	11.9	47% V□O_{2peak}
	14	OB	Postpubertal	M	14.6	45% V□O_{2peak}
Zunquin et al. (2009b)	17	OB	Pubertal	M	12.1	47% V□O_{2peak}
	13	NO	Pubertal	M	12.0	55% V□O_{2peak}
Lazzer et al. (2010)	20	OB	Pubertal and postpubertal	M	14-16	42% V□O_{2peak}

OB – obese, M – male, F – female, W_{max}th – theoretical maximal aerobic power, V□O_{2peak} – peak oxygen uptake, NO – non-obese, EP – early pubertal, MP – mid-pubertal, LP – late-pubertal, T1 – Tanner 1, T2 – Tanner 2, T3 – Tanner 3, T4 – Tanner

4 (Tanner, 1962), ACSM – prediction equations (ACSM, 1995), V□O_{2peak}W – prediction equations (Wasserman et al., 1987).

2.6 Breakfast consumption and fat oxidation

It is well established that fat oxidation is maximised in the fasted state, increasing in direct proportion to the duration of fasting (Montain et al., 1991) and being suppressed by pre-exercise CHO consumption (Achten and Jeukendrup, 2003b; Horowitz et al., 1997). In young people, exogenous CHO utilisation lowers the contribution of fat oxidation to energy expenditure during exercise (Riddell et al., 2000; Timmons et al., 2003; 2007a; 2007b). Furthermore, Fatmax appears to be reduced following CHO consumption in adults (Achten and Jeukendrup, 2003b). The mechanisms responsible for the reduction in fat oxidation following CHO consumption relate to the rise in insulin that inhibits lipolysis and FFA availability (Horowitz et al., 1997) and the increase in blood glucose uptake and, therefore, CHO oxidation, which inhibits the rate of FFA entry into the mitochondria (Coyle et al., 1997; Sidossis et al., 1996).

Although it is clear that exercise in the fasted state is preferential for augmenting fat oxidation, this may not be practical for young people and several lines of evidence support the promotion of regular breakfast consumption for health (Albertson et al., 2009; Panagiotakos et al., 2008; Timlin et al., 2008). Benefits associated with regular breakfast consumption in young people include reduced mental distress and improved academic performance (Lien, 2007), nutritional benefits such as increased vitamin and mineral intake (Barton et al., 2005; Nicklas et al., 1993; Song et al., 2006), higher physical activity levels, higher cardiorespiratory fitness and lower levels of obesity (Sandercock et al., 2010). Indeed, a plethora of associated lifestyle behaviours may explain the relationship between breakfast consumption and health, including overeating later in the day (Dubois et al., 2009), increased snacking (Sjöberg et al., 2003) and reduced physical activity (Sandercock et al., 2010).

It has also been suggested that the observed relationships between breakfast consumption and health may not be due to consumption *per se*, but rather breakfast composition (Deshmukh-Taskar et al., 2010). Ready-to-eat cereals are commonly consumed for breakfast in westernised countries (Albertson et al., 2003; Ruxton et al., 1996; Song et al., 2006). However, the nutritional content of these cereals varies considerably and there are concerns that the majority of ready-to-eat cereals marketed

to children fail to meet national nutrition standards; they are typically higher in energy, sugar and sodium, but lower in fibre and protein compared with cereals not marketed specifically for children (Schwartz et al., 2008). It has been suggested that the association between ready-to-eat cereal consumption and health may be attributed to whole-grain and not refined-grain cereals. Indeed, there has been considerable interest in the health benefits of whole-grain, fibre-rich, low-energy-dense breakfasts that contain LGI CHO (Kochar et al., 2007; Kosti et al., 2010), with a recent review recommending these types of breakfasts for weight management (Kosti et al., 2010).

2.7 Glycaemic index

The concept of GI was introduced as a method of classifying different CHO-rich foods according to their effect on postprandial glycaemia. It is defined as the incremental area under the two hour blood glucose curve following ingestion of 50 g available CHO as a percentage of the corresponding area following an equivalent amount of CHO from a standard reference product (glucose or white bread) (Jenkins et al., 1981). Values for GI range from 1 to 100 and CHOs can be classified as high (≥70), moderate (56 to 69) or low (≤55). Foods classified as HGI include refined grain products, white bread and potato, whereas LGI foods include whole grain products, legumes and fruits. Numerous published tables now contain GI values for a variety of foods, including The International Tables of Glycaemic Index (Atkinson et al., 2008). As the extent of postprandial glycaemia depends on both the GI and the amount of CHO consumed, the glycaemic load (GL) was later proposed to provide an indication of the total glycaemic effect of the diet and is calculated as the product of the GI and total dietary CHO divided by 100 (Salmerón et al., 1997). Critically, the consumption of mixed meals composed of commonly consumed foods more closely reflects 'real world' situations than assessing single CHO-containing foods. The GI of mixed meals can be predicted from the GI values of the component CHO foods. The weighted mean of the individual GI values is based on the percentage of the total meal CHO provided by each food and the predicted response is strongly correlated (r=0.987) with the actual glucose response (Wolever and Jenkins, 1986; Wolever et al., 2006).

Various food factors influence the GI of CHO-containing foods, which are affected by the method of preparation, processing, variety, origin, maturation and degree of ripeness (Englyst et al., 2003; Pi-Sunyer, 2002). The term 'available carbohydrate'

represents parts of the CHO that can be digested and absorbed, excluding dietary fibre. The type of monosaccharide (glucose, fructose, galactose) affects the GI, with fructose having a relatively low GI (Englyst et al., 2003; Foster-Powell et al., 2002). The ratio of amylose/amylopectin in starch is an important factor; the branched amylopectin is more rapidly digested than the unbranched amylose and results in a higher GI (Granfeldt et al., 1995). The macronutrient content of foods also affects the GI, with protein and fat reducing the glycaemic response (Ercan et al., 1994; Nuttall et al., 1984; 1985). However, these effects are negligible compared with the effect of CHO content on GI. Wolever and Bolognesi (1996) fed five meals varying in energy, fat, protein, CHO and GI to healthy participants and concluded the CHO source accounted for 85 to 94% of the variability of the glucose and insulin response. Controversy surrounds the effect of dietary fibre on GI (Pi-Sunyer, 2002). Insoluble fibre has little impact on the glycaemic response compared with soluble fibre, which may lower the GI, possibly by acting as a physical barrier and delaying access of digestive enzymes and water to the starch within the cereal grain (Jenkins et al., 1981; Nuttall, 1993).

2.7.1 Glycaemic index and insulin

In general, glycaemic and insulinaemic responses to consumed CHO are well-related (Wolever et al., 2006). However, a potential criticism of GI is that it does not necessarily indicate the insulin response of all foods (insulinaemic index). In certain foods, although the GI can predict the glucose response to a meal it does not necessarily predict the insulin response (Pi Sunyer, 2002). Indeed, the addition of protein to a meal increased the insulin response without affecting glucose (van Loon et al., 2000). The unexpectedly high insulinaemic index of milk (Ostman et al., 2001) may be important when considering postprandial metabolism following breakfasts that typically contain milk.

Although GI is believed to directly reflect the rate of digestion and glucose entry into the systemic circulation, the blood glucose concentration reflects both entry and removal of glucose from the blood. Schenk et al. (2003) reported a higher GI in corn flakes compared with bran cereal in men, but no difference in the rate of appearance of glucose. It was suggested that the earlier rise in postprandial insulin caused a 31% increase in the rate of disappearance of glucose following the bran cereal compared with the corn flakes, thus an earlier increase in insulin may result in increased glucose

uptake and reduce blood glucose concentrations (Schenk et al., 2003). In this study, however, the LGI bran cereal contained almost four times more protein than the HGI corn flakes, which may have increased insulin secretion, leading to a reduction in the GI of that meal (Nuttall et al., 1984). This highlights the importance of considering macronutrient content when examining the independent effect of GI.

2.7.2 Dietary glycaemic index and health in young people

The rate of glucose entry into the bloodstream and duration of elevated blood glucose concentration induce hormonal and metabolic changes that may affect health; mounting evidence suggests that the postprandial state contributes to the development of chronic disease (Heine et al., 2004). A recent review concluded that there is now a large body of evidence providing robust support for LGI diets in the prevention of obesity, diabetes, and cardiovascular disease (Brand-Miller et al., 2009). Although a relatively small number of interventions have assessed the impact of GI on health markers in young people compared with adults, the available evidence from a variety of studies indicates that reduced GI diets may have implications for lowering BMI, metabolic syndrome and cardiovascular risk factors, hyperglycaemia, fasted glucose and insulin and hunger in young people (Fajcsak et al., 2008; Rovner et al., 2009; Spieth et al., 2000). Moreover, a reduced-GL diet may be more effective at improving BMI and insulin sensitivity compared with a reduced-fat diet (Ebbeling et al., 2003). Encouragingly, health benefits of reducing dietary GI may be achieved by targeting the breakfast meal only (Pal et al., 2008). However, conflicting evidence has shown that dietary GI may not influence health markers in children (Cheng et al., 2009a). Potential health-enhancing effects of reduced GI diets in young people are, therefore, encouraging but require greater research attention.

2.8 Acute effect of breakfast glycaemic index on metabolism and satiety

Diets rich in LGI foods may reduce metabolic disease risk through both direct metabolic effects and reductions in body weight. Plausible mechanisms may arise from the contrasting metabolic responses to HGI and LGI foods, which have been investigated using randomised controlled trials in adults and to a lesser extent in young people.

2.8.1 Glucose and insulin

Numerous studies in adults have shown that LGI compared with HGI mixed breakfast meals attenuate postprandial glycaemia and insulinaemia (Stevenson et al., 2006; 2009) and a few studies in adolescents have provided similar findings (Ball et al., 2003; Ludwig et al., 1999). In adults, a LGI breakfast (Liljeberg and Björck, 2000) or evening meal (Stevenson et al., 2005b; 2008) may also reduce postprandial glucose and insulin responses to subsequent standard meals; this is known as the 'second meal effect'. Reduced blood glucose decreases the quantity of insulin required to clear glucose from the blood, which may up-regulate insulin receptors on cells and increase insulin sensitivity (Song et al., 2000). Furthermore, the reduced postprandial glycaemic and insulinaemic response to LGI breakfasts has been proposed to affect substrate metabolism and satiety.

2.8.2 Fat oxidation during rest and subsequent exercise

It is plausible that the attenuated postprandial insulin response to LGI breakfast consumption can augment subsequent fat oxidation (Wu et al., 2003). In this respect, LGI breakfast consumption may serve as a compromise between no breakfast (fasting) and HGI breakfast consumption. Several studies have investigated the effect of breakfast GI on fat oxidation during rest and subsequent exercise in adults; Table 2.2 provides details of the most relevant studies.

In adults, increased fat oxidation during the immediate postprandial rest period has been reported following a LGI compared with HGI breakfast (Stevenson et al., 2009). However, the majority of studies have not supported this finding (Díaz et al., 2005; Stevenson et al., 2006; Wee et al., 2005). It was suggested that the lower CHO load in the Stevenson et al. (2009) study compared with other studies reporting no effect of breakfast GI may have facilitated the reported differences in resting fat oxidation. When individuals consumed a HGI or LGI breakfast and lunch, higher resting fat oxidation was reported following the LGI meals after lunch only (Stevenson et al., 2005a). However, the consumption of a HGI compared with LGI evening meal did not influence fat oxidation following a standard HGI breakfast the next morning in men (Stevenson et al., 2005b) and women (Stevenson et al., 2008). Studies examining the more prolonged effect of GI on substrate oxidation have reported no difference in resting fat oxidation over 10 hours when obese women consumed a HGI or LGI breakfast and lunch (Díaz et al., 2005) and manipulation of dietary GI did not affect 24

h fat oxidation (McDevitt et al., 2000). However, glucose and insulin responses have not always been measured to confirm the expected differences between HGI and LGI meals (McDevitt et al., 2000). Furthermore, consuming two HGI compared with LGI meals for five consecutive days actually resulted in higher fat oxidation in trained men (Cocate et al., 2011). In line with this finding, resting fat oxidation was higher after high glucose (HGI) compared with high fructose (LGI) meals in obese adults, despite greater glucose and insulin responses to the high glucose meal (Tittelbach et al., 2000), suggesting fat oxidation may depend on the type of LGI CHO consumed.

Unlike resting fat oxidation, the majority of studies support the finding that LGI compared with HGI breakfast consumption results in higher fat oxidation during exercise performed 45 min to 3 h after breakfast (Sparks et al., 1998; Wee et al., 2005; Stevenson et al., 2006; 2009). These observations have typically been accompanied by higher plasma FFA and glycerol concentrations following LGI breakfasts (Sparks et al., 1998; Stevenson et al., 2006; Wee et al., 1999; Wu and Williams, 2006). However, some studies have found no effect of breakfast GI on fat oxidation during exercise (Bennard and Doucet, 2006; Febbraio and Stewart, 1996) and a recent study even reported higher fat oxidation during a cycling time trial performance when a HGI breakfast was consumed 45 min before exercise (Moore et al., 2010). The relationship between GI and fat oxidation is further complicated by findings that breakfast GI does not affect fat oxidation during exercise when comparing a moderate GI (MGI) and HGI breakfast (Backhouse et al., 2007) and no difference in fat oxidation was reported when exercise was preceded by two LGI or HGI meals rather than breakfast alone (Stevenson et al., 2005a). Furthermore, exercise fat oxidation was not affected when a LGI or HGI meal was provided the evening before (Stevenson et al., 2005b; 2008); this suggests that the 'second meal effect' does not apply to fat oxidation.

The effect of GI on fat oxidation remains unclear and much of these data are based on findings from studies including small samples of trained or recreationally active adults (Table 2.2). Moreover, LGI breakfasts typically contain a higher proportion of fat, protein and fibre; some of these studies have failed to match meals for macronutrient content (Bennard and Doucet, 2006; DeMarco et al., 1999; Febbraio et al., 2000; Kirwan et al., 2001) and have provided meals of a set absolute size rather than relative to body size (Bernnard and Doucet, 2006; Kirwan et al., 2001). The majority of this

research has been conducted after an overnight fast (e.g. Stevenson et al., 2009; Wee et al., 2005), whereas some has not (Moore et al., 2010; Sparks et al., 1998). Therefore, these findings require further examination using well-controlled studies that have carefully matched the breakfasts for macronutrient content and included larger more diverse samples, such as young people and overweight individuals.

It has been suggested that the reduced fat oxidation following HGI breakfasts is largely due to the higher insulin response, which increases muscle glycogen stores and utilisation, resulting in higher CHO and lower fat oxidation (Wee et al., 2005). Indeed, Wee et al. (2005) reported increased muscle glycogen concentration 3 h following a HGI breakfast, with no change following the LGI breakfast, and greater muscle glycogen utilisation during subsequent exercise in the HGI trial. This increased muscle glycogen utilisation following HGI breakfast consumption has been reported previously (Febbraio et al., 2000), but not consistently (Febbraio and Stewart, 1996). Contrasting findings may have been due to major differences in study design and, in particular, differences in the timing of the muscle biopsy, which has been obtained 30 min (Wee et al., 2005) or 2 h (Febbraio and Stewart, 1996) after exercise. Differences in FAT/CD36 gene expression following HGI and LGI CHO consumption may be another underlying mechanism controlling differences in fat oxidation. In men, FAT/CD36 mRNA and protein levels were down-regulated 3 h after the consumption of a HGI post-exercise meal, but were unchanged when an isoenergetic LGI meal with similar macronutrient content was consumed (Cheng et al., 2009b). Conversely, muscle glucose transporter type 4 (GLUT-4) expression was reduced to the same extent following both meals, suggesting that this is not implicated in the relationship between GI and substrate oxidation. The effect of GI on FAT/CD36 expression may also be mediated through differences in the insulin response to meals differing in GI (Luiken et al., 2002; Smith et al., 2007).

In general, evidence in adults supports the finding that a LGI compared with HGI mixed breakfast meal augments fat oxidation during exercise, although the effect of breakfast GI on resting fat oxidation remains unclear. Discrepancies between studies may be due to differences in breakfast size and composition, exercise mode, intensity and duration, the time interval between breakfast and exercise and participant characteristics (Table 2.2). It should be highlighted that the majority of these studies

have used endurance trained or recreationally active non-overweight adults as participants and a greater understanding of these responses in different populations may have more relevance for disease prevention. Moreover, similar studies in young people do not appear to be available.

Table 2.2 Experimental studies examining the effect of glycaemic index (GI) on fat oxidation

Author(s)	Participants	Experimental design	Effect of GI on fat oxidation
Backhouse et al. (2007)	6 women	HGI or MGI breakfast consumed 3 h before 60 min walk at 50% V□O_{2peak}	Rest and exercise: no effect
Bennard and Doucet (2006)	8 men	HGI or LGI breakfast (1.67 MJ (400 kcal), 80 g CHO) consumed 1 h before 1.67 MJ (400 kcal) treadmill exercise at Fatmax	Exercise: no effect
DeMarco et al. (1999)	10 trained men	HGI or LGI breakfast (1.5 g CHO·kg BM^{-1}) consumed 30 min before 2 h cycle at 70% V□O_{2peak}	Exercise: Lower RER in LGI
Febbraio and Stewart (1996)	6 trained men	HGI or LGI breakfast (1 g CHO·kg BM^{-1}) consumed 45 min before 2 h cycle at 70% V□O_{2peak}	Exercise: No effect on RER
Febbraio et al. (2000)	8 trained men	HGI or LGI breakfast (2 g CHO·kg BM^{-1}) consumed 30 min before 2 h cycle at 70% V□O_{2peak}	Exercise: Tendency for higher fat oxidation in LGI
Kirwan et al. (2001)	6 men	HGI or MGI breakfast (75g CHO) consumed 45 min before cycle to exhaustion at 60% V□O_{2peak}	Exercise: No effect
Moore et al. (2010)	10 trained men (cyclists)	HGI or LGI breakfast (1 g CHO·kg BM^{-1}) consumed 45 min before 40 km cycling time trial	Exercise: Higher fat oxidation in HGI
Sparks et al. (1998)	8 trained men	HGI or LGI breakfast (1 g CHO·kg BM^{-1}) consumed 45 min before 50 min cycle at 67% V□O_{2peak}	Exercise: Lower RER in LGI
Stevenson et al. (2005a)	9 men	HGI or LGI breakfast and lunch (2 g CHO·kg BM^{-1}) consumed, each followed by 3 h postprandial period; 60 min run at 70% V□O_{2peak} 3 h after lunch	Rest: higher fat oxidation after lunch in LGI; no effect after breakfast Exercise: no effect

Stevenson et al. (2005b)	5 active men	HGI or LGI evening meal consumed the day before HGI breakfast (2 g CHO·kg BM^{-1}); 60 min run at 65% $V\square O_{2peak}$ performed 3 h after breakfast	Postprandial rest and exercise: no effect
Stevenson et al. (2006)	8 active women	HGI and LGI breakfast (2 g CHO·kg BM^{-1}) consumed 3 h before 60 min run at 65% $V\square O_{2peak}$	Rest: No effect Exercise: higher fat oxidation in LGI
Stevenson et al. (2008)	7 active women	HGI or LGI evening meal consumed the day before HGI breakfast (all meals contained 2 g CHO·kg BM^{-1}); exercise performed 3 h after breakfast	Rest and exercise: no effect
Stevenson et al. (2009)	8 sedentary women	HGI or LGI breakfast (1 g CHO·kg BM^{-1}) consumed 3 h before 60 min walk at 50% $V\square O_{2peak}$	Rest and exercise: higher fat oxidation in LGI
Thomas et al. (1998)	8 trained men	HGI or LGI breakfast (1 g CHO·kg BM^{-1}) consumed 1 h before cycle to exhaustion at 67% $V\square O_{2peak}$	Exercise: higher fat oxidation in LGI
Wee et al. (1999)	8 adults (5 men, 3 women)	HGI or LGI breakfast (2 g CHO·kg BM^{-1}) consumed 3 h before run to exhaustion at 70% $V\square O_{2peak}$	Rest: No effect (but higher CHO oxidation in HGI) Exercise: higher fat oxidation in LGI
Wee et al. (2005)	7 trained men	HGI or LGI breakfast (2.5 g CHO·kg BM^{-1}) consumed 3 h before 30 min run at 71% $V\square O_{2peak}$	Rest: No effect Exercise: higher fat oxidation in LGI
Wong et al. (2008)	8 trained men	HGI or LGI breakfast (1.5 g CHO·kg BM^{-1}) consumed 2 h before 5k run at 70% $V\square O_{2peak}$ followed by 16 km performance run	Rest: No effect Exercise: higher fat oxidation in LGI
Wu et al. (2003)	9 trained men	HGI or LGI breakfast (2 g CHO·kg BM^{-1}) consumed 3 h before 60 min run at 65% $V\square O_{2peak}$	Rest: No effect Exercise: higher fat oxidation in LGI
Wu and Williams (2006)	8 trained men	HGI or LGI breakfast (2 g CHO·kg BM^{-1}) consumed 3 h before run to exhaustion at 70% $V\square O_{2peak}$	Rest: No effect Exercise: higher fat oxidation in LGI

GI – glycaemic index, HGI – high glycaemic index, MGI – moderate glycaemic index, LGI – low glycaemic index, $V\square O_{2peak}$ – peak oxygen uptake, CHO – carbohydrate.

2.8.3 Satiety

Much of the interest surrounding GI and body weight regulation has stemmed from the finding that LGI foods have satiating properties that may reduce subsequent food

intake. Importantly, there is some evidence to support these claims in young people (Ball et al., 2003; Ludwig et al., 1999; Warren et al., 2003). In a well-controlled study, Warren et al. (2003) reported lower lunchtime energy intake and hunger ratings after LGI, and LGI with added sugar, breakfasts compared with HGI and habitual breakfasts (which were also HGI) in girls and boys. In support, Henry et al. (2007) found a tendency towards a reduced energy intake at lunch following a LGI breakfast compared with HGI breakfast in preadolescent children, although the mean difference was low (75 kJ, 18 kcal) and mainly confined to boys. However, the actual glucose and insulin responses to the breakfasts were not determined in these studies, thus it is not possible to confirm whether the breakfasts differing in GI induced the expected metabolic responses (Henry et al., 2007; Warren et al., 2003). Despite these concerns, studies that have determined postprandial glucose and insulin responses support these findings; voluntary energy intake and hunger ratings were greatest after a HGI, followed by a MGI and lowest after a LGI breakfast in obese adolescent boys (Ludwig et al., 1999). However, although the HGI and MGI breakfasts were matched for key variables, the LGI breakfast contained less CHO, more protein and more fat than the HGI breakfast, possibly confounding the GI comparison (Ludwig et al., 1999). In contrast, another study reported similar energy intake and hunger ratings when comparing a LGI meal replacement, LGI whole-food meal and HGI meal replacement in overweight adolescents (Ball et al., 2003). Encouragingly, however, time to request additional food was prolonged following the LGI breakfast, indicating that overweight and obese adolescents are satisfied for a longer time period after LGI compared with HGI breakfast consumption (Ball et al., 2003; Ludwig et al., 1999). The lower energy intake and prolonged satiety following LGI breakfast consumption suggests that breakfasts rich in LGI CHO could have direct implications for weight management and may partly explain the relationship between dietary GI and BMI (Du et al., 2009; Spieth et al., 2000). In turn, reduced BMI may contribute to other health benefits associated with LGI diets, including increased insulin sensitivity and reduced cardiovascular risk factors (see section 2.7.2).

Differences in glycaemia might underpin the relationship between GI and satiety, as the lower glucose concentration following a LGI compared with HGI breakfast explained much of the lower voluntary food intake later in the day in obese adolescent boys (Ludwig et al., 1999). Indeed, the rapid absorption of glucose following HGI breakfast

consumption stimulates insulin release, which promotes glucose uptake by the liver, skeletal muscle and adipose tissue, while suppressing both lipolysis in adipocytes and the release of glucose from the liver into the circulation. Subsequently, blood glucose concentration decreases rapidly. The decreased circulating concentrations of metabolic fuels following HGI breakfast consumption would be expected to result in increased hunger and food intake as the body attempts to restore energy homeostasis. In contrast, the attenuated glucose response following LGI breakfast consumption stimulates more subtle hormonal responses and the prolonged and continued absorption of nutrients means that the fasted state is reached much later. The hunger response is, thus, prolonged following LGI breakfast consumption, which would promote longer term satiety (Brand-Miller et al., 2002; Ludwig et al., 1999).

2.8.4 Participant characteristics

Glycaemic responses depend on several factors, including age, sex, BMI, ethnicity and insulin resistance, thus a concern of GI is that values may vary between individuals (Pi-Sunyer, 2002). However, measuring the glycaemic response (as the area under the glucose concentration over time curve, AUC) is not the same as measuring the GI (food AUC relative to reference food AUC). Thus, factors affecting glycaemia will not affect the GI if these factors influence the AUC for the test food and the reference food to the same extent.

Participant characteristics such as age, sex, BMI, ethnicity and insulin sensitivity are generally not believed to influence GI (Wolever et al., 2003; Lan-Pidhainy and Wolever, 2011; Perälä et al., 2011), although the insulinaemic index may depend upon the glycaemic control and insulin sensitivity of the individual (Lan-Pidhainy and Wolever, 2011). There is, however, some evidence supporting a role of training status on GI. The GI of breakfast cereals was lower in endurance trained compared with sedentary men due to the similar glucose response to the reference solution coupled with the lower glucose response to the test food (cereal) in the trained men (Mettler et al., 2007). However, the glucose response to both the test and reference food was lower in endurance trained compared with sedentary women, resulting in similar GI values between these groups (Mettler et al., 2008). Similarly, no difference in the GI of raisins and snack bars was found in mixed-sex groups of endurance trained and sedentary adults (Kim et al., 2008; Trompers et al., 2010). It has been suggested that the effect of

individual characteristics on postprandial glucose may depend on the specific food. Wolever et al. (2009) investigated the effect of several factors, including age, sex, ethnicity, BMI, waist circumference and fasting insulin on the GI values of three foods and reported the GI for fruit leather was lower in those with a high waist circumference, while fasting insulin and ethnicity affected the GI for white bread. Therefore, it remains possible that participant characteristics can affect GI values, although this effect may be specific to certain foods.

2.8.5 Conclusions

Experimental studies have shown that LGI compared with HGI breakfast consumption results in a reduced postprandial glucose and insulin response and, consequently, increased satiety and possibly fat oxidation. These findings may explain relationships between reduced dietary GI and improved health markers, such as BMI, insulin resistance and cardiovascular risk factors. However, further research in young people is required to extend and confirm findings from the adult-based literature. In particular, the effect of breakfast GI on fat oxidation does not appear to have been investigated in young people, despite a plethora of studies in adults (Table 2.2). Furthermore, research directly comparing the metabolic responses of HGI and LGI breakfasts in different populations is sparse. Substituting a HGI breakfast for a LGI breakfast may be particularly beneficial for overweight individuals through increased glycaemic control, fat oxidation and satiety. Moreover, differences in insulin resistance between overweight and non-overweight individuals (see section 2.1) suggests that it may be worthwhile comparing the metabolic responses to HGI and LGI breakfasts in these populations. In addition, this research may be best focused on girls, as physical activity levels are lower in girls compared with boys (Riddoch et al., 2007) and girls are less likely to eat breakfast daily (Timlin et al., 2008). Methodological considerations for future work include matching breakfasts for macronutrient content and determining the actual glucose and insulin responses to breakfasts differing in GI in young people.

2.9 Acute effect of exercise on metabolism

In addition to LGI breakfast consumption, exercise may represent another effective strategy to attenuate the adverse metabolic responses associated with HGI breakfasts. Although the metabolic benefits of exercise training in young people are well documented (Ben Ounis et al., 2009, Shaibi et al., 2008), the effect of acute exercise has

received little attention. This is somewhat surprising, since it is widely recognised in adults that many of the metabolic improvements associated with exercise stem from the most recent exercise session. Moreover, exercise training improves insulin sensitivity in young people independent of changes in body composition, thus we know that weight loss is not required for these metabolic improvements to be manifested (Bell et al., 2007; Nassis et al., 2005). Therefore, the following section draws upon the literature in adults to review the acute effect of exercise on glucose, insulin and fat metabolism.

2.9.1 Glucose and insulin

It is well established in adults that a single exercise bout results in a transient increased insulin-mediated whole-body glucose uptake for up to 72 h post-exercise, but returns to baseline values thereafter (Horowitz, 2007; King et al., 1995). Accordingly, numerous studies in adults have assessed the effect of exercise on metabolism the next morning (12 to 16 h post-exercise). Reduced fasting (Horowitz et al., 2005) and postprandial insulin concentrations (Burton et al., 2008; Brestoff et al., 2009; Kokalas et al., 2005) the morning after a single bout of aerobic exercise compared with no exercise have been shown in healthy (Brestoff et al., 2009; Newsom et al., 2010), overweight/obese (Burton et al., 2008) and type 2 diabetic (Manders et al., 2010) adults. The acute effect of exercise on glucose is less clear, with studies reporting no effect (Brestoff et al., 2009; Burton et al., 2008), reductions (Kokalas et al., 2005; Mitchell et al., 2008) or even a trend for higher (Gill and Hardman, 2000) postprandial glucose concentrations the morning after exercise. Nevertheless, reductions in insulin concentrations with unchanged glucose after exercise indicate improved glucose control. Indices of insulin sensitivity, including the homoeostasis model assessment for insulin resistance (HOMA-IR) and insulin sensitivity index, may also improve the morning after exercise (Brestoff et al., 2009; Kokalas et al., 2005), although some have observed no change (Burton et al., 2008; Holtz et al., 2008). It is possible that more sensitive measures of insulin resistance (e.g., glucose clamp methods, minimal model method) may be required to detect potential changes in some studies. Only one study appears to have measured glucose and insulin concentrations the morning after acute exercise in young people. MacEneaney et al. (2009) reported 2.51 MJ (600 kcal) of exercise did not affect fasting and postprandial glucose or insulin in overweight or non-overweight adolescent boys. However, this study was not specifically designed to examine glucose or insulin and this was reflected in the study design, which included a high-fat breakfast meal and

the measurement of only three blood samples during the immediate 2 h postprandial period (MacEneaney et al., 2009). The inclusion of a high CHO breakfast containing HGI CHO to induce greater glucose and insulin responses and more frequent blood sampling may be more appropriate to detect potential differences between conditions and groups.

Glycogen repletion following exercise occurs in two distinct phases: an early insulin-independent period of rapid glycogen resynthesis (lasting approximately 1 h post-exercise) and a subsequent period (up to 72 h post-exercise) of slow insulin-dependent glycogen resynthesis (King et al., 1995; Price et al., 1999). Insulin-mediated glucose uptake is, therefore, elevated after exercise to facilitate glycogen synthesis (Holloszy, 2005), which may ultimately reduce postprandial insulin concentrations. Indeed, the increase in insulin sensitivity after exercise has been found to be directly proportional to the magnitude of muscle glycogen depletion during exercise (Cartee et al., 1989; Wojtaszewski et al., 2000). Although the underlying cellular mechanisms controlling post-exercise increases in insulin-mediated glucose uptake remain elusive, increased GLUT-4 translocation from intracellular storage sites to the plasma membrane is thought to play a crucial role; GLUT-4 was translocated to the plasma membrane 3.5 h after exercise in rodents (Hansen et al., 1998) and immediately after exercise in adults (Kennedy et al., 1999; Thorell et al., 1999). Moreover, there was a progressive post-exercise increase in GLUT-4 protein concentration that peaked at 16 h in rodents, although measurements were not taken after this time (Kuo et al., 1999), and GLUT-4 mRNA was increased up to 22 h after exercise in adults (Stephens et al., 2010). Post-exercise alterations in GLUT-4 may not be completely controlled by glycogen depletion, as isoenergetic exercise at 40 and 80% $V\square O_{2peak}$ increased GLUT-4 mRNA and GLUT-4 protein to a similar extent in human skeletal muscle, despite greater glycogen degradation after the higher intensity exercise (Kraniou et al., 2006). In more recent years, other cellular mechanisms have been proposed to mediate post-exercise increases in insulin sensitivity (Maarbjerg et al., 2011), which relate to increased glycogen synthase activity (Koval et al., 1998), increased hexokinase II (HK II) mRNA (Koval et al., 1998, Stephens et al., 2010) and reduced muscle malonyl-CoA content (Frøsig et al., 2009).

2.9.2 Fat oxidation

It is also well documented that fasting and postprandial fat oxidation are increased the morning after a bout of exercise in healthy (Schenk and Horowitz, 2007; Votruba et al., 2002) and overweight/obese (Burton et al., 2008; Holtz et al., 2008) adults. Moreover, lower RER values (Burton et al., 2008) and increased fat oxidation as a percentage of total energy expenditure (Horton et al., 1998) suggest these post-exercise increases in fat oxidation are not solely due to increased total energy expenditure. However, similar studies in young people do not appear to be available. It has been postulated that the high metabolic priority for muscle glycogen synthesis following exercise could explain the concomitant elevated fat oxidation (Kiens and Richter, 1998). Indeed, lower post-exercise insulin concentrations could augment fat oxidation (Horowitz et al., 1997).

More recently, the contention that the elevated fat oxidation after exercise could contribute to enhancements in insulin sensitivity has received much attention and increasing support (Horowitz, 2007). Accumulation of fatty acid metabolites within the muscle can induce insulin resistance (see section 2.1). Schenk and Horowitz (2007) demonstrated that a single exercise bout protected against fatty acid induced insulin resistance in women, which was accompanied by increased IMTG synthesis, reduced accumulation of fatty acid metabolites within skeletal muscle and suppressed activation of proinflammatory pathways known to impair insulin action. Thus, FFA entering the muscle cell after exercise were preferentially partitioned toward IMTG synthesis rather than toward the accumulation of the more damaging fatty acid intermediates that impair insulin sensitivity. Further support for this comes from studies in rat skeletal muscle (Thrush et al., 2011). Additionally, reducing exercise fat oxidation through the ingestion of a lipolysis inhibitor abolished the exercise-induced reduction in postprandial insulin in men, highlighting the importance of promoting fat oxidation during exercise (Malkova et al., 1999). However, it cannot be discounted that the lowered muscle glycogen concentration the day after exercise may have contributed to enhancements insulin sensitivity (Schenk and Horowitz, 2007). Previous work demonstrated that raising IMTG concentration the morning after exercise via increased post-exercise dietary fat intake (Fox et al., 2004) or overnight lipid infusion (Schenk et al., 2005) did not alter insulin sensitivity. On the surface, this implied that post-exercise muscle glycogen content is more critical than IMTG in regulating exercise-induced changes in insulin sensitivity and conflicted with the notion that IMTG accumulation is

related to insulin resistance; however, this relationship is not functional (see section 2.1). The higher IMTG content may have actually reflected an increase in the partitioning of FFA toward IMTG synthesis and a reduced accumulation of fatty acid metabolites, which is in agreement with the study of Schenk and Horowitz (2007).

Possible underlying cellular mechanisms mediating the enhanced fat oxidation after acute exercise include increased abundance of FAT/CD36 at the mitochondrial membrane (Campbell et al., 2004), increased muscle LPL activity (Kiens and Richter, 1998), reduced activity of pyruvate dehydrogenase (PDH, a key regulator of CHO metabolism; Kimber et al., 2003), reduced acetyl CoA, acetylcarnitine and pyruvate concentrations (Kimber et al., 2003) and increased adenosine monophosphate (AMP)-activated protein kinase (AMPK) activity (Ruderman et al., 2003). Increased AMPK activity may lower malonyl-CoA content, facilitating the transport of FFA into the mitochondria, and post-exercise reductions in malonyl-CoA were correlated with improved insulin-stimulated glucose uptake in men (Frøsig et al., 2009), supporting the link between increased fat oxidation and insulin sensitivity after exercise.

2.9.3 Energy and carbohydrate balance

With the crucial mechanistic role of muscle substrate stores, energy and/or CHO balance appears to be the main factor governing changes in metabolism after exercise. This can be affected by exercise energy expenditure (intensity, duration, mode, participant characteristics) and post-exercise energy and macronutrient intake. The maintenance of an energy deficit following exercise augmented the exercise-induced reduction in postprandial insulin, increase in fat oxidation and decrease in RER the next morning in overweight men (Burton et al., 2008). It has been suggested that the relationship between exercise energy expenditure and reduction in HOMA-IR is curvilinear, with a relatively high threshold of 3.77 MJ (900 kcal) for improvements to be manifested in active men (Magkos et al., 2008). However, this study did not measure energy and CHO balance and participants only completed one exercise condition, thus differences between participants could have confounded the results.

Studies directly comparing the independent effect of energy and CHO deficit suggest that the latter may be more important in explaining post-exercise increases in insulin sensitivity. Newsom et al. (2010) reported that the maintenance of an energy deficit

after exercise did not augment post-exercise enhancements in insulin sensitivity, whereas CHO deficit prevented muscle glycogen restoration and increased insulin sensitivity the next morning. In support, despite post-exercise energy replacement, the maintenance of a CHO deficit augmented the increased non-oxidative glucose disposal (glucose storage) and reduced glucose oxidation 12 h after exercise compared with energy and CHO replacement. Moreover, the magnitude of change in insulin action the day after exercise was directly proportional to the magnitude of the CHO deficit (Holtz et al., 2008). When dietary CHO and protein were kept constant, the addition of energy to post-exercise meals in the form of fat (Fox et al., 2004) or overnight lipid infusion (Schenk et al., 2005) did not alter postprandial glucose, insulin or insulin sensitivity the next day, despite the substantially higher energy intake, providing further evidence that post-exercise CHO rather than energy intake appears to control changes in insulin sensitivity. However, these studies did not include a control condition with no exercise (Fox et al., 2004; Holtz et al., 2008; Schenk et al., 2005). At the cellular level, energy or CHO replacement following exercise facilitates muscle glycogen restoration, lowers GLUT-4 and HK II mRNA and blunts the increased plasma membrane GLUT-4 protein (Cheng et al., 2005; Chou et al., 2005), supporting the importance of post-exercise energy and CHO intake.

In contrast, energy balance may be a more important factor driving post-exercise fat oxidation than CHO balance. Post-exercise energy deficit increased fat oxidation the day after exercise independent of CHO intake (Horowitz et al., 2005) and to a larger degree than CHO deficit (Newsom et al., 2010). Furthermore, post-exercise CHO deficit with energy replacement did not augment exercise-induced enhancements in fat oxidation (Holtz et al., 2008). In support, others have shown that exercise did not increase 24 h fat oxidation when exercise energy expenditure was replaced immediately post-exercise, although these studies did not include a CHO deficit condition (Dionne et al., 1999; Melanson et al., 2009). Energy deficit increases FFA availability, increases PDH kinase-4 mRNA expression and suppresses CHO oxidation even when dietary CHO content is not reduced (Horowitz et al., 2005), supporting the contention that energy rather than CHO deficit is paramount in this relationship.

A potential limitation of studies attempting to examine the independent effects of energy and CHO deficit is that, relative to when the post-exercise meal restored energy

and CHO balance, the fat content of the meal was reduced to create a state of energy deficit with CHO balance and increased to create a state of CHO deficit with energy balance (Horowitz et al., 2005; Newsom et al., 2010). Thus, an alternative interpretation may be that the observed findings were a direct result of differences in fat intake between conditions, rather than energy or CHO deficit. However, as discussed, the ingestion of fat following exercise does not appear to affect changes in insulin sensitivity (Fox et al., 2004; Schenk et al., 2005).

Importantly, it should be highlighted that exercise can induce favourable alterations in insulin and fat oxidation the next day even when energy and CHO balance is maintained, suggesting that energy and/or CHO deficit can augment these changes but is not a requirement (Burton et al., 2008; Newsom et al., 2010). Indeed, insulin sensitivity may continue to be elevated following exercise until glycogen is raised above pre-exercise levels and glycogen supercompensation is achieved (Cartee et al., 1989; Kawanaka et al., 1999). Furthermore, an exercise-induced, but not an equivalent dietary-induced, energy deficit lowered postprandial insulin concentrations in adults (Gill and Hardman, 2000) and exercise lowered the elevated postprandial insulin response induced by systematic overfeeding even when opposed by continued energy surplus (Hagobian et al., 2006), suggesting that energy deficit is not the sole determinant of exercise-induced changes in postprandial metabolism.

Although these studies enhance our understanding of the factors controlling post-exercise changes in metabolism, the prescribed post-exercise diet may not reflect the normal dietary habits of individuals. Compensatory increases in energy intake may occur in the post-exercise period and there is evidence of increased appetite during the later stages of recovery following acute exercise (Malkova et al., 2008). Studying the effect of a bout of exercise with the maintenance of habitual diet on subsequent metabolism may, therefore, more directly reflect a ‘real world’ situation. When an ad libitum breakfast was provided the morning after exercise, exercise-induced reductions in postprandial insulin and elevations in postprandial fat oxidation still occurred (Farah et al., 2010). It is noteworthy that these effects persisted despite participants consuming around 1.26 MJ (300 kcal) and 12 g CHO more the morning after exercise, although this difference was not significant. Therefore, these metabolic benefits of acute exercise appear to extend to ‘real world’ settings where food intake is not carefully controlled.

2.9.4 Exercise characteristics

There is some evidence that exercise intensity can affect post-exercise improvements in insulin. When compared with endurance exercise (75% V□□O_{2peak}), sprint intermittent exercise (125% V□O_{2peak}) did not improve insulin sensitivity or reduce postprandial insulin, perhaps due to the longer exercise duration of the endurance exercise and possibly higher energy expenditure (Brestoff et al., 2009). However, when comparing isoenergetic bouts of low (35% V□O_{2peak}) and high (70% V□O_{2peak}) intensity exercise, hyperglycaemia was only reduced following the low intensity exercise in type 2 diabetic men (Manders et al., 2010). This could be attributed to the high counter regulatory hormonal responses following high intensity exercise (Kjaer et al., 1990) or the eccentric load of sprint exercise and possible muscle damage (Del Aguila et al., 2000). Indeed, eccentric exercise that results in muscle damage is followed by a transient period of insulin resistance (Kirwan et al., 1992). Interestingly, the increased GLUT-4 mRNA and GLUT-4 protein after exercise was independent of exercise intensity and duration, but the increase in total crude membrane GLUT-4 protein was more pronounced 3 h after low intensity exercise at 39% V□□O_{2peak} (106% higher) compared with high intensity exercise at 83% V□□O_{2peak} (61% higher), although this difference was not significant (Kraniou et al., 2006). In addition, exercise utilising a greater amount of muscle tissue may augment post-exercise reductions in glucose and insulin; two-leg cycling resulted in lower 18 h post-exercise glucose and insulin responses compared with one-leg cycling with a similar energy expenditure, possibly due to elevated muscular blood flow during the two-leg cycling (Brambrink et al., 1997). Exercise intensity does not appear to affect the extent of the increase in post-exercise fat oxidation when exercise energy expenditure is constant, although the higher fat oxidation during moderate compared with high intensity exercise may result in a higher cumulative fat oxidation (Melanson et al., 2002; Votruba et al., 2002).

2.9.5 Obesity

Despite observations of post-exercise increases in fat oxidation and reductions in insulin concentrations in non-overweight, overweight and obese adults (section 2.9.1 and 2.9.2), studies directly examining the effect of weight status on exercise-induced changes in metabolism are sparse and have produced conflicting results. As discussed in section 2.9.1, exercise-induced changes in glucose and insulin were not observed in overweight or non-overweight adolescent boys (MacEneaney et al., 2009). In contrast,

exercise reduced postprandial insulin concentrations in both overweight and non-overweight women (Mitchell et al., 2008) and in obese, but not non-obese, men (Gill et al., 2004). A major flaw in these adult-based studies was that exercise intensity and duration were the same for all participants, and consequently, exercise energy expenditure was higher in the overweight/obese groups, confounding between-group comparisons (Gill et al., 2004; Mitchell et al., 2008). Indeed, the higher energy expenditure in the obese compared with non-obese men may explain why exercise-induced reductions in postprandial insulin were observed in the obese men only (Gill et al., 2004). Furthermore, as with the study in adolescent boys (MacEneaney et al., 2009), glucose and insulin have not necessarily been the main outcome variables in these adult studies, which have included high-fat breakfast meals and infrequent blood sampling during the immediate postprandial period (Gill et al., 2004). There is also some limited evidence that exercise-induced changes in substrate oxidation may depend on the weight status of the individual (Melanson et al., 2009). In lean endurance trained, lean sedentary and obese sedentary adults, exercise with energy replacement (energy balance maintained) did not increase 24 h fat oxidation, whereas 24 h CHO oxidation increased in the lean endurance-trained and lean sedentary, but not the obese sedentary adults (Melanson et al., 2009). However, exercise characteristics (energy expenditure and exercise mode) differed between individuals, possibly confounding between-group comparisons.

Obesity may affect the magnitude of exercise-induced changes in metabolism due to differences in fat and insulin metabolism between obese and non-obese individuals (see section 2.1). Obese adults have delayed adipose tissue blood flow, slower lipid mobilisation and lower plasma glycerol concentration 2 h post-exercise (Børsheim et al., 2000). Furthermore, patterns of subcellular distribution of GLUT-4 and FAT/CD36 at the plasma membrane appear to be opposed in obesity; GLUT-4 translocation from intracellular stores to the sarcolemma by insulin is inhibited, whilst more FAT/CD36 is located at the plasma membrane (Bonen et al., 2002; 2004). Given the potential role of substrate transporters in mediating post-exercise changes in metabolism, it is plausible that potential metabolic improvements could depend on the weight status of the individual.

2.9.6 Conclusions

Evidence in adults indicates that a single bout of exercise performed ~16 h prior to breakfast consumption can reduce postprandial insulin concentrations and increase fat oxidation, although the effect on glucose is less clear. These exercise-induced metabolic benefits may be augmented by maintaining the exercise-induced energy and/or CHO deficit. Possible mechanisms may relate to muscle glycogen depletion, GLUT-4 translocation and a reduced accumulation of fatty acid metabolites. Although it is clear that exercise training can increase insulin sensitivity and fat oxidation in young people, studies have not yet examined the acute effect of exercise on these health markers. The clinical relevance of this work may be particularly important for overweight or obese individuals (see section 2.1), thus it would be worthwhile to assess the acute effect of exercise on postprandial glucose, insulin and fat oxidation in both overweight and non-overweight young people.

2.10 Summary

The high prevalence of overweight and obesity in young people has prompted a need for evidence-based interventions aimed at improving metabolism and weight control in the paediatric population. Fat oxidation has been implicated in the development of insulin resistance, the most common metabolic alteration associated with obesity. Exercise increases fat oxidation and there has been growing interest in the exercise intensity that promotes maximal fat oxidation (Fatmax) in young people. However, little is known regarding the exercise characteristics that can be manipulated to augment increases in fat oxidation or whether the consumption of different types of breakfast meals affects this relationship. There has been considerable interest in the health benefits of LGI diets and exercise training, although studies have not yet examined the acute effect of breakfast GI or exercise on glucose, insulin and fat metabolism in young people. Based on the adult literature, it is plausible that acute manipulation of breakfast GI and a single exercise bout can induce favourable health outcomes in young people, relating to fat oxidation and insulin resistance. Overall, it is clear that our understanding of exercise metabolism in young people is severely lacking relative to that in adults. A better understanding of the exercise characteristics required to increase fat oxidation and reduce insulin resistance would provide valuable information for maximising the important metabolic benefits of each exercise session in young people.

Chapter 3

General Methods

The purpose of this chapter is to describe the methods used in the experimental studies that follow. These methods were common between studies; methods that specifically relate to individual studies are described within the individual experimental chapters (Chapters 4 to 7).

3.1 Participants

After gaining approval from the University Ethical Advisory sub-Committee, participants were recruited from local schools in Loughborough. Written informed consent was obtained from the primary carer and the participants provided their written "willingness to participate". Participants were screened using a health history questionnaire (Appendix 1). Exclusion criteria included: known congenital heart disease, musculoskeletal problems, uncontrolled exercise-induced asthma, diabetes and epilepsy.

3.2 Anthropometry

Anthropometric characteristics were assessed and recorded prior to all experimental trials. Stature was measured using a stadiometer (Holtain, Holtain Limited, Dyfed, UK) to the nearest 0.01 m and body mass (BM) was measured using a beam balance scale (Seca Model 888, Hamburg, Germany) to the nearest 0.1 kg. Body mass index (BMI) was calculated as body mass (kg) divided by stature squared (m^2). Skinfold thickness was determined from three different sites (triceps, subscapular and medial calf) on the right hand side of the body using a Harpenden skinfold calliper to the nearest 0.2 mm (Baty International, England). Each site was measured three times by the same investigator and the median value for each site was used to estimate percentage body fat (% BF) according to Slaughter et al. (1988). Fat free mass (FFM) in kg was estimated subsequently using the following equation:

$$FFM = BM \cdot (1-(\%BF/100))$$

Waist circumference was measured midway between the 10th rib and the iliac crest (McCarthy et al., 2005) using a Guilick tape measure (Creative Health Products, Plymouth, MI). With the assistance of a primary home-based carer (parent/guardian), participants provided a self-assessment of their physical maturation using secondary sexual characteristics, as discussed in section 2.5.1 (Tanner, 1962; Appendix 2).

3.3 Gas exchange during rest and exercise

Gas exchange was sampled continuously for all exercise tests and for 5 min periods during rest after the participant had rested on a bed in a supine position for 20 min. Ventilatory variables were measured on a breath-by-breath basis and displayed on-line; the K4 b^2 (Cosmed, Rome, Italy) was used in Chapter 4 and 6 and the Metalyzer 3B (Cortex, Leipzig, Germany) was used in Chapters 5 and 7. The flow meter was attached to a facemask (Hans Rudolf, Shawnee, USA) of an appropriate size with a dead space volume of 32 to 40 mL, which was fitted carefully to the face and checked for leaks prior to each test. Gas calibration was performed according to the manufacturer's recommendations using well ventilated room air and a bottled gas mixture containing 5% CO_2, 16% O_2, balance N_2 (K4 b^2: Scott Medical Products, Plumsteadville, PA or Metalyzer 3B: Cranlea and Company, Birmingham, UK). The flow meter was calibrated using a bi-directional 3.0 L volume calibration syringe (Hans Rudolf, Shawnee, USA). All calibration procedures were carried out prior to each experimental test.

3.4 Heart rate

Heart rate (HR) was monitored and recorded continuously during exercise tests using short-range telemetry (Polar Vantage, Polar, Kempele, Finland).

3.5 Exercise tests

Exercise tests were performed on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands) or a treadmill (RunRace, TechnoGym, Gambettola, Italy). Prior to testing, participants were habituated to the laboratory environment, equipment and exercise protocols. In particular, they practiced exercising on the cycle ergometer at 60 revs·min^{-1} and walking and running on the treadmill at various speeds whilst breathing through the facemask. The seat height and pedal cranks were adjusted for each child and replicated during subsequent

measurements using the cycle ergometer. Participants were asked to pedal at 60 revs·min^{-1} for all exercise trials.

3.5.1 Peak exercise test

Participants completed a peak exercise test to volitional exhaustion for each of the experimental studies. All participants were asked to avoid strenuous exercise and caffeine on the day and food intake 2 h prior to testing. The exercise protocols used were as follows:

Cycling: Following a 2 min warm-up of unloaded pedalling, the work rate increased by 8 or 10 W·min^{-1} (dependent on body size) to attain a test duration of 10 to 12 min.
Treadmill: Participants ran at an individual pre-determined fixed speed chosen to attain a test duration of 10 to 12 min. Following a 2 min warm-up at 0% gradient, the gradient increased by 1% each minute.

In the absence of a plateau in $V\square O_2$, maximal effort was considered to have been reached if the following secondary criteria were achieved: HR levelling off at approximately 200 beats·min^{-1} and RER ≥1.05, in addition to the participant demonstrating clear subjective symptoms of fatigue (Armstrong et al., 1996). The highest 30 s moving average during the exercise test was recorded as $V\square O_{2peak}$.

3.6 Indirect calorimetry

Breath-by-breath ventilatory variables were interpolated into 1 second (s) intervals for all tests. Breath-by-breath responses occasionally contain values that are clearly artifactual, which may result from swallowing or coughing (Lamarra et al., 1987). Therefore, individual $V\square O_2$ and $V\square CO_2$ values that were >3 standard deviations (SDs) from the mean were removed (Lamarra et al., 1987), as were RER values >1. Indirect calorimetry does not provide a valid estimation of substrate oxidation for exercise intensities >80 to 85% $V\square O_{2peak}$ in adults (Romijn et al., 1992) or during the non-steady state (Frayn, 1983). Data >80% $V\square O_{2peak}$ were, therefore, not used. Subsequently, average values for $V\square O_2$ and $V\square CO_2$ were calculated and used for analyses.

Fat oxidation rates were estimated using the following stoichiometric equation with the assumption that the urinary nitrogen excretion rate was negligible (Frayn, 1983):

Fat oxidation ($mg \cdot min^{-1}$) = 1.67 x $V\square O_2$ ($mL \cdot min^{-1}$) - 1.67 x $V\square CO_2$ ($mL \cdot min^{-1}$)

3.7 Blood sampling and analysis

Capillary blood samples were obtained from a pre-warmed hand by finger prick using the Unistik 2 single-use lancing device (Owen Mumford, Oxford, UK) into Microvette CB300 ethylenediaminetetraacetic acid (EDTA) coated capillary blood collection tubes (Sarstedt Ltd, Leicester, UK). Capillary rather than venous blood sampling is preferred for reliable GI testing (Wolever et al., 2003). Duplicate 25 μL aliquots of whole blood were deproteinised in 250 μL of ice cooled perchloric acid (PCA; 2.5%), centrifuged for 4 min at 2415·g and stored at -20°C for blood glucose analysis. The remaining whole blood was centrifuged for 4 min at 2415·g. Plasma was then extracted and stored at -20°C for insulin analysis.

Blood glucose concentration was determined spectrophotometrically using the glucose oxidase method (GOD-PAP, Randox, Crumlin, Ireland). Plasma insulin was measured in duplicate using an enzyme-linked immunosorbent assay (ELISA, Mercodia, Uppsala, Sweden). The total 120 min area under the curve (TAUC) for blood glucose and plasma insulin were calculated using the trapezium rule (Wolever and Jenkins, 1986). HOMA-IR was calculated from fasted glucose and insulin concentrations using the following equation (Matthews et al., 1985):

HOMA-IR = fasting plasma insulin ($mU \cdot L^{-1}$) x fasting plasma blood ($mmol \cdot L^{-1}$) / 22.5

3.8 Perceived hunger

Perceptions of hunger, satisfaction, fullness and prospective food consumption were assessed using 100 mm visual analogue scales (Flint et al., 2000). Following breakfast, participants were also asked to rate how much they liked the breakfast for the assessment of breakfast palatability (Appendix 3).

Chapter 4

Exercise protocols to estimate Fatmax and maximal fat oxidation in children

Abstract

Consensus on the exercise protocol used to estimate Fatmax (the exercise intensity corresponding to maximal fat oxidation (MFO)) in children has not been reached. The present study compared Fatmax estimated using the 3 min incremental cycling protocol (3-INC) and a protocol consisting of several 10 min constant work rate exercise bouts (10-CWR) in 26 prepubertal children (13 boys and 13 girls aged 9.5(0.5) y). Group Fatmax values were the same for 3-INC and 10-CWR (55% $V\dot{O}_{2peak}$, P=1.000) and 95% limits of agreement (LoA) were ± 7% $V\dot{O}_{2peak}$. The 95% LoA for Fatmax were small when considering the range of intensities where fat oxidation remained high. Group MFO values were similar between protocols (P=0.372), but 95% LoA were -94 to 113 $mg \cdot min^{-1}$ and indicated a large degree of within-participant variation. It can be concluded that a 3 min incremental exercise protocol and prolonged isolated exercise bouts result in comparable estimations of Fatmax in prepubertal children. The 3 min exercise protocol is, therefore, recommended for practical reasons. However, caution should be maintained when estimating MFO in prepubertal children.

4.1 Introduction

The exercise intensity that promotes MFO has been termed Fatmax (Achten et al., 2002) and has received a recent surge in interest in young people (Aucouturier et al., 2009; Brandou et al., 2006; Lazzer et al., 2007; 2010; Riddell et al., 2008; Zunquin et al., 2009a). Studies in young people have identified Fatmax using incremental protocols with exercise stages of 3 (Riddell et al., 2008), 3.5 (Zunquin et al., 2009a), 4 (Aucouturier et al., 2009; Lazzer et al., 2010), 5 (Lazzer et al., 2007) and 6 (Brandou et al., 2006) min in duration. In contrast, others have continued to use a more traditional approach of isolated exercise bouts lasting 6 (Stephens et al., 2006) and 8 to 10 (Maffeis et al., 2005) min with standardised recovery periods to estimate fat oxidation at different intensities (see section 2.4).

The major advantage of using an incremental protocol with short stages is that fat oxidation can be estimated across a wide range of exercise intensities and in a single visit to the laboratory. Conversely, the use of longer duration exercise bouts can limit the estimation of fat oxidation to only three (Maffeis et al., 2005) or five (Stephens et al., 2006) different intensities, precluding a precise estimation of Fatmax. Furthermore, there is a trade-off between exercise stage duration and the number of exercise intensities; as stage duration increases, the number of exercise intensities may diminish (e.g., Brandou et al., 2006; Lazzer et al., 2007; Riddell et al., 2008) to such an extent that the advantage of the incremental protocol may be lost. Therefore, an incremental protocol with 3 min stages may be the preferred combination of stage duration and number of stages to estimate Fatmax.

Studies using incremental protocols to estimate Fatmax in young people have adapted the 3 min protocol originally validated by Achten et al. (2002) in trained adult males. The primary issues with a 3 min incremental protocol are whether a physiological steady state is attained before the onset of the sampling period and whether there is a residual (carry-over) effect from stage to stage as the increments progress that influence subsequent fat oxidation estimations (see section 2.3 and 2.4). Oxygen and carbon dioxide kinetics research has shown that children attain a steady state faster than adults and $V\dot{O}_2$ time constant values indicate the attainment of steady state within 2 min (Fawkner et al., 2002; Welsman et al., 2001). However, the time constant may be longer for $V\dot{C}O_2$ (Cooper et al., 1990; Welsman et al., 2001) and, consequently, the

attainment of a steady state may be delayed. Furthermore, V□O_2, but not V□CO_2, kinetics may become progressively slower at higher work rate steps during incremental exercise with 3 min stages (Zhang et al., 1991). To the author's knowledge, no studies have systematically examined the potential residual effect of incremental exercise on fat oxidation in children. Yet, in adults, it has been demonstrated that prior bouts of exercise may increase fat oxidation during subsequent exercise when compared with a single bout of prolonged exercise (Goto et al., 2007) and active warm-up may influence fat oxidation during a subsequent exercise bout, possibly by increasing acetylcarnitine (Gray et al., 2002, Odland et al., 1998) and reducing blood and muscle lactate concentrations (Boyd et al., 1974; Robergs et al., 1991).

Research investigating the potential influence of sex on Fatmax in children appears to be limited to just one study (Lazzer et al., 2007) and the vast majority of this research is based on boys (e.g., Brandou et al., 2006; Riddell et al., 2008). Consequently, there is a need for studies to estimate Fatmax in girls and also examine any potential between-sex differences.

Evidently, there is a lack of consensus on the type of protocol that should be used to estimate Fatmax in young people and inconsistencies in the methods employed limits inter-study comparisons (e.g., Riddell et al., 2008; Stephens et al., 2006). Considering the recent interest in Fatmax and potential clinical relevance of increasing fat oxidation (Ben Ounis et al., 2008; Holloway et al., 2009), it is important to systematically evaluate protocols suitable for estimating Fatmax in children specifically. A criterion ('gold standard') method for the measurement of Fatmax does not exist; a study comparing different protocols to estimate Fatmax would, therefore, provide valuable information. The aim of the present study was to compare Fatmax estimated using an incremental protocol with 3 min stages (3-INC) and several 10 min constant work rate (10-CWR) exercise bouts in prepubertal children. In addition, inclusion of girls and boys allowed an exploration of an independent sex effect.

4.2 Methods

4.2.1 Participants

The sample consisted of 30 prepubertal children (15 boys and 15 girls) aged 8 to 10 y (26 were included in the final analyses). Anthropometric characteristics were assessed

and recorded prior to experimental trials, as described in the General Methods (section 3.2). None of the girls had started the menstrual cycle.

4.2.2 Apparatus

All exercise tests were performed on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). Gas exchange was measured on a breath-by-breath basis and displayed on-line using a portable metabolic cart (K4 b^2, Cosmed, Rome, Italy). Calibration procedures were performed according to the manufacturer's recommendations prior to every test (see section 3.3).

4.2.3 Experimental design

In this cross-sectional study, participants were asked to visit the laboratory on five separate occasions ~7 days apart.

Visit 1: Cycling $V\dot{O}_{2peak}$ measurement

The children were habituated to the laboratory environment, equipment and exercise protocols. Subsequently, an incremental test was completed to volitional exhaustion for the measurement of $V\dot{O}_{2peak}$ (see section 3.5.1).

Visits 2, 3 and 4: Fatmax exercise trials

Participants reported to the laboratory at 08:00 following a 12 h overnight fast. With the assistance of a primary home-based carer (parent/guardian), the children were asked to record their food and drink intake in the 24 h period prior to visit 2 and replicate this before visits 3 and 4 (Appendix 4). Participants also minimised their physical activity on the day prior to exercise testing. A healthy breakfast was provided on the completion of exercise.

Visit 2 (incremental exercise test): Participants completed a submaximal incremental exercise protocol (3-INC) for the determination of Fatmax. The work rate began at 0 W and increased by 6 or 8 W every 3 min (work rate increment dependent on body size). The test was terminated when the RER was ~0.95 or the participant was exercising above 80% $V\dot{O}_{2peak}$. The average number of stages completed was nine (range 8 to 11), which corresponded to a total exercise duration of 27 min.

Visit 3 and 4 (constant work rate exercise bouts): Participants completed 6 x 10 min constant work rate exercise bouts (10-CWR) at exercise intensities corresponding to those in 3-INC, for which Fatmax had been identified previously. The bouts were performed in a randomly assigned counter-balanced order over the two visits (three bouts per visit) and were each separated by a 15 min rest period. The completion of this protocol resulted in an exercise duration of 60 min spanning ~2 h in total.

Visit 5: Repeat measurement of cycling $V\dot{O}_{2peak}$

Participants performed a second exercise test for the confirmation of $V\dot{O}_{2peak}$. The highest $V\dot{O}_{2peak}$ value from either visit 1 or 5 for each child was used for data analyses. The $V\dot{O}_{2peak}$ determined during visit 5 was higher than that determined during visit 1 for the group (P=0.003, ES: 0.56).

4.2.4 Indirect calorimetry and Fatmax calculations

Ventilatory variables during the final min of each stage (3-INC) or bout (10-CWR) were used for data analyses. As detailed in the General Methods, fat oxidation rates were calculated according to Frayn (1983) and data >80% $V\dot{O}_{2peak}$ were not used (see section 3.6). Confirmation of a steady state was achieved by checking the slope of the linear regression line for $V\dot{O}_2$ and $V\dot{C}O_2$ plotted against time during the final 5 min of each exercise bout for 10-CWR. The exercise bout was not included in data analyses if a steady state could not be confirmed (arbitrary slope >0.2). On average, nine exercise stages (3-INC) and five bouts (10-CWR) were included in data analyses for each child.

For each individual, the results from 3-INC and 10-CWR were used to construct a 2nd order polynomial curve of fat oxidation rate against exercise intensity, expressed as % $V\dot{O}_{2peak}$. The curve was used to estimate Fatmax (% $V\dot{O}_{2peak}$), MFO ($mg \cdot min^{-1}$) and the 5% Fatmax zone (range of exercise intensities with fat oxidation rates within 5% of MFO) (Achten et al., 2002). The HR corresponding to Fatmax was calculated using the relationship between % $V\dot{O}_{2peak}$ and HR. The mean(SD) r^2 values for the polynomial curves of fat oxidation against % $V\dot{O}_{2peak}$ were 0.80(0.18) for 3-INC and 0.81(0.21) for 10-CWR. An example of a graph and polynomial fit used to estimate Fatmax using both protocols for a participant is displayed in Figure 4.1.

A simple visual method was also employed to identify Fatmax and MFO (Riddell et al., 2008) to confirm results from the modelled data, where MFO was taken as the highest recorded fat oxidation rate and Fatmax was the corresponding exercise intensity.

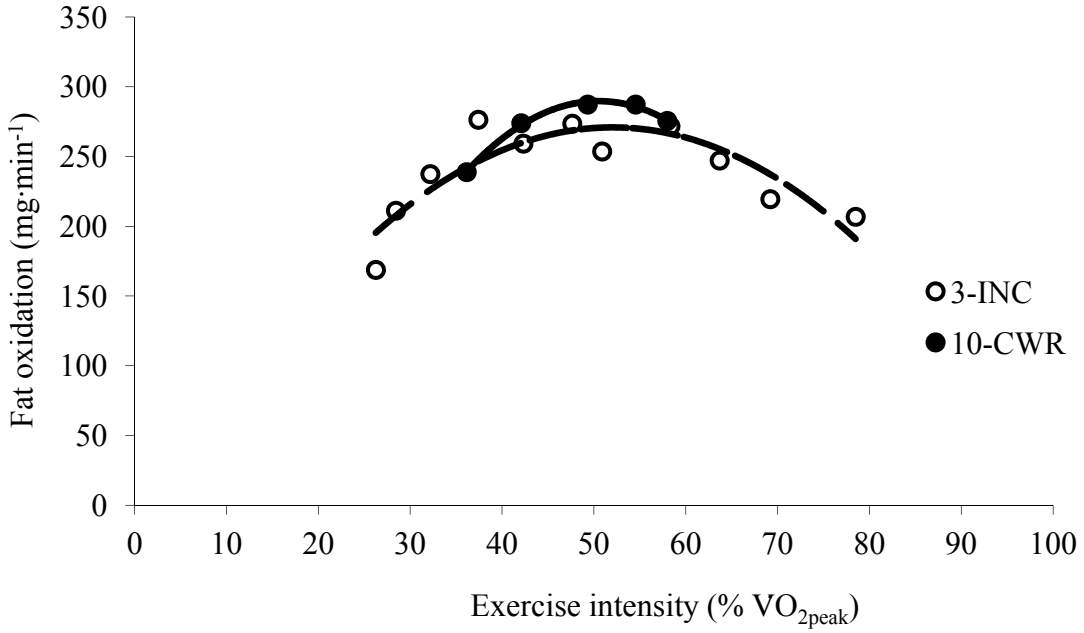


Figure 4.1 Example of a graph of fat oxidation ($mg \cdot min^{-1}$) against exercise intensity (% $V\dot{O}_{2peak}$) used to estimate Fatmax and maximal fat oxidation (MFO) from the 3 min incremental protocol (3-INC) and 10 min constant work rate bouts (10-CWR)

4.2.5 Statistical analyses

Statistical analyses were completed using SPSS software version 16.0 for Windows (SPSS Inc, Chicago, IL, USA). Shapiro-Wilk tests were used to confirm normal distribution and Levene's tests were used to confirm homogeneity of variance. Separate 2 x 2 mixed measures analysis of variance (ANOVA) repeated for protocol were used to examine the data for Fatmax and MFO. Student's independent t-tests were used to compare the girls' and boys' characteristics. Values are expressed as mean(SD), unless stated otherwise, and effect sizes (ES) were calculated (Rosenthal, 1991). Statistical significance was accepted at $P \leq 0.05$.

Limits of agreement (LoA) were used to compare 3-INC and 10-CWR at the individual level (Bland and Altman, 1986). Systematic error (SE), or bias, was determined by calculating the mean difference between 3-INC and 10-CWR and the random error

(RE) was the SD of the paired differences, as outlined by Bland and Altman (1986). The LoA were then calculated by determining a 95% limit above and below the mean difference (bias ± (1.96 × RE)). Student's paired t-tests were used to examine the correlation between the residuals and the mean (proportional error check) and the absolute residuals and the mean (random error check). The 95% LoA for Fatmax were compared with estimated values for the 5% Fatmax zone (range of exercise intensities with fat oxidation rates within 5% of MFO) to determine their practical importance.

4.3 Results

4.3.1 Participant characteristics

Complete data for 26 children (13 girls and 13 boys) were available for analyses. The four children excluded had r^2 values below the arbitrarily chosen threshold of 0.5 for the polynomial models or less than four 10 min CWR bouts available for analyses. The boys had a lower BMI and % body fat compared with the girls ($P \leq 0.05$). The physical characteristics of the participants are displayed in Table 4.1.

Table 4.1 Participant characteristics

	Girls *n*=13	Boys *n*=13	Combined *n*=26
Age (y)	9.3(0.6)	9.8(0.4)	9.5(0.5)
Body mass (kg)	35.1(6.1)	32.3(5.3)	33.7(5.7)
Stature (m)	1.37(0.06)	1.39(0.06)	1.38(0.06)
BMI ($kg \cdot m^{-2}$) [a]	18.6(2.6)	16.5(1.6)	17.6(2.3)
Body fat (%) [a]	21.7(4.7)	15.8(4.5)	18.8(5.4)
FFM (kg)	27.2(3.5)	27.0(3.3)	27.1(3.3)
Waist circumference (cm)	60.5(7.8)	59.7(4.9)	60.1(6.3)
Tanner (pubic hair) †	1(0.5)	1(0.5)	1(0.5)
$V\dot{O}_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) [a]	42(6)	51(7)	47(8)

BMI – body mass index, FFM – fat free mass, Tanner stage – estimation of secondary sexual characteristics (Tanner, 1962), $V\dot{O}_{2peak}$ – peak oxygen uptake.
[a] between sex significant difference ($P<0.05$)
† median (interquartile range)

4.3.2 Peak exercise responses

Peak V□O_2 was higher in the boys compared with girls (P=0.002, ES: 0.57; Table 4.1). All participants included in the analysis achieved the criteria for the attainment of maximal effort. The mean(SD) peak responses for secondary criteria were RER 1.07(0.07) and HR 201(7) beats·min^{-1} or 96(4)% age-predicted HR_{max} (220-age).

4.3.3 Fatmax

Group Comparison: At the group level, Fatmax (% V□O_{2peak}) was the same for 3-INC and 10-CWR (P=1.000, ES: 0.000) and this was independent of sex (P=0.481, ES: 0.14). Furthermore, the main effect for sex (P=0.262, ES: 0.23) showed that small differences in Fatmax between the girls and boys were not meaningful (Table 4.2).

Table 4.2 Group comparisons of Fatmax and maximal fat oxidation (MFO) for the 3 min incremental protocol (3-INC) and 10 min constant work rate bouts (10-CWR)

	Girls *n*=13		Boys *n*=13		Combined *n*=26	
	3-INC	10-CWR	3-INC	10-CWR	3-INC	10-CWR
Fatmax (% V□O_{2peak})	54(6)	53(6)	57(9)	57(9)	55(7)	55(8)
Fatmax (HR, beats·min^{-1})	141(15)	140(14)	140(12)	134(14)	141(13)	137(14)
Fatmax % HR_{max}	71(7)	71(8)	70(7)	67(8)	70(7)	69(8)
MFO (mg·min^{-1})	255(45)	253(33)	265(54)	286(43)	260(49)	270(41)
MFO (mg·kgFFM^{-1}·min^{-1})	9.4(1.4)	9.4(1.7)	9.9(2.3)	10.8(2.2)	9.7(1.9)	10.1(2.0)

V□O_{2peak} – peak oxygen uptake, HR – heart rate, MFO – maximal fat oxidation, FFM – fat free mass.

Individual comparison: Individual paired data provided a systematic bias ± random error of 0 ± 4% V□O_{2peak}, resulting in 95% LoA of ± 7% V□O_{2peak}. Furthermore, 18 of the 26 participants had paired Fatmax values that were within 3% V□O_{2peak} of each

other when comparing 3-INC and 10-CWR (Figure 4.2). Proportional bias was not evident from examination of the residuals and random errors were homoscedastic from examination of the absolute residuals (Table 4.3).

The 5% Fatmax zone spanned 45(6) to 65(9)% V□O_{2peak} for 3-INC and 47(6) to 63(9)% V□O_{2peak} for 10-CWR. Therefore, the 95% LoA were within the 5% Fatmax zone, suggesting that 3-INC provides a practically useful surrogate measure of 10-CWR (Figure 4.3).

Table 4.3 Bias and limits of agreement for Fatmax and maximal fat oxidation (MFO) estimated using the 3 min incremental protocol (3-INC) and 10 min constant work rate bouts (10-CWR)

	Bias ± RE	95% LoA	Residual check		Absolute residual check	
			R value	P value	R value	P value
Fatmax (% V□O_{2peak})	0 ± 4	-7 to +7	0.10	0.626	0.12	0.545
MFO ($mg \cdot min^{-1}$)	9 ± 53	-94 to +113	-0.19	0.364	-0.08	0.701

RE – random error, LoA – limits of agreement, V□O_{2peak} – peak oxygen uptake, MFO – maximal fat oxidation.

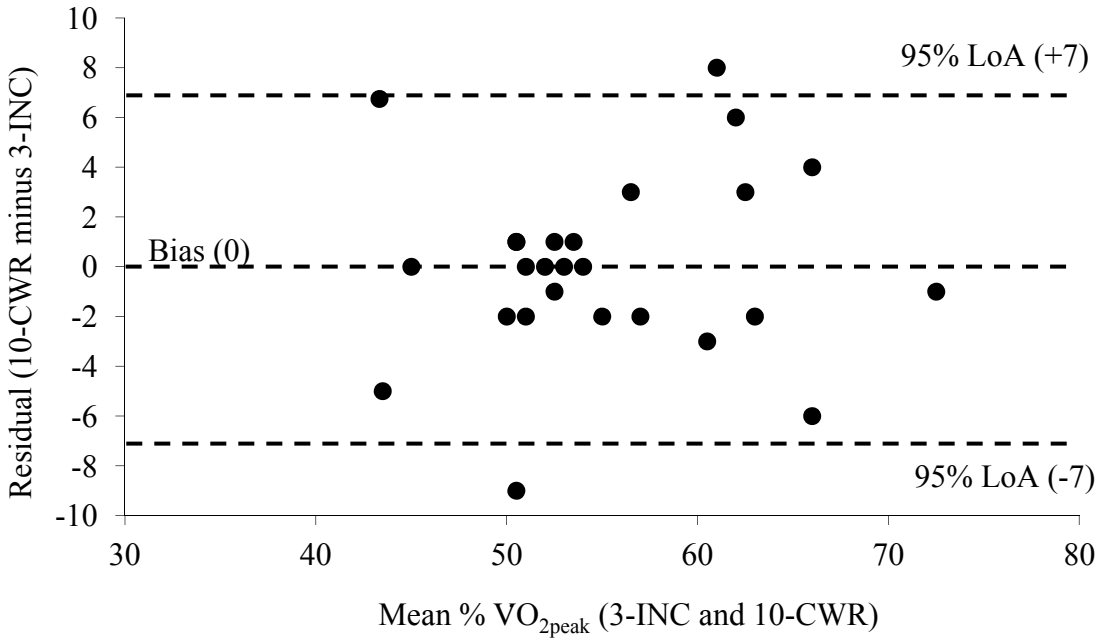


Figure 4.2 Bland-Altman plot of Fatmax (%V□O_{2peak}) for the 3 min incremental protocol (3-INC) and 10 min constant work rate bouts (10-CWR)

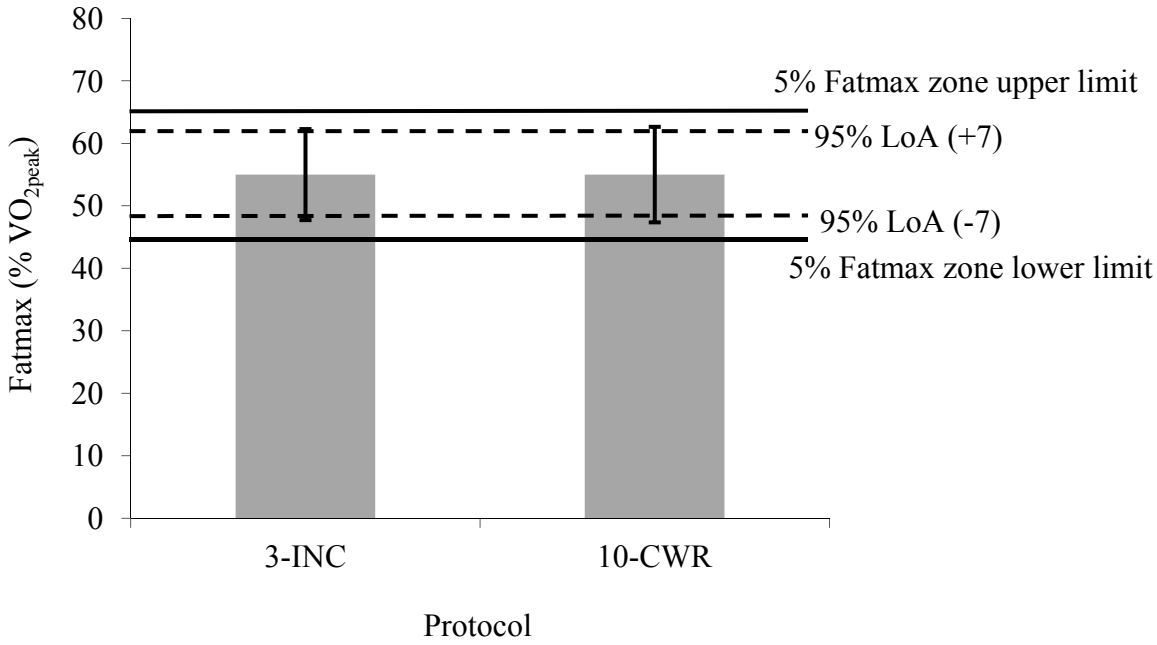


Figure 4.3 Visual representation of Fatmax 95% limits of agreement (LoA) fitting within the 5% Fatmax zone using group Fatmax values

4.3.4 MFO

Group comparison: Group comparisons revealed similar MFO for 3-INC and 10-CWR when expressed as mg·min^{-1} (P=0.372, ES = 0.18) and mg·FFM^{-1}·min^{-1} (P=0.290, ES: 0.21). The sex by protocol interactions for both absolute (P=0.271, ES: 0.22) and scaled MFO (P=0.316, ES: 0.20) again indicated that the small between protocol effects were independent of sex. In addition, the main effect for sex for absolute (P=0.138, ES: 0.30) and scaled MFO (P=0.171, ES: 0.28) showed that differences between the girls and boys were not meaningful (Table 4.2).

Individual comparison: For absolute MFO, the systematic bias ± random error was 9 ± 53 mg·min^{-1} and, subsequently, 95% LoA were -94 to 113 mg·min^{-1}, showing considerable individual variability when comparing 3-INC and 10-CWR (Figure 4.4). Proportional bias was not evident from examination of the residuals and random errors were homoscedastic from examination of the absolute residuals (Table 4.3).

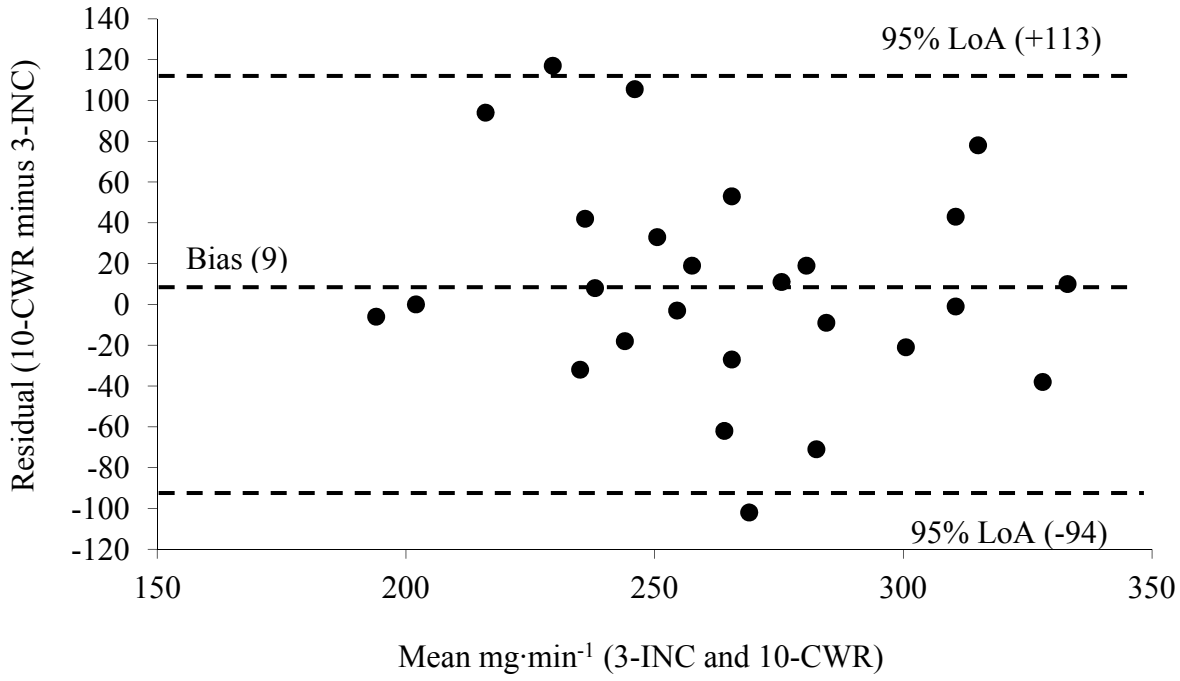


Figure 4.4 Bland-Altman plot of maximal fat oxidation (MFO; mg·min^{-1}) for the 3 min incremental protocol (3-INC) and 10 min constant work rate bouts (10-CWR)

4.3.5 Visual analyses

Visual analyses were consistent with results from the polynomial modelled data. Fatmax values for 3-INC (55(10)% V□O_{2peak}) and 10-CWR (56(8)% V□O_{2peak}) were similar on a group basis (P=0.361, ES: 0.19). Individual analysis showed that systematic bias ± random error was 1 ± 5, resulting in 95% LoA of -9 to +11% V□O_{2peak} for Fatmax. Absolute MFO for 3-INC (271(47) mg·min^{-1}) and 10-CWR (277(40) mg·min^{-1}) were similar again on a group basis (P=0.534, ES: 0.13). Systematic bias ± random error was 6 ± 53, resulting in 95% LoA of -97 to +109 mg·min^{-1} for absolute MFO.

4.4 Discussion

To the author's knowledge, this was the first study to compare Fatmax estimated using different exercise protocols in children. Although the majority of studies have used incremental protocols to estimate Fatmax in children (e.g., Brandou et al., 2006; Riddell et al., 2008), there are two primary issues with a 3 min incremental protocol: (1) whether a physiological steady state is attained before the onset of the sampling period; and (2) whether there is a residual effect from stage to stage as the increments progress that influence subsequent fat oxidation estimations. Therefore, 10 min isolated bouts (10-CWR) were selected to potentially minimise these issues (Rowlands and Hopkins, 2002) and Fatmax for each individual was compared between 3-INC and 10-CWR. This study has systematically demonstrated that a 3 min incremental exercise protocol and prolonged isolated exercise bouts result in comparable estimations of Fatmax in prepubertal girls and boys, indicating that a 3 min exercise protocol can be used to estimate Fatmax in this population.

Fatmax for the group was identical when comparing the two protocols and individual analysis revealed that the 95% LoA were ± 7% V□O_{2peak}. These limits are small enough to recommend the use of 3-INC for the estimation of Fatmax for various reasons. Firstly, fat oxidation rates were within 5% of MFO (mg·min^{-1}) from 45 to 65% V□O_{2peak} (3-INC) and 47 to 63% V□O_{2peak} (10-CWR), thus the 95% LoA were within the limits of the 5% Fatmax zone. Furthermore, fat oxidation remained high (within 5% of MFO) over a wide range of exercise intensities, thus a small under- or over-estimation in Fatmax is only likely to have negligible effect on absolute fat oxidation rates. A major strength of 3-INC is the estimation of fat oxidation at around ten

different exercise intensities using a single test lasting approximately 30 min. Although the 2nd order polynomial r^2 values (0.80 for 3-INC, 0.81 for 10-CWR) indicated a moderate to good goodness of fit for both protocols, it was not possible to estimate fat oxidation over the same number of exercise intensities for 3-INC and 10-CWR. Indeed, pilot work indicated a residual effect of over three repeated 10 min bouts on fat oxidation in a single fasted session similar to the up-regulation reported during prolonged steady state exercise (Delamarche et al., 1992). In this respect, 3-INC is undoubtedly practically advantageous when compared with 10-CWR, with the latter requiring multiple visits to the laboratory and the replication of food intake and physical activity in the days preceding each measurement.

The findings of the present study are in agreement with Achten et al. (2002), where it was reported that a 3 min incremental protocol can be used to identify Fatmax in trained adult males when compared with longer isolated bouts. A more recent study comparing Fatmax using two incremental protocols in sedentary adults reported an average underestimation of 2 W when using 3 compared with 6 min stages and a maximum difference of 8 W (Bordenave et al., 2007). Given the small magnitude of these between protocol differences, the practical implications may be negligible. Therefore, the results of the present study support previous findings in adults that suggest only small differences are evident when comparing short and long exercise stages to estimate Fatmax (Achten et al., 2002; Bordenave et al., 2007). Furthermore, the $V\square O_2$ and $V\square CO_2$ kinetic response to moderate intensity exercise is faster in children compared with adults (Fawkner et al., 2002; Welsman et al., 2001), supporting the use of short 3 min stages in children, although fat oxidation was not estimated in these studies.

Fatmax for the group occurred at 55% $V\square O_{2peak}$ (3-INC and 10-CWR), corresponding to heart rates of 141 (3-INC) and 137 (10-CWR) beats·min^{-1}. Similarly, Fatmax values reported previously in prepubertal boys have ranged from 49 to 56% $V\square O_{2peak}$ (Brandou et al., 2006; Riddell et al., 2008; Zunquin et al., 2009a). However, the majority of these studies have included obese participants (Brandou et al., 2006; Zunquin et al., 2009a), with only one study including non-obese boys (Riddell et al., 2008). Moreover, this study has extended some of these findings to non-obese girls, a population where Fatmax values do not appear to be available. The wide limits of the 5% Fatmax zone suggest that fat oxidation remained high over a large range of exercise

intensities, a finding that is also in agreement with Achten et al. (2002). Therefore, prescribing exercise within the Fatmax zone rather than Fatmax specifically may be sufficient to promote high fat oxidation rates. However, individual prescription is required for exercise at Fatmax (or within the Fatmax zone) due to the large inter-individual variability observed in the present study (40 to 73% V□O_{2peak}) and other studies with adults (Meyer et al., 2007). The 3 min incremental protocol provides a practical method for providing this individual exercise prescription. The large inter-individual variation also suggests that fat oxidation should be assessed at exercise intensities as high as 73% V□O_{2peak} in some children (Fatmax occurred above 60% V□O_{2peak} in 6 of the 26 participants), although previous studies have only identified fat oxidation rates up to 60% V□O_{2peak} (Brandou et al., 2006; Zunquin et al., 2009a).

The findings related to MFO in the present study are less clear. On a group level, MFO was similar for 3-INC and 10-CWR (260 vs. 270 mg·min^{-1}, respectively). However, the large 95% LoA (-94 to +113 mg·min^{-1}) indicate considerable intra-individual variability when comparing protocols. Achten et al. (2002) also reported similar group fat oxidation rates between a 3 min incremental protocol and prolonged steady state bouts, but the correlation data provided did not allow insight at the individual level. Data from the present study suggests that the estimation of MFO depends on the protocol employed. However, the exercise protocol may only be partially responsible for these differences, as the residuals between 3-INC and 10-CWR were randomly distributed above and below the small bias. Although participants were asked to consume the same diet and to minimise physical activity the day preceding each Fatmax exercise test, this may not have been adequate to control for inherent day to day variations in RER and fat oxidation (Bagger et al., 2003); even controlling food intake 36 h before trials may not be sufficient (Meyer et al., 2007). Initial muscle glycogen content and dietary fat intake are both determinants of resting and exercise metabolism; therefore, variations in either or both may have influenced the results (Cameron-Smith et al., 2003; Goedecke et al., 2002). When considering the clear practical applications of estimating Fatmax and the practical advantages of 3-INC, the discrepancy between the two protocols concerning MFO is not clear enough to recommend the use of prolonged isolated exercise bouts. Moreover, 3-INC may be used to estimate MFO in comparative studies. Importantly, studies estimating Fatmax should acknowledge the issues highlighted within this study

and caution should be maintained when reporting MFO values and making inter-study comparisons.

A further finding of the present study was that sex did not influence Fatmax or MFO in prepubertal children. However, differences in $V\dot{O}_{2peak}$, BMI and % body fat between the girls and boys may have affected the between sex comparison. In contrast to the present study, higher absolute fat oxidation has been reported in obese pubertal boys compared with girls (Lazzer et al., 2007). Differences between studies may have resulted from puberty, which has been shown to influence Fatmax and fat oxidation (Riddell et al., 2008). Further research is required to examine the effect of sex on Fatmax and fat oxidation during exercise in young people when these factors have been carefully matched.

Possible limitations of the present study include the use of a 5% Fatmax zone to interpret the LoA rather than a previously defined clinical anchor. However, it is a reasonable assumption that a 5% reduction in MFO will have a small effect on total fat oxidation and thus the potential health benefits of exercising at MFO will continue to be promoted. Although steps were taken to increase the validity of indirect calorimetry for fat oxidation estimations (e.g., excluding data >80% $V\dot{O}_{2peak,}$ checking for a steady state in $V\dot{O}_2$ and $V\dot{C}O_2$), we assumed that the urinary nitrogen excretion rate was negligible and did not account for an increase in non-respiratory carbon dioxide excretion that may have resulted in an underestimation of fat oxidation at some of the higher exercise intensities (Rowlands, 2005). Furthermore, estimations of FFM were based on % body fat values from skinfold measurements, which could introduce a source of error in MFO values expressed relative to FFM. It should also be acknowledged that fat oxidation increases with exercise duration in children (Delamarche et al., 1992), thus the fat oxidation values reported using short duration exercise stages are likely to underestimate fat oxidation during prolonged exercise. Finally, the present study only included healthy prepubertal children. Consequently, it is not possible to recommend the use of 3-INC for children with conditions that may affect substrate oxidation or slow $V\dot{O}_2$ or $V\dot{C}O_2$ kinetics. Indeed, slower $V\dot{C}O_2$ kinetics in obese compared with non-obese children (Cooper et al., 1990) suggests that slightly longer stages (~4 min) may be preferred in this population (Aucouturier et al., 2009; Lazzer et al., 2010). However, research in this area is inconclusive (Cooper et al.,

1990; Unnithan et al., 2007). Future research investigating the validity of 3-INC in obese children is, therefore, warranted.

In conclusion, an incremental exercise test with 3 min stages provided a similar estimation of Fatmax compared with several 10 min constant work rate exercise bouts in prepubertal children. However, caution should be maintained when estimating MFO in these children. The 3 min incremental protocol is, therefore, recommended to provide an estimation of Fatmax using a wide range of intensities and for practical reasons. The estimation of Fatmax using a practical protocol should ensure optimal exercise prescription for maximising fat oxidation during exercise that may help to manage obesity, insulin resistance and other health-related conditions.

Chapter 5

Comparison of Fatmax and fat oxidation over a range of intensities during treadmill and cycling exercise in children

Abstract

Exercise mode and intensity are two of the main factors influencing fat oxidation during exercise. A direct comparison of fat oxidation over a range of exercise intensities and the estimation of Fatmax (exercise intensity that elicits maximal fat oxidation) during treadmill (TM) and cycling exercise (CE) does not appear to be available in children. Fat oxidation and Fatmax were compared during TM and CE in 22 pre- to early pubertal children (9 girls and 13 boys aged 9.9(0.8) y). Fat oxidation was higher for TM compared with CE over a range of absolute ($V\square O_2$, $L \cdot min^{-1}$) and relative (% $V\square O_{2peak}$) exercise intensities and this difference was more pronounced at higher intensities ($P<0.05$). Fat oxidation was higher in the boys compared with girls at similar relative, but not absolute intensities ($P<0.05$). Fatmax was higher during TM compared with CE and higher in boys compared with girls ($P<0.05$). The 5% Fatmax zone (range of exercise intensities where fat oxidation was within 5% of maximal fat oxidation) spanned a wider range of intensities for TM compared with CE ($P<0.05$). Collectively, these findings suggest that exercise programmes aimed at promoting high rates of fat oxidation in pre- to early pubertal children should include TM rather than CE regardless of the exercise intensity. Furthermore, Fatmax values indicate that brisk walking or slow running promotes maximal fat oxidation rates in this population.

5.1 Introduction

Exercise mode and intensity are two of the main factors influencing fat oxidation during exercise and should, therefore, be considered when implementing interventions to promote high rates of fat oxidation (Achten et al., 2003). Fatmax (the intensity corresponding to MFO) has received increasing attention in young people and can be determined by estimating fat oxidation over a range of intensities in children (Chapter 4). Cycling prescription at Fatmax has been shown to improve exercise fat oxidation and a number of health markers in young people (Ben Ounis et al., 2008; 2009). It is possible that walking or running may help to further optimise these effects through the recruitment of a larger active muscle mass and subsequent elevation of fat oxidation. However, studies comparing Fatmax between treadmill and cycling exercise in children do not appear to be available. Moreover, the adult literature is equivocal, with some showing Fatmax is similar during treadmill and cycling exercise (Achten et al., 2003; Glass et al., 1999) and another suggesting Fatmax is higher during treadmill exercise (Chenevière et al., 2010).

Several considerations must be taken into account when comparing fat oxidation between exercise modes. Traditionally, studies examining the effect of exercise mode on fat oxidation have used a small number of exercise intensities corresponding to the exercise mode-specific $V\dot{O}_{2peak}$ (Houmard et al., 1991; Mácek et al., 1976). However, $V\dot{O}_{2peak}$ is typically 7 to 10% higher for treadmill compared with cycling exercise in untrained children and adults (Mácek et al., 1976; Millet et al., 2009), thus the higher absolute $V\dot{O}_2$ during treadmill exercise may explain differences in fat oxidation between exercise modes. Therefore, a comparison of fat oxidation over a wide range of both relative (% mode-specific $V\dot{O}_{2peak}$) and absolute ($V\dot{O}_2$, $L \cdot min^{-1}$) exercise intensities is needed. Indeed, more recent work using incremental exercise protocols to estimate fat oxidation over a range of intensities has reported that treadmill exercise promotes higher fat oxidation rates compared with cycling at comparable absolute $V\dot{O}_2$ values in obese adolescent boys (Lafortuna et al., 2010) and trained adults (Achten et al., 2003). In contrast, no difference in MFO during treadmill and cycling exercise was reported in moderately trained men and women (Chenevière et al., 2010).

To the author's knowledge, only one study has compared fat oxidation over a range of exercise intensities between treadmill and cycling exercise in young people, but Fatmax

was not estimated and this study was limited to obese adolescent boys (Lafortuna et al., 2010). Similar studies involving girls and non-obese children appear to be unavailable and the influence of puberty on fat oxidation must be considered (Riddell et al., 2008). Therefore, the primary aim of the present study was to compare Fatmax and fat oxidation over a range of intensities during treadmill (TM) and cycling exercise (CE) in pre- to early pubertal children. A secondary aim of the study was to examine potential between-sex differences in Fatmax and fat oxidation.

5.2 Methods

5.2.1 Participants

After gaining ethical approval from the University Ethical Advisory sub-Committee, 22 pre- to early pubertal children (13 boys and 9 girls) aged 8 to 11 y volunteered to participate in the study. Anthropometric characteristics were assessed and recorded prior to experimental trials (see General Methods, section 3.2). None of the girls had started the menstrual cycle.

5.2.2 Experimental design

In this cross-sectional study, participants were asked to visit the laboratory on five separate occasions ~7 days apart. Following the habituation session, participants completed the treadmill trials ($V\dot{O}_{2peak}$ then Fatmax exercise test) and cycling trials ($V\dot{O}_{2peak}$ then Fatmax exercise test) in a counter-balanced order. Exercise tests were performed on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands) and motorised treadmill (RunRace, Technogym, Gambettola, Italy). Expired air was sampled continuously and displayed online using the Metalyzer 3B (Cortex, Leipzig, Germany). Calibration procedures were carried out prior to each experimental test (see General Methods, section 3.3).

5.2.3 Experimental trials

Participants performed incremental exercise tests to voluntary exhaustion for the measurement of TM and CE $V\dot{O}_{2peak}$ (see section 3.5.1). On separate days, the children then completed the Fatmax exercise trials (TM Fatmax and CE Fatmax). Participants reported to the laboratory at 08:00 following a 12 h overnight fast. With the assistance of a primary home-based carer, the children were asked to record their food and drink intake in the 24 h period prior to the first Fatmax test and replicate this before

the second Fatmax test (Appendix 4). Participants also minimised physical activity on the day prior to exercise testing. A healthy breakfast was provided after the exercise trials.

The children performed a TM and CE submaximal incremental exercise protocol with 3 min stages for the estimation of Fatmax:
TM Fatmax exercise test: The speed began at 3 $km \cdot h^{-1}$ and increased by 0.5 $km \cdot h^{-1}$ every 3 min at a constant gradient of 0%.
CE Fatmax exercise test: The work rate began at 0 W and increased by 6 or 8 W every 3 min (dependent on body size).

Tests were terminated when the RER exceeded 0.95 or the participant was exercising above 80% $V\dot{O}_{2peak}$. Expired air, HR and ratings of perceived exertion (RPE) were recorded during the final min of each 3 min stage. The average number of stages completed was 10(1) for both the TM and CE Fatmax tests, which corresponded to an exercise duration of 30 min.

5.2.4 Indirect calorimetry and Fatmax calculations

As detailed in the General Methods, ventilatory variables were collected on a breath-by-breath basis and interpolated into 1 s intervals for all tests; $V\dot{O}_2$ and $V\dot{C}O_2$ values during the final min of each stage of the Fatmax exercise tests were edited (Lamarra et al., 1987), averaged and used for data analyses (see section 3.6). Data > 80% $V\dot{O}_{2peak}$ were removed. Fat oxidation was then calculated according to Frayn (1983). The average number of exercise stages included in data analyses for each participant was 10(2) for TM and 9(1) for CE.

For each individual, graphs of fat oxidation against % $V\dot{O}_{2peak}$ and $V\dot{O}_2$ ($L \cdot min^{-1}$) were used to compare fat oxidation over a range of intensities between exercise modes. Fatmax (% $V\dot{O}_{2peak}$), MFO ($mg \cdot min^{-1}$) and the 5% Fatmax zone (range of exercise intensities with fat oxidation rates within 5% of MFO) were estimated using individual 2^{nd} order polynomial curves of fat oxidation rate against % $V\dot{O}_{2peak}$. The HR corresponding to Fatmax was calculated using the relationship between % $V\dot{O}_{2peak}$ and HR. The measured RPE value at Fatmax (% $V\dot{O}_{2peak}$) was recorded for each

individual. Average r^2 values for the polynomial curves of fat oxidation vs. % $V\square O_{2peak}$ were 0.74(0.21) for TM and 0.71(0.17) for CE.

5.2.5 Statistical analysis

Statistical analyses were completed using SPSS software version 16.0 for Windows (SPSS Inc, Chicago, IL, USA). Shapiro-Wilk tests were used to confirm normal distribution and Levene's tests were used to confirm homogeneity of variance. Separate 2 x 2 (mode by sex) mixed measures ANOVA repeated for mode were used to examine the data for fat oxidation, Fatmax and Fatmax zone. Student's independent t-tests were used to compare anthropometric characteristics by sex (Table 5.1). Pearson's product moment correlation analyses were used to examine bivariate relationships between Fatmax, MFO, Fatmax zone, $V\square O_{2peak}$ and anthropometric measures. Values are expressed as mean(SD), unless stated otherwise, and ES were calculated. Statistical significance was accepted at $P \leq 0.05$.

5.3 Results

5.3.1 Participant characteristics

Complete data for 22 participants (13 boys and 9 girls) were available for analyses (Table 5.1). The girls were older than the boys (P=0.018, ES: 0.50) and, according to the self-assessment of pubic hair, 5 of the 9 girls but only 3 of the 13 boys had entered puberty (Tanner stage 2) (P=0.150, ES: 0.37).

5.3.2 Peak exercise responses

Treadmill $V\square O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) was ~15% higher than CE ($P \leq 0.0005$, ES: 0.87) and this difference was independent of sex (P=0.529, ES: 0.14). The boys' $V\square O_{2peak}$ values were higher than the girls' by the same margin as the mode-related differences, although the ES was smaller (P=0.011, ES: 0.53; Table 5.1).

All participants included in analysis achieved the criteria for the attainment of maximal effort. For the TM peak test, average peak responses for secondary criteria were RER 1.08(0.06) and HR 204(7) $beats \cdot min^{-1}$ or 97(3) % age-predicted HR_{max}. For the CE peak test, average peak responses for secondary criteria were RER 1.15(0.09) and HR 196(7) $beats \cdot min^{-1}$ or 93(3) % age-predicted HR_{max}.

Table 5.1 Participant characteristics

	Girls *n*=9	Boys *n*=13	Combined *n*=22
Age (y) [a]	10.3(0.8)	9.6(0.6)	9.9(0.8)
Body mass (kg)	35.1(4.8)	34.7(7.9)	34.9(6.7)
Stature (m)	1.42(0.08)	1.43(0.09)	1.43(0.09)
BMI ($kg \cdot m^{-2}$)	17.3(1.3)	16.9(2.3)	17.1(1.9)
Body fat (%)	20.4(3.2)	16.8(6.3)	18.3(5.4)
FFM (kg)	27.9(3.2)	28.5(4.5)	28.2(3.9)
Waist circumference (cm)	58.2(4.7)	61.3(5.9)	60.1(5.6)
Tanner (pubic hair) †	2(1)	1(0)	1(1)
TM $V\dot{O}_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) [a]	53(4)	59(5)	57(6)
CE $V\dot{O}_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) [a]	45(8)	53(6)	49(8)

BMI – body mass index, FFM – fat free mass, Tanner stage – estimation of secondary sexual characteristics (Tanner, 1962), TM – treadmill, $V\dot{O}_{2peak}$ – peak oxygen uptake, CE – cycle ergometer.
[a] between sex significant difference ($P \leq 0.05$)
† median (interquartile range)

5.3.3 Fatmax

Group mean values for parameters corresponding to Fatmax are displayed in Table 5.2. Fatmax (% $V\dot{O}_{2peak}$) was higher for TM compared with CE (P=0.005, ES: 0.57). Although this difference was independent of sex (P=0.117, ES: 0.34), Fatmax was higher in the boys compared with girls (P=0.019, ES: 0.50) and the difference was marked for TM (12% $V\dot{O}_{2peak}$) compared with CE (4% $V\dot{O}_{2peak}$). The RPE at Fatmax was higher for TM compared with CE (P=0.029, ES: 0.47). Peak $V\dot{O}_2$ explained 44% of the variation in Fatmax for TM (r^2=0.44), but only 4% for CE (r^2=0.04). Peak $V\dot{O}_2$ explained 32% of the variation in FFM relative MFO for TM (r^2=0.32) and 20% of the variation in absolute MFO for CE (r^2 = 0.20). The relationship

between TM Fatmax and absolute MFO was strong (r^2=0.53), but only moderate for CE (r^2=0.28).

5.3.4 Fatmax zone

The 5% Fatmax zone was wider for TM compared with CE (P=0.002, ES: 0.62) and the sex by exercise mode interaction was not meaningful (P=0.254, ES: 0.25). The 5% Fatmax zone extended over a greater range of exercise intensities in the boys compared with the girls (P=0.020, ES 0.49). There was a moderate correlation between Fatmax zone and $V\dot{O}_{2peak}$ for TM (r^2=0.20) but not CE (r^2=0.03).

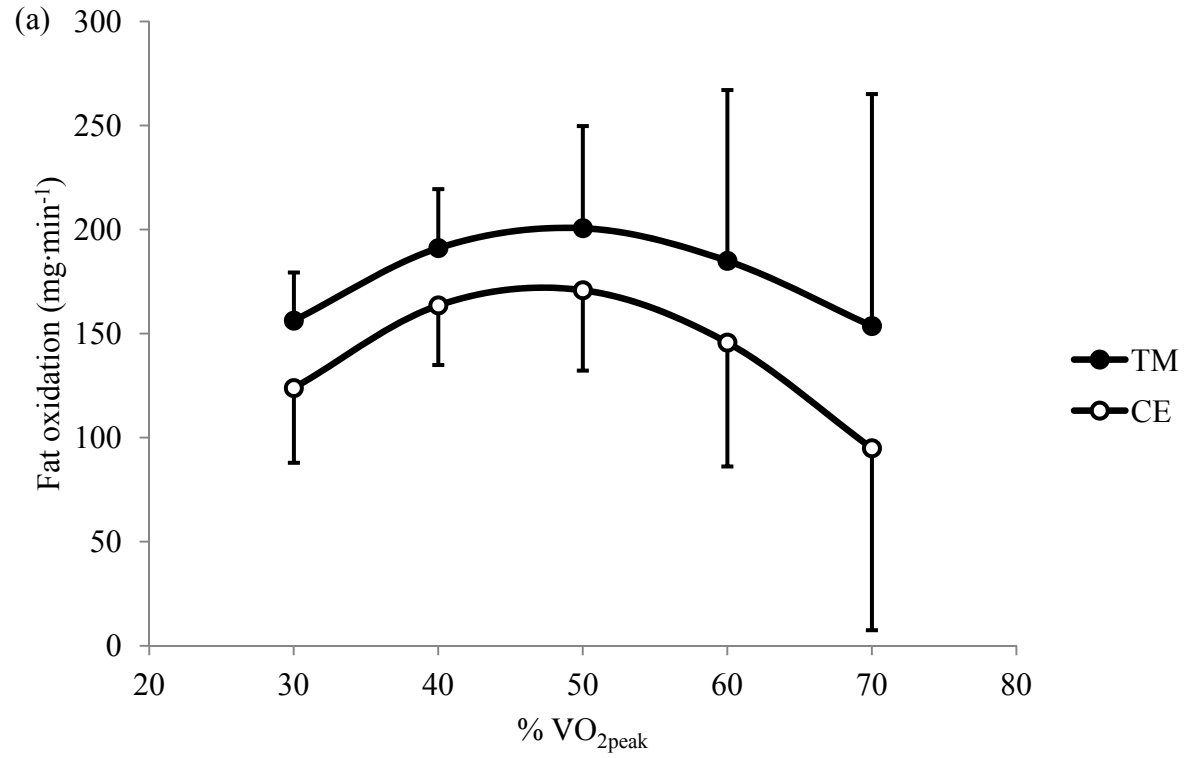
Table 5.2 Group comparisons of Fatmax and maximal fat oxidation (MFO) for treadmill (TM) and cycling exercise (CE)

	Girls *n*=9		Boys *n*=13		Combined *n*=22	
	TM	CE	TM	CE	TM	CE
Fatmax						
% $V\dot{O}_{2peak}$ [a,b]	52(13)	49(8)	64(10)	53(5)	59(13)	51(7)
% HRmax [a,c]	70(11)	67(8)	79(8)	67(6)	75(10)	67(7)
TM Speed ($km \cdot h^{-1}$) [b]	5.6(1.3)		7.2(1.4)		6.5(1.6)	
CE Work rate (W)		31(11)		40(10)		36(11)
RPE [a]	12(3)	12(2)	12(3)	10(2)	12(3)	11(2)
5% Fatmax zone (%$V\dot{O}_{2peak}$) [a,b]	20(6)	17(6)	26(6)	19(4)	24(6)	18(5)
MFO ($mg \cdot min^{-1}$) [a]	217(60)	176(36)	262(61)	191(55)	243(63)	185(47)
MFO ($mg \cdot kgFFM^{-1} \cdot min^{-1}$) [a]	7.9(2.5)	6.4(1.6)	9.3(2.3)	6.9(2.4)	8.8(2.5)	6.7(2.1)

$V\dot{O}_{2peak}$ – peak oxygen uptake, HR – heart rate, TM – treadmill, CE – cycle ergometer, RPE – rating of perceived exertion, MFO – maximal fat oxidation, FFM – fat free mass.
[a] between mode significant difference
[b] between sex significant difference
[c] sex by mode interaction (P<0.05)

5.3.5 Fat oxidation at relative and absolute exercise intensities

The relationship between relative and absolute exercise intensity and fat oxidation is displayed in Figure 5.1 and 5.2, respectively. Fat oxidation was higher for TM compared with CE at similar absolute (P=0.009, ES: 0.54) and relative (P=0.004, ES: 0.59) intensities. These exercise mode differences in fat oxidation were proportional to exercise intensity (P≤0.0005), but independent of sex (P≥0.503). Fat oxidation was higher for the boys compared with girls at similar relative (P=0.037, ES: 0.45), but not absolute (P=0.184, ES: 0.29) exercise intensities.



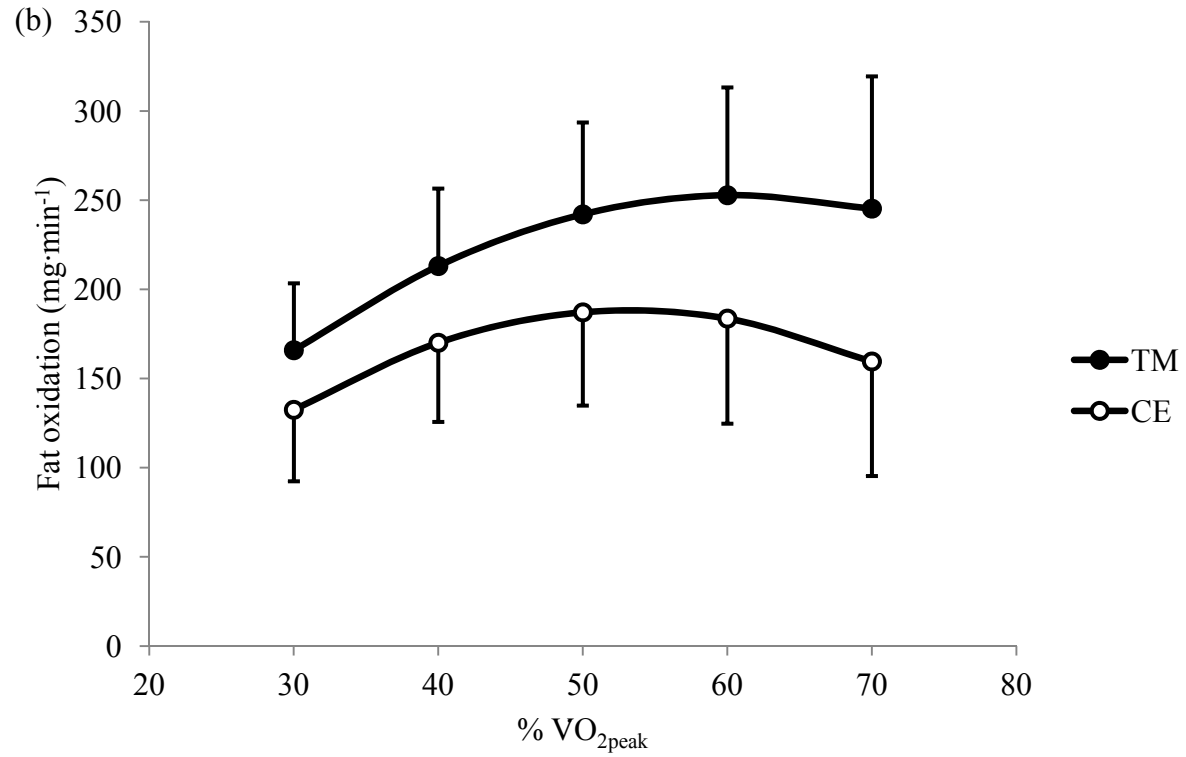
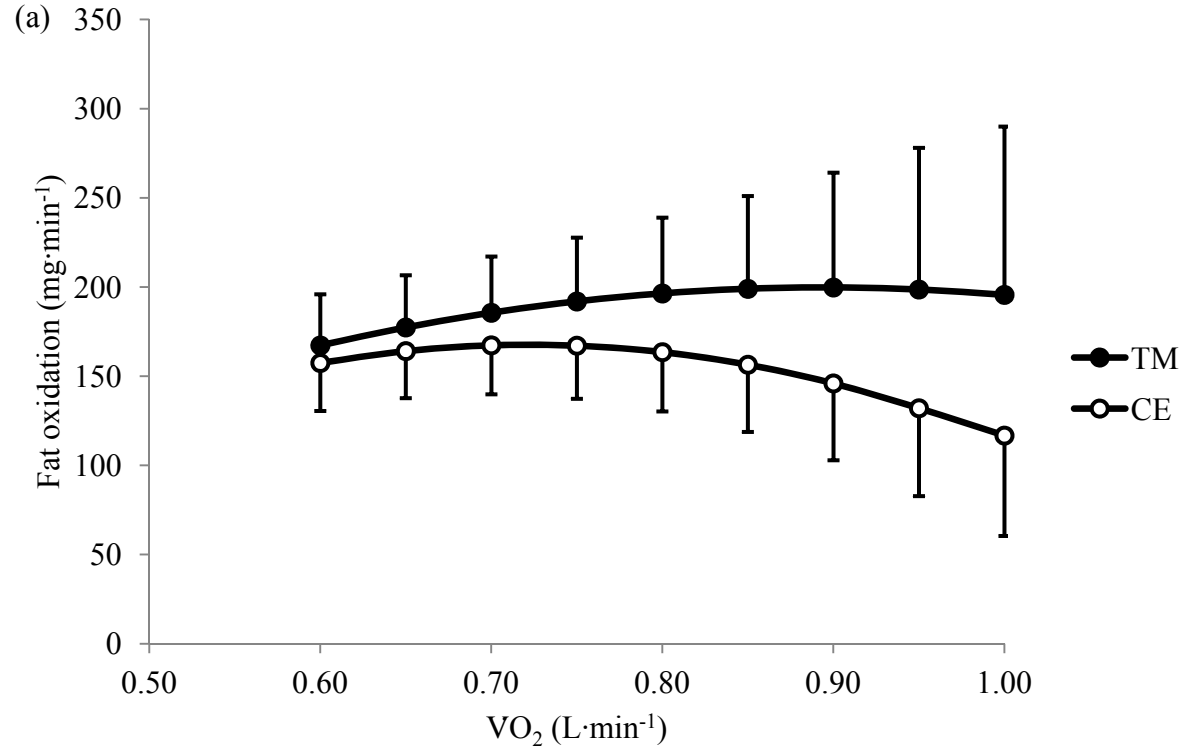


Figure 5.1 Comparison of fat oxidation rates between treadmill (TM) and cycling exercise (CE) at 30 to 70% V□O_{2peak} for girls (a) and boys (b)



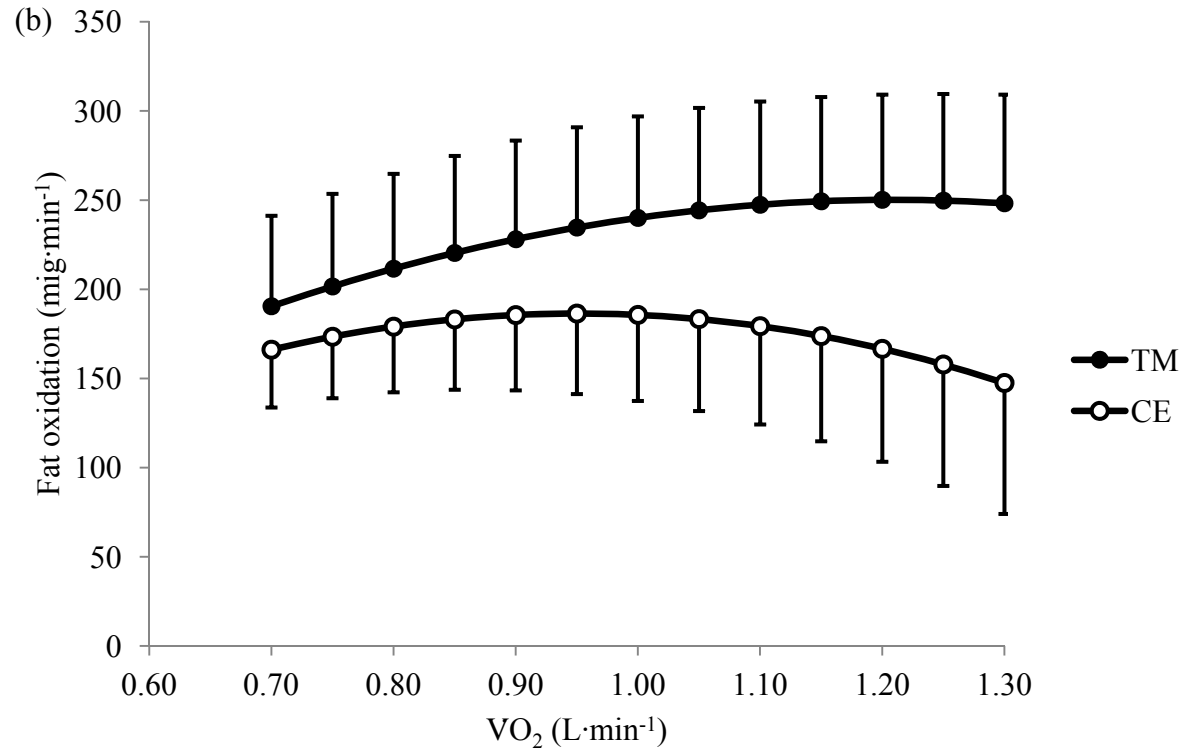


Figure 5.2 Comparison of fat oxidation rates between treadmill (TM) and cycling exercise (CE) at absolute V□O_2 values for girls (a) and boys (b)

5.4 Discussion

The main finding from the present study was that fat oxidation was higher during treadmill compared with cycling exercise over a range of absolute and relative intensities in pre- to early pubertal girls and boys. Furthermore, Fatmax was higher and fat oxidation remained high (within 5% of MFO) over a wider range of intensities for treadmill exercise. These findings strongly suggest that treadmill exercise is preferential for promoting high rates of fat oxidation in children.

Studies comparing fat oxidation between exercise modes have traditionally used a small number of intensities corresponding to the exercise mode-specific V□O_{2peak} (Houmard et al., 1991; Mácek et al., 1976). However, comparing relative exercise intensities often results in a higher absolute V□O_2 for treadmill exercise (Mácek et al., 1976; Millet et al., 2009) and, therefore, difficulties comparing exercise modes. Importantly, fat oxidation was higher during treadmill exercise over a range of both absolute and relative exercise intensities in the present study, indicating the higher fat oxidation during treadmill exercise was not due to a higher absolute V□O_2. These findings are in agreement with studies in obese adolescent boys (Lafortuna et al., 2010) and trained

adults (Achten et al., 2003), as well as studies in adults that have compared relative intensities only (Capostagno and Bosch, 2010; Glass et al., 1999). The higher fat oxidation during treadmill compared with cycling exercise was more pronounced at higher intensities, which is also in line with work in adults (Achten et al., 2003), but appears to be a novel finding in young people. Not only were fat oxidation rates higher during treadmill compared with cycling exercise, they remained high over a wider range of intensities, with the 5% Fatmax zone spanning 24% V□O_{2peak} for treadmill and 18% V□O_{2peak} for cycling exercise. In agreement, Chenevière et al. (2010) reported greater dilation (widening) of the treadmill compared with cycling fat oxidation curve, indicating a wider Fatmax zone. These findings suggest that treadmill exercise is preferable for exercise programmes aimed at promoting high rates of fat oxidation, particularly when it is difficult to monitor exercise intensity over prolonged periods of time.

The higher Fatmax during treadmill compared with cycling exercise (59 vs. 51% V□O_{2peak}) is another novel finding in children. Cycling Fatmax values are similar to those reported in the existing paediatric literature (see Chapter 2, Table 2.1), although data for treadmill Fatmax do not appear to be available in children. A similar study investigating fat oxidation over a range of seven intensities during treadmill and cycling exercise in obese adolescents did not estimate Fatmax, although graphical representations of fat oxidation support the findings of the present study indirectly (Lafortuna et al., 2010). In moderately trained adults, Chenevière et al. (2010) also reported a higher Fatmax for treadmill compared with cycling exercise. In contrast, other studies with adults have shown no difference in Fatmax between these exercise modes in moderately trained male cyclists (Achten et al., 2003) or untrained men and women (Glass et al., 1999). However, treadmill exercise intensity was increased via changes in gradient rather than speed (Achten et al., 2003). The comparison of cycling with uphill walking, two exercises dominated by concentric muscle contractions, rather than running, which has an eccentric component, may explain the difference in findings between the present study and Achten et al. (2003). Furthermore, when walking and running are performed at the same speed (above the walk-run transition), higher RER values have been reported during walking (Monteiro and de Araújo, 2009), which may contribute to a decline in fat oxidation and lower Fatmax during uphill walking compared with running. In addition, it is not possible to directly compare findings from

children and adults due to differences in the mechanisms controlling fat oxidation in these populations (Riddell, 2008; see section 2.5.1).

Collectively, these findings indicate that brisk walking or slow running (5.2 to 7.6 km·h^{-1}) may provide both an effective and convenient means of promoting fat oxidation in pre- to early pubertal children. Increasing fat oxidation may have clinical relevance for the prevention and treatment of obesity, insulin resistance and other metabolic disorders (see section 2.1). Although Fatmax was higher for treadmill exercise, it still occurred at a moderate intensity. Therefore, treadmill exercise at Fatmax may also be feasible for overweight or obese children, especially since Fatmax appears to be lower in this population (Zunquin et al., 2009b).

Studies with adults may provide some insight regarding possible explanations for the higher fat oxidation and Fatmax during treadmill compared with cycling exercise, as the mechanisms controlling fat oxidation in children are yet to be determined. The reduced muscle mass during cycling compared with running performed at the same relative intensity has a long standing history (Hermansen and Saltin, 1969). This would result in higher energy expenditure relative to active muscle mass in cycling and the recruitment of more type II muscle fibres, inducing a higher contribution from CHO and, consequently, a lower contribution of fat to energy expenditure. Furthermore, studies suggest the release of catecholamines is proportional to exercising muscle mass (Lewis et al., 1983). As catecholamines are potent stimulators of lipolysis, the larger muscle mass during treadmill exercise may elicit a larger catecholamine response and thus increased FFA mobilisation and oxidation (Martin, 1996). It has also been speculated that the eccentric muscle action in running may delay peripheral fatigue and reduce the recruitment of type II motor units during running compared with cycling for the same relative exercise intensity (Carter et al., 2000). In line with this, higher blood lactate and lower arterial pH and bicarbonate concentrations have been reported during cycling compared with treadmill exercise at comparable metabolic rates (Miles et al., 1980). Hydrogen ion accumulation in the sarcoplasm may inhibit CPT-1 activity (enzyme controlling the transport of FFA into the mitochondria), resulting in decreased fat oxidation (Starritt et al., 2000). Moreover, the higher fat oxidation during treadmill exercise coincided with lower blood lactate concentrations in the obese boys (Lafortuna

et al., 2010), indicating blood lactate accumulation may be a possible mechanism limiting fat oxidation during cycling exercise in young people.

The LIAB coincides with Fatmax in children (Tolfrey et al., 2010) and adults (Achten and Jeukendrup, 2004). This implies that a higher LIAB during treadmill compared with cycling exercise could partially explain the higher Fatmax during treadmill exercise. Although blood lactate was not measured in the present study, the lactate threshold occurs at a higher intensity in treadmill compared with cycling exercise in children (Machado et al., 2009) and adults (Carter et al., 2000). The trained cyclists in the study by Achten et al. (2003) had similar blood lactate concentrations during treadmill and cycling exercise at a given intensity, possibly explaining the similar Fatmax values between exercise modes in this population. However, this would not explain the higher fat oxidation rates during treadmill compared with cycling exercise in the trained cyclists, indicating that other factors are likely to contribute to differences in fat oxidation.

Sex did not influence fat oxidation at similar absolute intensities; the higher absolute $V\dot{O}_2$ in the boys may explain the higher fat oxidation observed when comparing relative intensities. Interestingly, the difference in Fatmax between the girls and boys was markedly higher for treadmill (12% $V\dot{O}_{2peak}$ higher for the boys) compared with cycling exercise (4% $V\dot{O}_{2peak}$ higher for the boys). Furthermore, treadmill, but not cycling, Fatmax was related to $V\dot{O}_{2peak}$. Research in adults is equivocal with some showing that Fatmax is higher in those with higher $V\dot{O}_{2peak}$ values during cycling (Nordby et al., 2006), whilst others have reported no relationship between Fatmax and $V\dot{O}_{2peak}$ during treadmill (Lima-Silva et al., 2010) or cycling (Stisen et al., 2006) exercise. In addition, $V\dot{O}_{2peak}$ was correlated with MFO for both treadmill and cycling exercise, a finding consistent with studies in adults (Lima-Silva et al., 2010). Indeed, cardiorespiratory fitness confers several health benefits which might enhance fat oxidation in children (Ortega et al., 2008). Higher $V\dot{O}_{2peak}$ values in the boys compared with girls along with the correlation between Fatmax and $V\dot{O}_{2peak}$ for treadmill (but not cycling) exercise may partially explain the higher treadmill (but not cycling) Fatmax values in the boys. It is also possible that the slightly higher Tanner stage in the girls may have contributed to the lower Fatmax in this group (Riddell et al., 2008). However, the difference in Tanner stage between groups was small and

decreases in Fatmax and fat oxidation occur during mid- to late-puberty (Riddell et al., 2008; Stephens et al., 2006). Therefore, differences in puberty between pre- and early pubertal children may not be sufficient to account for differences in Fatmax. Furthermore, as detailed in section 2.5.1, problems associated with self-report data and Tanner stages may mean that the measure of maturation was not accurate enough to assess small differences in puberty in the present study (Baxter-Jones et al., 2005) and the small sample size may limit between-sex comparisons in particular.

Possible limitations of the present study include the use of indirect calorimetry to estimate fat oxidation. Although steps were taken to increase the validity of indirect calorimetry, it was assumed that the urinary nitrogen excretion rate was negligible and an increase in non-respiratory carbon dioxide excretion may have resulted in an underestimation of fat oxidation at some of the higher exercise intensities (Rowlands, 2005). The exercise protocol employed does not appear to affect the estimation of cycling Fatmax. However, the MFO values reported may not be comparable to those estimated from more prolonged exercise bouts (Chapter 4) and a similar study involving treadmill exercise does not appear to be available. Despite these concerns, research with adults suggests that the V□O_2 kinetic response to moderate intensity exercise is similar between running and cycling and a steady state is attained within two minutes (Carter et al., 2000).

In conclusion, treadmill exercise is preferable for promoting fat oxidation compared with cycling due to the higher fat oxidation over a range of absolute and relative exercise intensities, higher Fatmax and wider Fatmax zone. Consequently, Fatmax values suggest brisk walking or slow running may provide both an effective and convenient means of promoting fat oxidation in pre- to early pubertal children. These recommendations relating to exercise mode and intensity could have considerable clinical relevance for guiding interventions designed to increase fat oxidation, which may be useful for preventing conditions such as obesity and insulin resistance.

Chapter 6

Effect of breakfast glycaemic index on postprandial glucose, insulin and fat oxidation during rest and exercise in overweight and non-overweight girls

Abstract

The metabolic responses to mixed breakfast meals with different glycaemic indexes (GI) and their effects on substrate metabolism during exercise in adolescent girls have not been examined. The interaction with weight status also warrants investigation. The present study investigated the effect of mixed breakfast meals containing high GI (HGI) or low GI (LGI) carbohydrates on metabolic responses and fat oxidation during rest and exercise in overweight (OW; aged 12.6(0.5) y) and non-overweight (NO; aged 13.1(0.4) y) girls. Eight OW and 12 NO adolescent girls consumed an isoenergetic HGI (GI=73) or LGI (GI=44) breakfast 120 min before completing a 30 min treadmill walk at 50% $V\dot{O}_{2peak}$. Peak blood glucose concentration was higher for HGI compared with LGI in OW (P=0.023), but not NO (P=0.741) girls. Blood glucose total area under the curve (TAUC) was 13% higher in HGI compared with LGI in OW (P=0.006), but only 4% higher in NO (P=0.072) girls. Plasma insulin data were $\log_e$ transformed (lninsulin). Plasma lninsulin concentrations were not different between HGI and LGI (P>0.05). Peak plasma lninsulin concentration (P=0.016) and TAUC (P=0.001) were greater in OW than NO girls. Fat oxidation during postprandial rest and exercise was not different between breakfasts (P>0.05). The elevated glycaemic response following HGI compared with LGI breakfast consumption was more pronounced in the OW girls, suggesting a reduced ability to cope with the metabolic demands of the HGI, but not LGI, breakfast in this population. Manipulation of breakfast GI did not alter fat oxidation during rest or subsequent moderate intensity exercise in OW and NO adolescent girls.

6.1 Introduction

It is well established that fat oxidation is maximised by exercising in the fasted state (Horowitz et al., 1997; Timmons et al., 2007a), but this may not be a practical option for young people. Moreover, several lines of evidence have shown benefits associated with regular breakfast consumption in young people, relating to academic performance (Lien, 2007), nutrition (Barton et al., 2005; Song et al., 2006), cardiorespiratory fitness and obesity (Sandercock et al., 2010). However, the relationship between breakfast and health benefits may not be due to consumption *per se*, but rather breakfast composition (Cho et al., 2003). There are concerns that ready-to-eat cereals commonly eaten by children and adolescents (Song et al., 2006) fail to meet national nutrition recommendations (Schwartz et al., 2008). In contrast, there has been considerable interest in potential health benefits of breakfasts containing LGI CHO (Ludwig et al., 1999; Willett et al., 2002).

Manipulation of the GI of a mixed breakfast meal affects postprandial glucose and insulin responses in young people (Ludwig et al., 1999) and adults (Stevenson et al., 2009). Evidence that breakfasts rich in LGI CHO promote satiety in obese adolescents (Ball et al., 2003; Ludwig et al., 1999) suggest that LGI breakfast consumption could have direct implications for paediatric weight management. In adults, the reduced glucose and insulin response to a LGI compared with HGI breakfast can also result in increased fat oxidation during rest (Stevenson et al., 2009) and subsequent exercise (Stevenson et al., 2006; 2009; Wee et al., 2005; see section 2.8.2). This suggests that LGI breakfast consumption be a compromise between promoting breakfast consumption and fasted exercise for fat oxidation. However, breakfast GI does not affect fat oxidation during rest or exercise when comparing a MGI and HGI breakfast (Backhouse et al., 2007) or when exercise is preceded by two LGI meals rather than breakfast alone (Stevenson et al., 2005a). A recent study even reported higher fat oxidation during a cycling time trial following a HGI compared with LGI breakfast (Moore et al., 2010). Therefore, the influence of GI on postprandial fat oxidation remains unclear.

Reductions in fat oxidation (Zunquin et al., 2009b), glucose tolerance (Sinha et al., 2002a), and insulin sensitivity (Weiss et al., 2004) have been shown in overweight and obese young people. Substituting a HGI breakfast for a LGI breakfast may, therefore,

be particularly beneficial for these individuals through increased glycaemic control (Willett et al., 2002), fat oxidation (Stevenson et al., 2009) and satiety (Ludwig et al., 1999). However, the majority of studies investigating the impact of GI on fat oxidation have included endurance trained or recreationally active adults as participants (Stevenson et al., 2006; Wu et al., 2003) and similar studies including overweight individuals or young people do not appear to be available, despite well recognised differences in metabolism between adolescents and adults (Riddell, 2008; Riddell et al., 2008). Therefore, the present study examined the effect of HGI and LGI mixed breakfast meals on metabolic responses during rest and subsequent exercise in overweight and non-overweight adolescent girls.

6.2 Methods

6.2.1 Participants

After gaining approval from the University Ethical Advisory sub-Committee, 8 OW and 12 NO girls aged 11 to 13 y participated in the study. Overweight status was defined using age and sex specific BMI reference points (Cole et al., 2000). Anthropometric characteristics were assessed and recorded prior to experimental trials, as described in the General Methods (section 3.2).

6.2.2 Preliminary measurements

Participants completed a peak (see section 3.5.1) and submaximal treadmill exercise test. The submaximal exercise test consisted of 4 x 4 min bouts at different speeds to determine the relationship between treadmill speed and V□O_2. Subsequently, the speed eliciting 50% V□O_{2peak} was determined for each participant.

6.2.3 Experimental protocol

Participants completed two experimental trials (HGI and LGI) in a counter-balanced order (Figure 6.1). Trials were conducted a maximum 48 h apart for the girls who had irregular menstruation to minimise the potential influence of menstrual cycle phase on within-participant comparisons (Oosthuyse and Bosch, 2010). Due to the sporadic nature of the menstrual cycle in young adolescent girls, other studies have not accounted for menstrual cycle phase (Timmons et al., 2007b). The girls consumed the same diet and minimised physical activity in the 24 h prior to experimental trials (Appendix 4).

Participants reported to the laboratory at 08:00 following a 12 h fast. Following fasted measures, the girls consumed a HGI or LGI breakfast (Table 6.1) within 15 min. Blood, expired air samples and subjective ratings of hunger were collected at regular intervals during the 120 min postprandial period. Subsequently, the girls completed a 30 min treadmill walk at 50% $V\square O_{2peak}$. Water was available ad libitum throughout the first trial and the girls drank the same volume during the second trial.

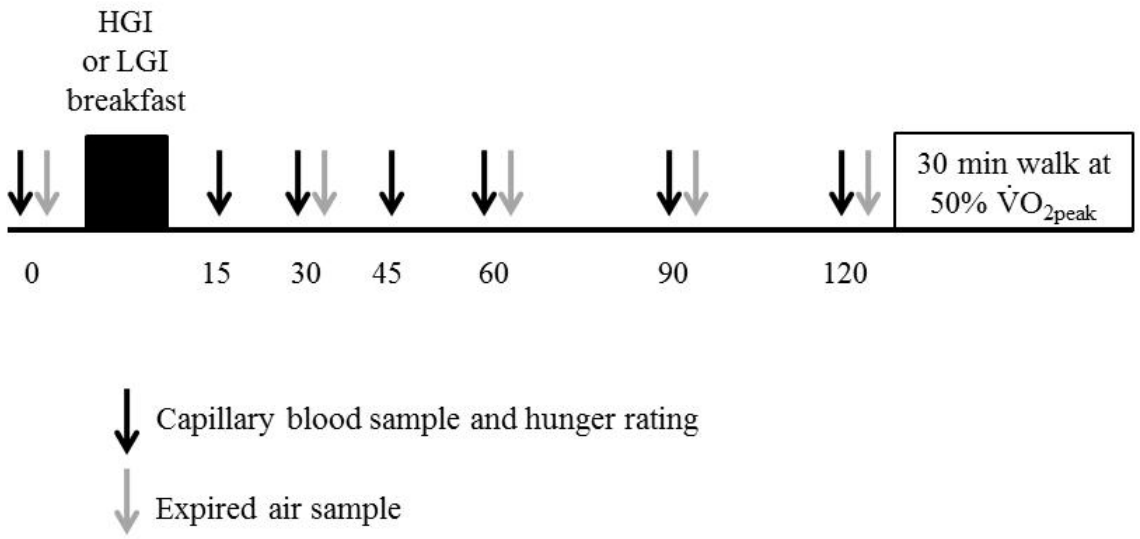


Figure 6.1 Schematic of protocol for the high glycaemic index (HGI) and low glycaemic index (LGI) experimental conditions

6.2.4 Test breakfasts

Participants were provided with a HGI or LGI breakfast containing 1.5 g CHO·kg BM^{-1} (Table 6.1). The breakfasts were matched for energy, macronutrients and fluid, but the LGI breakfast was heavier and contained more fibre ($P \leq 0.05$). The GI values for individual foods were taken from the International Table of Glycemic Index and Glycemic Load Values (Atkinson et al., 2008) and breakfast GI was calculated from the weighted means of the GI values for the component foods (Wolever and Jenkins, 1986). The calculated GI for the breakfasts were 73 (HGI) and 44 (LGI).

Table 6.1 Composition of test breakfasts for a 45 kg girl

Breakfast	Description	Macronutrient content
HGI	45 g Cornflakes[a] + 135 g skimmed milk, 44 g white bread, 4 g jam, 5 g margarine[a], 124 g water (total weight = 356 g)	1498 kJ energy, 68 g CHO, 4.2 g fat, 11.6 g protein, 2.7 g fibre GI = 73[b]
LGI	41 g muesli[a] + 90 g skimmed milk, 107 g apple, 169 g apple juice, 87 g yoghurt (total weight = 493 g[c])	1498 kJ energy, 67 g CHO, 4.3 g fat, 11.7 g protein, 5.3 g fibre[c] GI = 44[b]

HGI – high glycaemic index, CHO – carbohydrate, LGI – low glycaemic index.
[a] Cornflakes, Kellogg's; Flora original margarine spread, Unilever; Alpen no added sugar, Weetabix Ltd
[b] Calculated according to Wolever and Jenkins (1986) with GI values taken from Atkinson et al. (2008)
[c] Significant difference in total weight and fibre content between HGI and LGI ($P \leq 0.05$)

6.2.5 Blood sampling and analysis

Capillary blood samples were obtained by finger prick. Blood glucose and plasma insulin concentrations were then determined in duplicate, as detailed in the General Methods (section 3.7). Capillary rather than venous blood sampling is preferred for reliable GI testing (Wolever et al., 2003). Blood glucose and plasma insulin TAUC for the 120 min postprandial period were calculated using the trapezium rule (Wolever and Jenkins, 1986). HOMA-IR was also calculated (Matthews et al., 1985). The intra-assay CV for the duplicate samples was 2.4% for blood glucose and 6.3% for plasma insulin.

6.2.6 Expired air and indirect calorimetry

Breath-by-breath data were displayed online using a portable metabolic cart (K4 b^2, Cosmed, Rome, Italy). Calibration procedures were carried out prior to each experimental test (see section 3.3). Fat oxidation rates were calculated using stoichiometric equations, with the assumption that the urinary nitrogen excretion rate was negligible and a physiological steady-state had been attained (Frayn, 1983; see section 3.6). Fat oxidation TAUC for the 120 min rest period was calculated using the trapezium rule and included in subsequent analyses.

6.2.7 Perceived hunger

Perceptions of hunger, satisfaction, fullness, prospective food consumption and breakfast palatability were assessed using 100 mm visual analogue scales (see section 3.8).

6.2.8 Statistical analyses

Statistical analyses were completed using SPSS (v16 SPSS Inc, Chicago, IL, USA). The insulin data were transformed using a natural logarithm (lninsulin) to normalise them and homogenise the variances between the groups. Breakfast by time (2 x 7) repeated measures ANOVA were used to examine differences between HGI and LGI over time for glucose and lninsulin; these were conducted separately for OW and NO girls. Breakfast by group (2 x 2) mixed measures ANOVA with breakfast as the repeated factor were used to compare the two groups directly for glucose and lninsulin TAUC. For resting and exercise fat oxidation, breakfast by group (2 x 2) mixed measures analysis of covariance (ANCOVA) with estimated FFM as the covariate was used. Homogeneity of regression slopes was confirmed prior to each ANCOVA. Paired sample t-tests with Bonferroni correction were used to compare glucose and lninsulin concentrations at different time points and to follow-up significant two-way interactions. Values are expressed as mean(SD), unless stated otherwise, and ES were calculated. Statistical significance was accepted at $P \leq 0.05$.

6.3 Results

6.3.1 Participant characteristics

Complete data for 8 OW and 12 NO girls were available for analyses (Table 6.2). Body mass, BMI, body fat, FFM, waist circumference and hip circumference were higher in the OW compared with NO girls ($P \leq 0.05$), whereas $V\square O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) was higher in the NO girls ($P \leq 0.0005$). Two of the OW girls were insulin resistant (HOMA-IR >3.16) (Keskin et al., 2005).

Table 6.2 Participant characteristics

	OW *n*=8	NO *n*=12
Age (y)	12.6(0.5)	13.1(0.4)
Body mass (kg) [a]	70.9(19.4)	45.5(8.4)
Stature (m)	1.61(0.08)	1.56(0.09)
BMI ($kg \cdot m^{-2}$) [a]	27.0(5.8)	18.5(2.0)
Body fat (%) [a]	35.7(6.0)	19.2(3.9)
FFM (kg) [a]	45(9)	37(6)
Waist circumference (cm) [a]	84.6(13.5)	63.0(4.7)
Hip circumference (cm) [a]	99.5(12.3)	82.1(8.2)
Tanner (pubic hair)†	3(1)	3(1)
$V\dot{O}_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) [a]	32(7)	45(6)

OW – overweight, NO – non-overweight, BMI – body mass index, FFM – fat free mass, Tanner stage – estimation of secondary sexual characteristics (Tanner, 1962), $V\dot{O}_{2peak}$ – peak oxygen uptake.
[a] significant difference between OW and NO (P≤0.05)
†median (interquartile range)

6.3.2 Blood glucose concentration

Following breakfast, blood glucose concentrations increased and peaked at a median (interquartile range) time of 30(0) min for all trials, except in the OW HGI trial where it peaked at 45 min in 4 girls (median 37.5(15) min) (Figure 6.2). Breakfast by time interactions were found for OW (P≤0.001) and NO (P=0.001) girls; concentrations were higher in HGI compared with LGI at 45 (P=0.004) and 60 (P≤0.0005) min in OW girls and at 90 (P=0.006) and 120 (P=0.001) min in NO girls.

There were no differences in fasting or postprandial glucose between OW and NO girls at any time points (P>0.05). However, breakfast by group interactions for peak blood glucose (P=0.053, ES: 0.44) and TAUC (P=0.026, ES: 0.50) were found. Peak blood glucose was higher for HGI compared with LGI in OW (6.1 vs. 5.5 $mmol \cdot L^{-1}$; P=0.023,

ES: 0.74), but similar between breakfasts in NO girls (5.8 vs. 5.9 mmol·L^{-1}; P=0.741). There were no between group differences in peak blood glucose after the HGI (P=0.404) or LGI (P=0.122) breakfasts. Blood glucose TAUC was 13% higher in HGI compared with LGI in OW (P=0.006, ES: 0.82), but only 4% higher in NO girls (P=0.072, ES: 0.51). Moreover, HGI TAUC was 9% higher in OW compared with NO girls (P=0.070, ES: 0.41), but LGI TAUC was similar between the groups (P=0.831, ES: 0.05). Similarly, the pattern of blood glucose over time differed between the OW and NO girls for HGI (P=0.047, ES: 0.24), but not LGI (P=0.119).

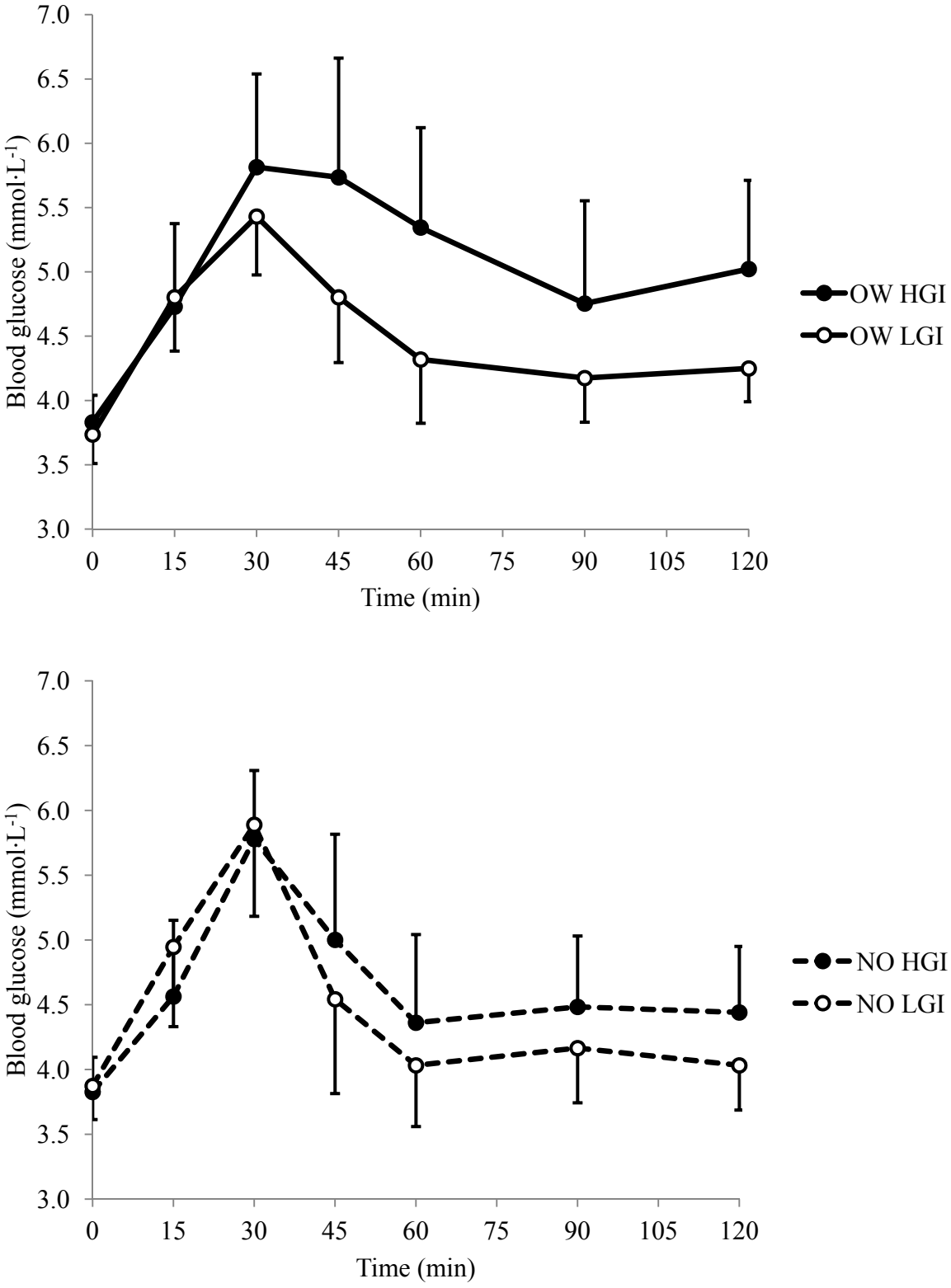


Figure 6.2 Blood glucose response to the high glycaemic index (HGI) and low glycaemic index (LGI) breakfasts for overweight (OW) and non-overweight (NO) girls. Breakfast was consumed between 0 and 15 min. Blood glucose concentration was higher in HGI compared with LGI at 45 and 60 min in OW girls and at 90 and 120 min in NO girls (Bonferroni correction; significance was $P \leq 0.025$).

6.3.3 Plasma insulin concentration

Following breakfast, plasma lninsulin concentration increased and peaked at a median (interquartile range) time of 30(15) and 30(0) min for HGI and LGI in NO and 30(15) min in the OW LGI trial, but 45(15) min in the OW HGI trial (Figure 6.3). Breakfast by time interactions were found for OW (P=0.012) and NO (P≤0.005); however, pairwise comparisons only revealed a single significant difference in NO girls at 120 min (P=0.001). Neither the main effect for breakfast nor the breakfast by group interaction for lninsulin TAUC were significant (P>0.05). Although a strong statistical trend in fasting lninsulin between the OW and NO girls was found (P=0.054, ES: 0.45), pairwise analyses were not significant (P>0.025). Peak lninsulin (P=0.016) and TAUC (P=0.001) were higher in OW than NO. Whilst Bonferroni follow-up indicated peak lninsulin was only significantly different following the HGI breakfast (P≤0.025), it was clear that both breakfasts led to significant differences in TAUC (P≤0.025) between OW and NO. HOMA-IR was higher in OW compared with NO (3.2 vs. 2.9, P=0.054).

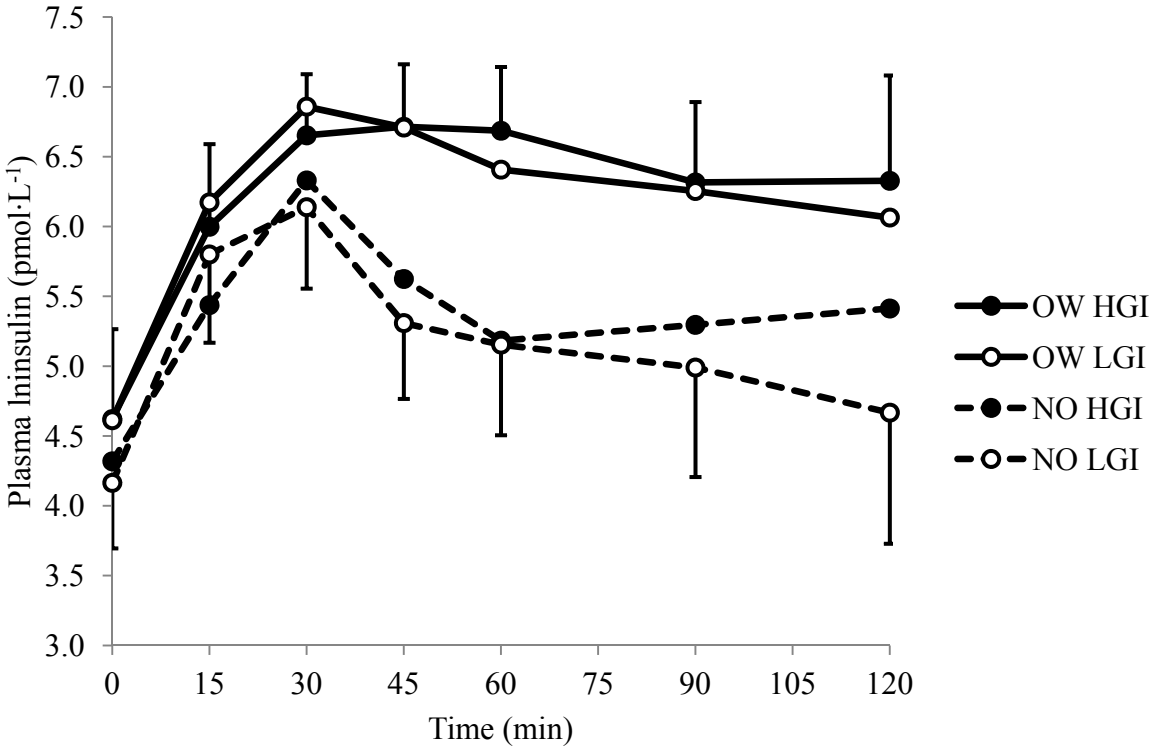


Figure 6.3 Plasma insulin response to the high glycaemic index (HGI) and low glycaemic index (LGI) breakfasts for overweight (OW) and non-overweight (NO) girls. Breakfast was consumed between 0 and 15 min. Plasma lninsulin concentration was higher in HGI compared with LGI at 120 min in NO girls (Bonferroni correction; significance was P≤0.025).

6.3.4 Fat oxidation

Resting and exercise fat oxidation results by group and breakfast are shown in Table 6.3. During both postprandial rest and subsequent exercise, absolute and ANCOVA FFM adjusted fat oxidation were not different between HGI and LGI breakfast conditions in either group of girls (P>0.05).

During the postprandial rest period, absolute fat oxidation was higher in the OW compared with NO girls in HGI (P=0.004, ES: 0.61) and LGI (P=0.005, ES: 0.60). However, once between group differences in FFM were accounted for, resting fat oxidation was similar in the two groups of girls (P>0.05). During subsequent exercise, absolute and ANCOVA FFM adjusted total fat oxidation were not different when comparing the OW and NO girls for the HGI and LGI conditions (P>0.05).

Table 6.3 Resting and exercise fat oxidation (area under curve over time): comparisons between breakfasts and groups

Group	Breakfast	Rest (g·120 min^{-1})		Exercise (g·30 min^{-1})	
		Absolute	FFM[1]	Absolute	FFM[1]
OW *n*=8	HGI	0.25(0.10)[a]	0.20(0.05)	5.05(1.53)	4.49(1.71)
	LGI	0.26(0.13)[b]	0.18(0.07)	6.03(3.49)	5.08(2.38)
NO *n*=12	HGI	0.15(0.04)	0.16(0.09)	4.76(1.83)	5.14(1.66)
	LGI	0.13(0.05)	0.14(0.07)	5.23(1.63)	5.86(2.31)

OW – overweight, NO – non-overweight, HGI – high glycaemic index, LGI – low glycaemic index.
[1] ANCOVA adjusted values with FFM (fat free mass) as the covariate
[a] Higher in OW compared with NO girls after consuming the HGI breakfast (P=0.004)
[b] Higher in OW compared with NO girls after consuming the LGI breakfast (P=0.005)

6.3.5 Hunger

Perceptions of hunger, satisfaction, fullness, prospective food consumption and breakfast palatability were similar between trials (P>0.05).

6.4 Discussion

The main finding of the present study was that the higher glycaemic response in HGI compared with LGI was more pronounced in OW than NO girls, possibly reflecting a reduced ability to cope with the metabolic demands of a HGI breakfast in OW girls. Breakfast GI did not affect fat oxidation during the 120 min postprandial rest period or subsequent moderate intensity exercise in OW and NO adolescent girls.

A novel finding was that the higher glycaemic response to HGI compared with LGI breakfast consumption was exaggerated in the OW girls, mainly due to the delayed decline in blood glucose following the postprandial peak. This may indicate a delayed blood glucose uptake up to 60 min following HGI breakfast consumption in OW girls. Previous work has reported higher postprandial glucose responses to HGI compared with LGI breakfasts in obese adolescents, but these studies did not include non-overweight participants for direct comparison (Ball et al., 2003; Ludwig et al., 1999). Furthermore, higher and more sustained postprandial glucose responses have been reported in obese compared with non-obese children (Sinha et al., 2002a). However, we were unable to locate another study that has investigated whether these differences between OW and NO young people are dependent on the GI of the consumed CHO. Perälä et al. (2011) recently reported that the higher glycaemic response to a HGI compared with LGI meal was similar in OW and NO 62 to 72 year olds. However, the meals only contained 50 g CHO and were not scaled to body mass. It is, therefore, difficult to directly compare the findings of the present study with those of Perälä et al. (2011) due to differences in study design and participants.

It is possible that the combination of readily absorbed glucose from the HGI (but not LGI) breakfast and higher insulin resistance (HOMA-IR) in the OW girls contributed to the larger glycaemic response in the OW HGI trial. Furthermore, plasma insulin peaked earlier and returned towards baseline values for LGI, but remained elevated for HGI in the OW girls; the earlier peak in plasma insulin may have contributed to the more rapid decline in blood glucose in LGI. Indeed, a study in adults reported that hyperinsulinemia occurred earlier following a LGI compared with HGI breakfast and was associated with a higher rate of glucose disappearance between 30 and 60 min of the LGI postprandial period, resulting in reduced plasma glucose concentrations (Schenk et al., 2003). However, the larger amount of protein in the LGI bran cereal

compared with HGI corn flakes (Schenk et al., 2003) may have caused the increased insulin secretion (Pi-Sunyer, 2002), whereas the macronutrient content of the breakfasts in the present study was similar.

Collectively, these findings indicate that LGI breakfasts may be beneficial for blood glucose control in OW girls. Furthermore, the elevated glycaemic response in the OW HGI trial may also increase voluntary food intake later in the day (Ludwig et al., 1999). Encouragingly, adults with higher postprandial glycaemic responses have a greater postprandial reduction when changing from HGI to LGI foods (Høstmark, 2007) and lowering breakfast GI for 21 days reduced fasting glucose and increased satiety in obese adults (Pal et al., 2008). Moreover, the similar palatability between the breakfasts in the present study indicates LGI breakfast promotion for OW girls may be feasible. As the higher fibre content in the LGI breakfast may have contributed to the lower glycaemic response to this breakfast (Pi-Sunyer, 2002), it may be more appropriate to recommend LGI high-fibre breakfasts (rather than LGI breakfasts) for OW girls. This is feasible since LGI foods typically contain more fibre than HGI foods. Nevertheless, confirmation of these results in larger groups of young people, including boys, is required.

Breakfast GI did not affect postprandial fat oxidation during rest or exercise in either group of girls. However, it is noteworthy that LGI resulted in 12% higher exercise fat oxidation (ANCOVA adjusted for FFM) in both groups on average, a finding that may have meaningful health-related implications since higher rates of fat oxidation may ameliorate the development of obesity and insulin resistance (Holloway et al., 2009; see section 2.1). During exercise, studies in adults have reported higher fat oxidation following LGI breakfasts (Stevenson et al., 2009; Wee et al., 2005), no effect of GI (Backhouse et al., 2007; Stevenson et al., 2005a) or even higher fat oxidation following a HGI breakfast (Moore et al., 2010). During rest, most have reported no effect of breakfast GI on fat oxidation (Díaz et al., 2005; Stevenson et al., 2006; Wee et al., 2005), although higher fat oxidation following LGI breakfast consumption has been shown (Stevenson et al., 2009). Inconsistencies between studies may be due to differences in breakfast size or composition, exercise mode, intensity and duration, postprandial time period and participant characteristics (see section 2.8.2; Table 2.2). However, higher exercise fat oxidation following LGI breakfasts has been reported 45

min to 3 h (Sparks et al., 1998; Stevenson et al., 2009) following breakfasts containing 1 to 2.5 g CHO·kg BM^{-1} during exercise lasting 60 or 30 min at 50-71% $V\square O_{2peak}$ (Stevenson et al., 2009; Wee et al., 2005). It is, therefore, difficult to ascertain which factors contribute specifically to the higher fat oxidation following LGI breakfasts in some adult studies. It is possible that the 1.5 g CHO·kg BM^{-1} breakfast, 120 min postprandial period and 30 min exercise duration at 50% $V\square O_{2peak}$ used in the present study was a sub-optimal combination to induce differences in fat oxidation between HGI and LGI. Furthermore, differences in fat metabolism between adolescents and adults (Riddell et al., 2008) may have resulted in discrepancies between the present study and some of the adult literature. Therefore, further examination of the relationship between breakfast GI and fat oxidation in young people is warranted.

The similar insulin response between HGI and LGI reported in the present study may have underpinned the similarity in fat oxidation (Horowitz et al., 1997). Furthermore, fructose has a lower GI than glucose, but results in higher blood lactate concentrations (Moore et al., 2000). It is possible that higher lactate concentrations compromised fat oxidation following the LGI breakfast through direct inhibition of adipose tissue FFA release (Boyd et al., 1974). Indeed, resting fat oxidation was lower after high fructose compared with high glucose meals in obese adults, despite lower glucose and insulin responses to the high fructose meal (Tittelbach et al., 2000). Although we did not measure blood lactate, higher postprandial lactate concentrations have been reported following LGI compared with HGI breakfasts (Stevenson et al., 2006). In addition, blood lactate can affect the validity of indirect calorimetry for fat oxidation estimations (Rowlands, 2005). However, it is unlikely that this was a factor in the present study since the girls exercised at a moderate intensity (50% $V\square O_{2peak}$) and additional steps were taken to increase the validity of indirect calorimetry (e.g., removing individual $V\square O_2$ and $V\square CO_2$ ≥3 SD's from the mean and verifying a steady state in $V\square O_2$ and $V\square CO_2$).

Although no difference in perceptions of hunger between breakfasts was reported, the 120 min postprandial period may have been too short for differences to emerge (Anderson and Woodend, 2003; Stevenson et al., 2009). Furthermore, prolongation of satiety based on time to request food was found >3 h following LGI compared with

HGI foods in obese adolescents, despite no difference using hunger scales (Ball et al., 2003).

In summary, the higher glycaemic response in HGI compared with LGI was more pronounced in OW girls, suggesting a reduced ability to cope with the metabolic demands of a HGI breakfast in this population. This provides further evidence for potential health benefits of LGI foods and suggests that LGI breakfast promotion for OW girls is warranted. Breakfast GI did not affect fat oxidation during rest or subsequent exercise in OW and NO adolescent girls, although further examination of this relationship young people is required.

Chapter 7

Acute effect of Fatmax exercise on postprandial glucose, insulin and fat oxidation in overweight and non-overweight girls

Abstract

Acute exercise can reduce postprandial insulin concentrations and increase fat oxidation in adults, which may have important implications for insulin resistance and weight control. However, similar studies with young people or comparing overweight and non-overweight individuals are sparse. Therefore, the acute effect of Fatmax exercise on glucose, insulin and fat oxidation was examined in 12 overweight (OW; aged 11.7(1.3) y) and 15 non-overweight (NO; aged 12.3(1.5) y) girls. Participants completed two 2-day conditions in a counter-balanced order. On day 1, participants either expended 2.09 MJ (500 kcal) during treadmill exercise at individual Fatmax (EX) or 0.47 MJ (112 kcal) during rest (CON). On day 2, capillary blood and expired air samples were taken in the fasted state and at regular intervals for 120 min after high glycaemic index (HGI) breakfast consumption. Subsequently, blood glucose and plasma insulin concentrations were determined and fat oxidation was estimated. Blood glucose was similar between conditions in both groups ($P \geq 0.05$). Fasting plasma insulin ($P=0.047$) and total area under the 120 min curve (TAUC, $P=0.049$) were reduced in EX compared with CON in NO, but not OW girls ($P \geq 0.05$). Fasting fat oxidation was higher in EX than CON for the NO girls ($P=0.036$) and fat oxidation TAUC was higher in EX for both the OW and NO girls ($P \leq 0.05$). A bout of Fatmax exercise performed ~16 h before HGI breakfast consumption reduced fasting and postprandial insulin concentrations in NO girls and increased fat oxidation in both OW and NO girls. The higher post-intervention energy and CHO intake or a degree of 'metabolic inflexibility' in the OW girls may have compromised potential exercise-induced reductions in insulin in this group.

7.1 Introduction

Mounting evidence has shown that high rates of fat oxidation may protect against insulin resistance and weight gain; this is currently a topic of great interest (DeLany et al., 2006; Holloway et al., 2009; see section 2.1). Chapter 6 highlighted that elevated insulin concentrations and insulin resistance have emerged as serious health concerns in young people, particularly those with high levels of adiposity (Sinha et al., 2002a; 2002b). Moreover, these adverse metabolic outcomes are exacerbated by the consumption HGI breakfasts, which induce exaggerated glucose and insulin responses (Ball et al., 2003; Ludwig et al., 1999), and Chapter 6 indicated that OW girls may manifest an inability to control blood glucose after a HGI breakfast in particular. Ultimately, this is concerning since the postprandial state contributes to the development of chronic disease (Heine et al., 2004). Therefore, interventions to reduce postprandial glucose and insulin concentrations and increase fat oxidation in young people could have considerable clinical relevance.

Exercise training can increase insulin sensitivity and fat oxidation in young people (Ben Ounis et al., 2009; Shaibi et al., 2008). In adults, however, it is also well established that such improvements in metabolism are largely a consequence of the acute effects of exercise rather than long-term training adaptations (Burton et al., 2008; King et al., 1995). Furthermore, studying the acute effect of exercise reflects the metabolic responses of individuals who do not participate in regular exercise training. Studies in adults have reported that a single bout of aerobic exercise can increase insulin sensitivity (Newsom et al., 2010), reduce postprandial insulin concentrations (Burton et al., 2008; Kokalas et al., 2005) and increase fat oxidation the next day (Burton et al., 2008; Holtz et al., 2008; Schenk and Horowitz, 2007), although the effect on glucose is less clear (Burton et al., 2008; Kokalas et al., 2005). Furthermore, improvements in fat oxidation and insulin sensitivity may be related; high rates of fat oxidation may reduce the accumulation of fatty acid metabolites (e.g., diacylglycerol, ceramides) within the muscle that interfere with insulin signalling (Holloway et al., 2009; see section 2.1). Markers of fatty-acid induced insulin resistance are present even in obese young people (Sinha et al., 2002b). Importantly, a single bout of exercise can protect against fatty acid-induced insulin resistance in adults by increasing the partitioning of fatty acids toward IMTG synthesis and reducing the accumulation of fatty acid metabolites (Schenk and Horowitz, 2007). In this respect, it is plausible that exercise at Fatmax (the

individual exercise intensity corresponding to peak fat oxidation) is particularly beneficial.

Despite concerns of overweight and insulin resistance in young people and the well-recognised improvements in metabolism after a single exercise bout in adults, studies examining the acute effect of exercise on glucose, insulin and fat metabolism in young people are not available. There are clear differences in metabolism between young people and adults (Riddell, 2008) and also between overweight (OW) and non-overweight (NO) young people (Aucouturier et al., 2011). Thus, the interaction between exercise-induced changes in metabolism and weight status in young people requires examination. The present study examined the acute effect of Fatmax exercise on blood glucose, plasma insulin and fat oxidation in the fasted state and after HGI breakfast consumption in OW and NO girls.

7.2 Methods

7.2.1 Participants

Twelve OW and 17 NO girls aged 9 to 14 y participated in the study (12 OW and 15 NO girls were included in final analyses). Overweight status was defined using age and sex specific BMI reference points (Cole et al., 2000). Anthropometric characteristics were assessed and recorded prior to experimental trials, as detailed in the General Methods (section 3.2).

7.2.2 Preliminary measurements

Participants were familiarised with treadmill walking and running before completing two preliminary tests. First, an uphill incremental treadmill test was used to measure $V\dot{O}_{2peak}$ (see section 3.5.1). On a separate occasion, the girls completed a 4 min incremental exercise test to determine Fatmax. The speed increased by 0.5 $km \cdot h^{-1}$ every 4 min (1% gradient). Tests were terminated when the RER exceeded 0.95 or the participant was exercising above 80% $V\dot{O}_{2peak}$. Average $V\dot{O}_2$ and $V\dot{C}O_2$ values from the final min of each stage were used to estimate fat oxidation. Subsequently, Fatmax (% $V\dot{O}_{2peak}$) was estimated using individual best-fit polynomial curves of fat oxidation rate against % $V\dot{O}_{2peak}$.

7.2.3 Experimental conditions

All participants completed two 2-day conditions in a counter-balanced order (Figure 7.1). On day 1, participants either expended ~2.09 MJ (500 kcal) during treadmill exercise at individual Fatmax (EX) or rested (CON). On day 2, the girls reported to the laboratory at 08:00 after a 12 h overnight fast. After fasted measures, the girls consumed a HGI mixed breakfast meal providing 1 g CHO·kg BM^{-1} within 15 min. The nutritional content of the breakfast was calculated from information provided by the manufacturer. For a 50 kg participant, the breakfast contained 31.4 g cornflakes, 100.0 g skimmed milk, 34.3 g white bread, 4.3 g margarine, 6.4 g jam and 114.3 g water (calculated GI=73). The GI values for individual foods were taken from the International Table of Glycemic Index and Glycemic Load Values (Atkinson et al., 2008) and the GI was calculated from the weighted means of the GI values for the component foods (Wolever and Jenkins, 1986). The 120 min postprandial period commenced immediately after breakfast consumption; during which, capillary blood, expired air samples and subjective ratings of hunger were collected at regular intervals.

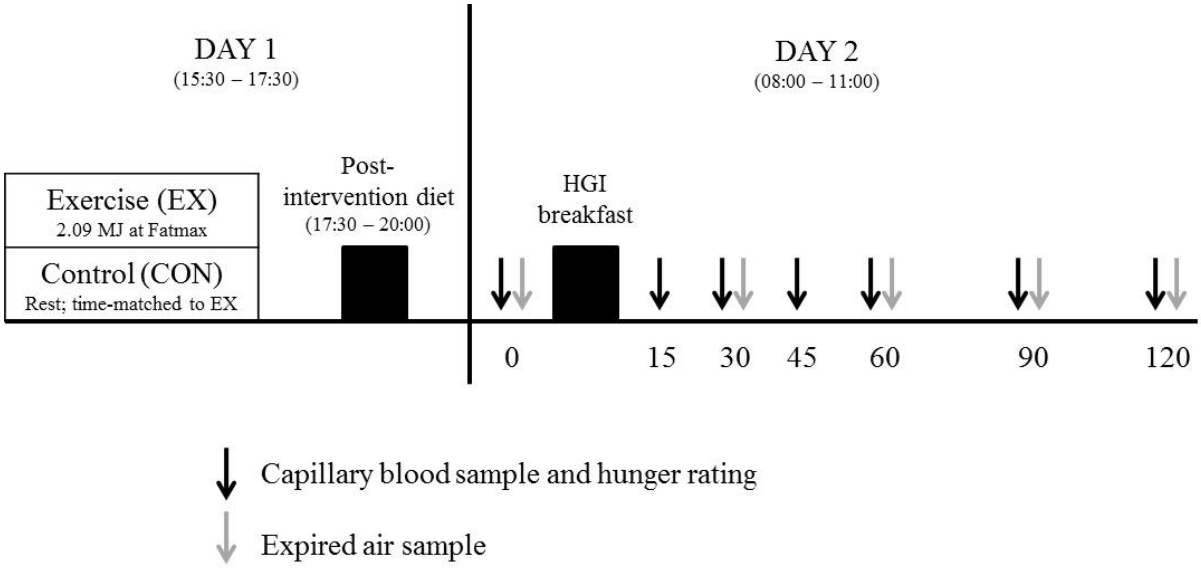


Figure 7.1 Schematic of 2-day protocol for experimental conditions

7.2.4 Control of diet and physical activity

With the assistance of a parent, the girls were asked to record their food and drink intake in the 48 h period prior to day 2 of the first condition and replicated this in the 48 h before the second condition (Appendix 4). As Fatmax is affected by pre-exercise

CHO consumption (Achten and Jeukendrup, 2003b), participants also consumed this diet in the 48 h prior to the Fatmax measurement in an attempt to prescribe Fatmax exercise in the same conditions that it was measured. The girls were asked to minimise physical activity in the 48 h before the Fatmax measurement and experimental conditions.

7.2.5 Menstrual cycle phase

Ten of the participants had commenced menstruation and completed their experimental conditions 48 h apart to minimise the potential influence of menstrual cycle phase on within-participant comparisons (Oosthuyse and Bosch, 2010). After asking the girls to complete a menstrual cycle diary, the author attempted to conduct experimental conditions during the early follicular phase (days one to seven) to reduce between-participant variability. However, five of the girls completed their experimental conditions during the early follicular phase, two during the late luteal phase and it was not possible to determine menstrual cycle phase in the remaining three girls due to irregularities in menstrual cycle.

7.2.6 Expired air and indirect calorimetry

Breath-by-breath data were displayed online using a gas analysis system (Metalyzer 3B, Cortex, Leipzig, Germany). Calibration procedures were carried out prior to each experimental test (see section 3.3). Fat oxidation rates were calculated using stoichiometric equations, with the assumption that the urinary nitrogen excretion rate was negligible and a physiological steady-state had been attained (Frayn, 1983; see section 3.6). Fat oxidation TAUC for the 120 min rest period was calculated using the trapezium rule and included in subsequent analyses.

7.2.7 Blood sampling and analysis

Capillary blood samples were obtained from a pre-warmed hand by finger prick and, subsequently duplicate blood glucose and plasma insulin concentrations were determined (see section 3.7). Blood glucose and plasma insulin TAUC for the 120 min postprandial period was calculated using the trapezium rule (Wolever and Jenkins, 1986) and HOMA-IR was calculated from fasted glucose and insulin (Matthews et al., 1985). The CV for the duplicate samples was 2.0 % for blood glucose and 5.8 % for plasma insulin.

7.2.8 Statistical analyses

Statistical analyses were completed using SPSS software version 18.0 for Windows (SPSS Inc, Chicago, IL, USA). Shapiro-Wilk and Levene's tests were used to verify normal distribution and homogeneity of variance, respectively. Greenhouse-Geisser correction was used when sphericity could not be assumed. Condition by time (2 x 7) repeated measures ANOVA were used to examine differences between EX and CON; these were conducted separately for OW and NO girls. To compare the two groups of girls directly, condition by group (2 x 2) mixed measures ANOVA with condition as the repeated factor were used to examine the fasting, peak and TAUC values for glucose, insulin and fat oxidation. Values are expressed as mean(SD), unless stated otherwise, and ES were calculated. Statistical significance was accepted at $P \leq 0.05$.

7.3 Results

7.3.1 Participant characteristics

Participant characteristics are displayed in Table 7.1. Body mass, BMI, body fat, waist circumference and hip circumference were higher in the OW compared with NO girls ($P<0.003$), whereas $V\dot{O}_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) ($P<0.0005$) and Fatmax (% $V\dot{O}_{2peak}$) ($P=0.024$) were higher in the NO girls. Two of the OW girls were insulin resistant (HOMA-IR >3.16) (Keskin et al., 2005).

Table 7.1 Participant characteristics

	OW *n*=12	NO *n*=15
Age (y)	11.7(1.3)	12.3(1.5)
Body mass (kg) [a]	61.2(20.3)	42.2(7.9)
Stature (m)	1.53(0.10)	1.54(0.09)
BMI ($kg \cdot m^{-2}$) [a]	25.8(5.2)	17.7(1.8)
Body fat (%) [a]	35.2(6.2)	19.1(5.1)
FFM (kg)	39.0(10.3)	34.0(5.5)
Waist circumference (cm) [a]	77.5(13.6)	61.7(4.9)
Hip circumference (cm) [a]	90.4(15.7)	74.9(8.5)
Tanner (pubic hair)†	3(1)	3(2)
$V\dot{O}_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$) [a]	41(6)	51(4)
Fatmax (% $V\dot{O}_{2peak}$) [a]	52(10)	63(12)

OW – overweight, NO – non-overweight, BMI – body mass index, FFM – fat free mass, Tanner stage – estimation of secondary sexual characteristics (Tanner, 1962), $V\dot{O}_{2peak}$ – peak oxygen uptake.
[a] significant difference between OW and NO ($P \leq 0.05$)
†median (interquartile range)

7.3.2 Energy expenditure and energy intake

During the 2.09 MJ (500 kcal) Fatmax exercise bout, the OW girls exercised at 54(8)% $V\dot{O}_{2peak}$ and the NO girls exercised at 63(12)% $V\dot{O}_{2peak}$ (P=0.039). Exercise duration was 73(20) min for the OW and for 67(18) min for the NO girls (P=0.422). During the time-matched resting condition, the OW girls tended to expend more energy than the NO girls (0.51(0.11) vs. 0.43(0.12) MJ; P=0.079).

Average daily energy and macronutrient intake in the 48 h prior to EX and CON was similar between the OW and NO girls (P>0.05). However, day 1 post-intervention (17:30 to 20:00) energy intake was higher in the OW compared with NO girls (4.11(1.87) vs. 2.96(0.84) MJ; P=0.042), whilst CHO (146(85) vs. 101(35) g; P=0.076)

and fat (31(12) vs. 23(10) g; P=0.079) intake tended to be higher. The counter-balanced assignment to EX and CON did not affect post-intervention energy or macronutrient intakes between OW and NO nor within OW and NO.

7.3.3 Blood glucose concentration

Blood glucose responses are shown in Figure 7.2. Postprandial blood glucose concentration increased and peaked at a median (interquartile range) time of 15(15) min for all conditions, except in the OW CON condition where it peaked at 30 min in 4 girls and 60 min in 1 girl (median 22.5(15) min). Fasting, peak and TAUC for blood glucose were similar between EX and CON in both groups of girls (P>0.05) and there were no differences in blood glucose concentration between the OW and NO girls (P>0.05) (Table 7.2).

7.3.4 Plasma insulin concentration

Plasma insulin responses are shown in Figure 7.3. Postprandial plasma insulin concentration increased and peaked at a median (interquartile range) time of 15(15) min for both conditions and groups. Fasting (P=0.047, ES: 0.50) and TAUC (P=0.049, ES: 0.50) for plasma insulin were lower for EX compared with CON in the NO girls, but there was no difference in peak postprandial plasma insulin (P=0.263, ES: 0.30). All measures of plasma insulin were similar between conditions in the OW girls (P>0.05). Although not significantly different between conditions, HOMA-IR was 15% lower for EX in the NO girls (P=0.125, ES: 0.40), but 9% higher in the OW girls (P=0.663, ES: 0.13). Fasting and postprandial plasma insulin concentrations (P≤0.0005) and HOMA-IR (P=0.022, ES: 0.44) were higher in the OW compared with NO girls (Table 7.2).

7.3.5 Fat oxidation

Fat oxidation ($mg \cdot kgFFM^{-1} \cdot min^{-1}$) for both conditions and groups is displayed in Figure 7.4. Fasting fat oxidation was higher (P=0.036, ES: 0.53) for EX compared with CON in the NO girls, but similar between conditions in the OW girls (P=0.790). Fat oxidation TAUC was higher for EX in the NO (P=0.005, ES: 0.66) and OW girls (P=0.04, ES: 0.57). Fat oxidation was similar between the groups (P>0.05). When expressed relative to total energy expenditure (% EE), fasting (P=0.024, ES: 0.56) and TAUC (P=0.021, ES: 0.57) for fat oxidation was higher in EX compared with CON in NO, but not OW girls (P>0.05) (Table 7.2).

Table 7.2 Summary of fasting and postprandial responses

	OW *n*=12		NO *n*=15	
	EX	CON	EX	CON
Glucose ($mmol \cdot L^{-1}$)				
Fasting	3.79(0.42)	3.81(0.32)	3.87(0.38)	3.75(0.25)
Peak	5.80(0.46)	5.83(0.61)	5.77(0.75)	5.73(0.60)
Average postprandial	4.88(0.53)	4.92(0.52)	4.78(0.54)	4.76(0.61)
TAUC	9.52(0.63)	9.60(0.62)	9.41(0.76)	9.38(0.87)
Insulin ($pmol \cdot L^{-1}$)				
Fasting [a]	96(112)	92(79)	26(13)	32(18) [b]
Peak [a]	745(377)	712(325)	274(116)	307(169)
Average postprandial [a]	419(253)	439(256)	144(66)	168(91) [b]
TAUC [a]	769(411)	809(451)	259(89)	304(128) [b]
HOMA-IR [a]	2.90(3.91)	2.65(2.34)	0.75(0.40)	0.89(0.54)
Fat oxidation ($mg \cdot kgFFM^{-1} \cdot min^{-1}$)				
Fasting	2.01(0.65)	1.96(0.78)	2.00(0.64)	1.77(0.80) [b]
Average postprandial	1.63(0.42)	1.41(0.39) [c]	1.69(0.49)	1.45(0.58) [b]
TAUC	6.19(2.86)	4.80(2.55) [c]	6.36(2.88)	5.07(3.35) [b]
Fat oxidation (% total EE)				
Fasting	42(12)	41(17)	41(13)	34(17) [b]
Average postprandial	31(10)	28(10)	32(9)	27(11) [b]

OW – overweight, NO – non-overweight, EX – exercise condition, CON – control condition, TAUC – total area under the curve, HOMA-IR – homeostasis model assessment for insulin resistance, FFM – fat free mass, EE – energy expenditure.
[a] significant difference between OW and NO ($P \leq 0.05$)
[b] significant difference between EX and CON within group for NO ($P \leq 0.05$)
[c] significant difference between EX and CON within group for OW ($P \leq 0.05$)

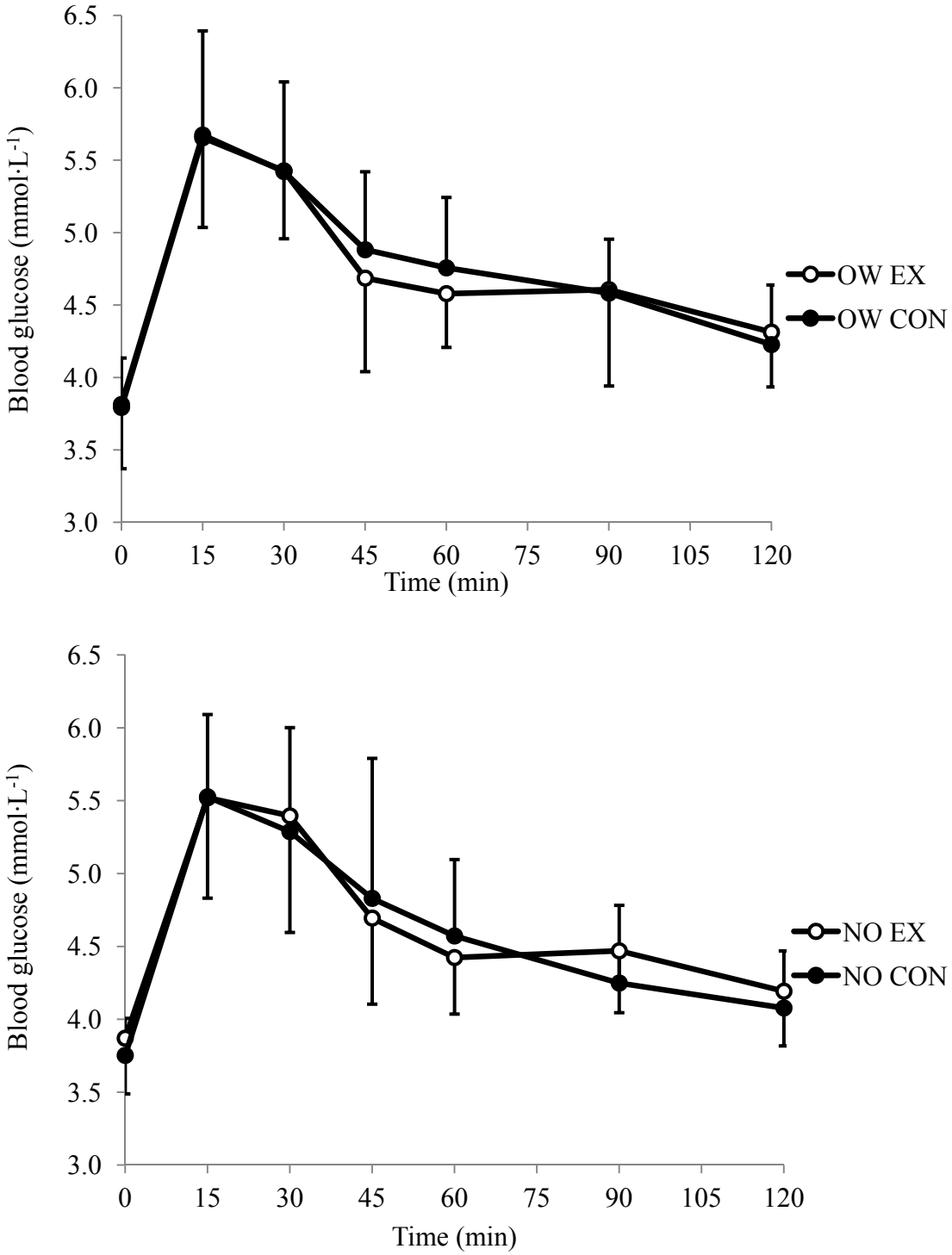


Figure 7.2 Blood glucose concentration the morning after the exercise (EX) and control (CON) conditions for the overweight (OW) and non-overweight (NO) girls. Breakfast was consumed between 0 and 15 min.

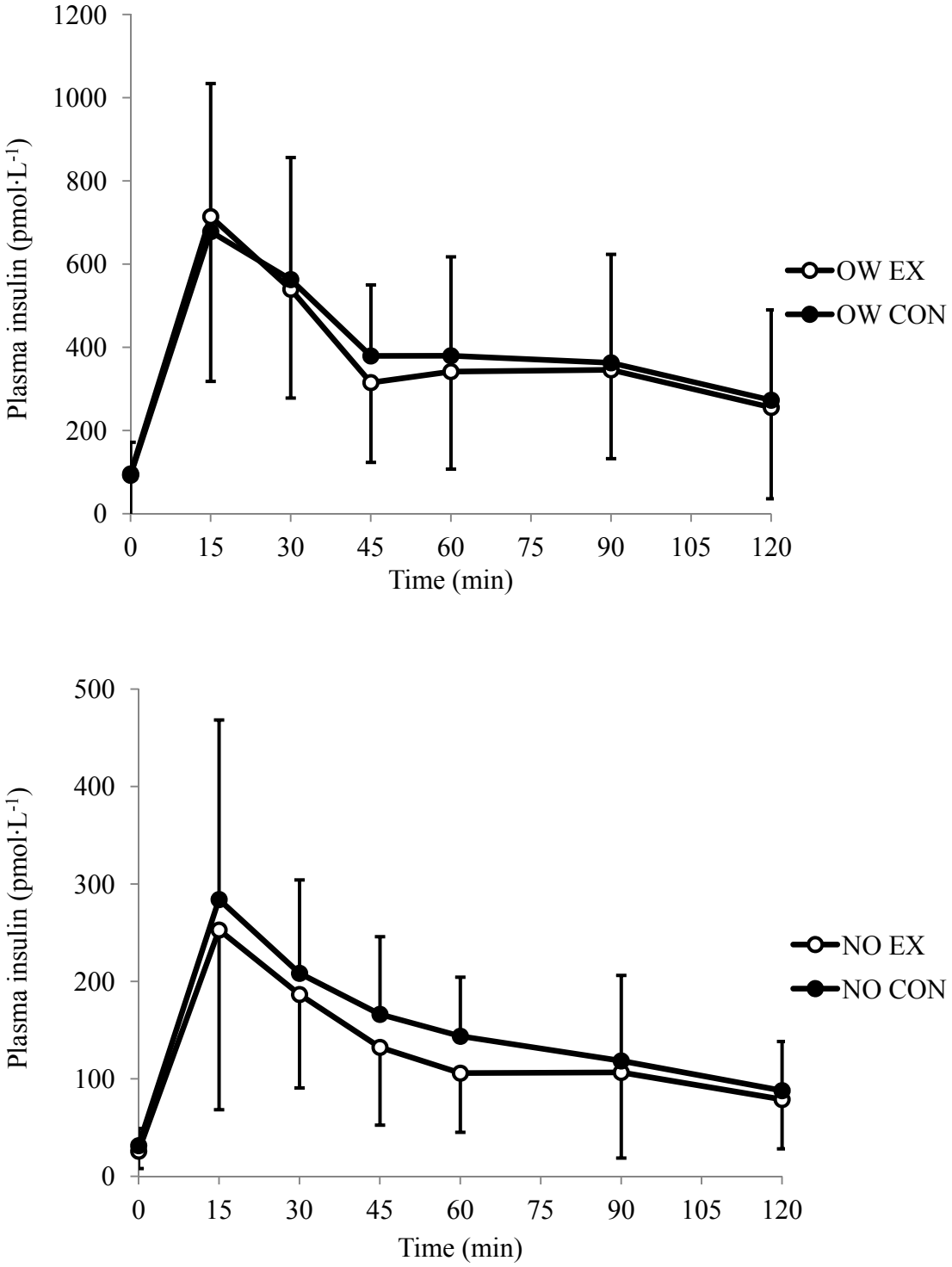


Figure 7.3 Plasma insulin concentration the morning after the exercise (EX) and control (CON) conditions for the overweight (OW) and non-overweight (NO) girls. Breakfast was consumed between 0 and 15 min.

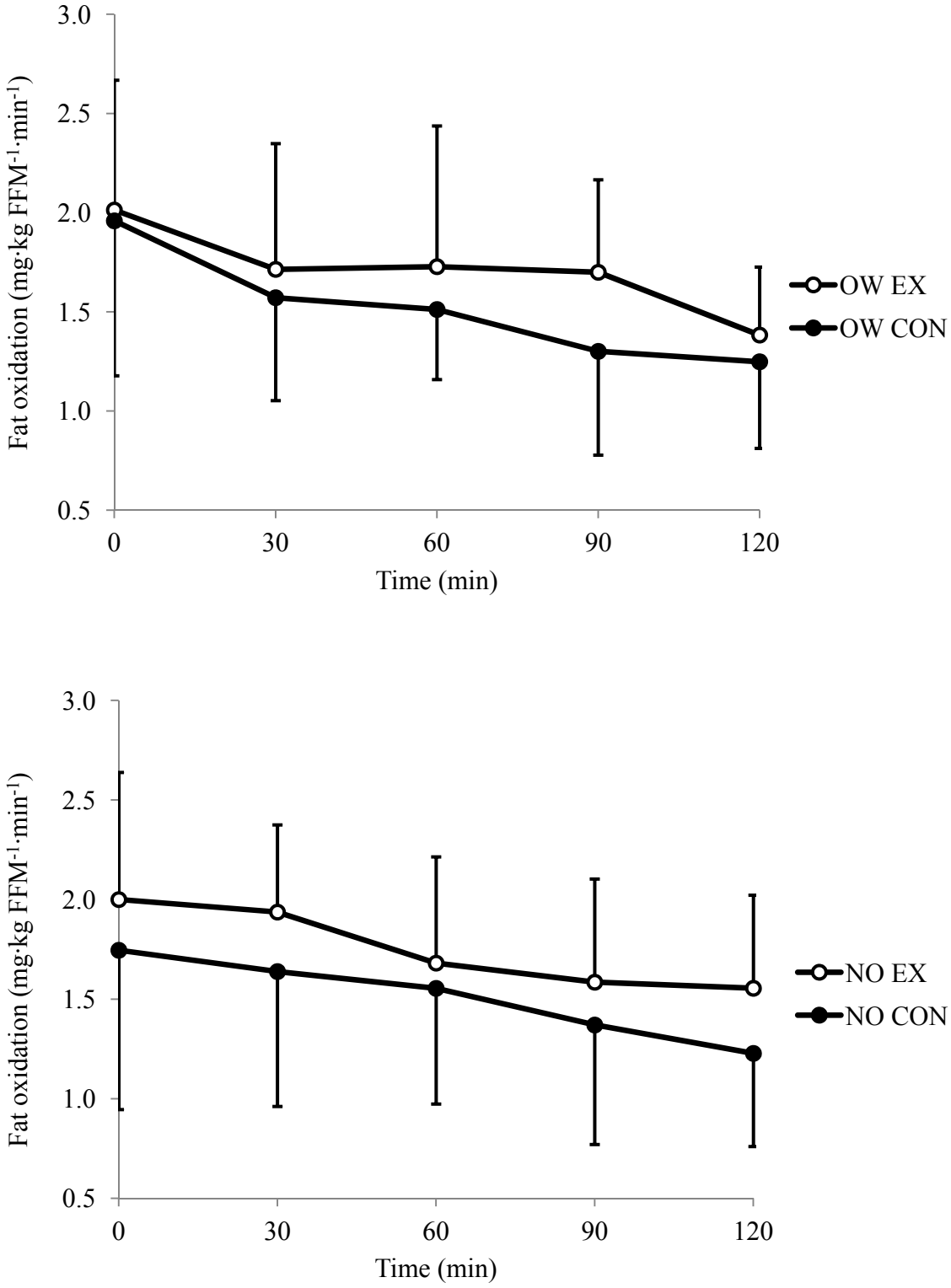


Figure 7.4 Fat oxidation the morning after the exercise (EX) and control (CON) conditions for overweight (OW) and non-overweight (NO) girls. Breakfast was consumed between 0 and 15 min.

7.4 Discussion

The main finding of the present study was that 2.09 MJ (500 kcal) of Fatmax exercise performed ~16 h before HGI breakfast consumption reduced fasting and postprandial insulin concentrations in NO girls, with no change in blood glucose concentrations. However, exercise did not affect glucose or insulin concentrations in the OW girls. Furthermore, an increase in fat oxidation the day after Fatmax exercise was observed in both the OW and NO girls. To the author's knowledge, this is the first study to demonstrate these acute exercise-induced metabolic effects in young people.

Reductions in insulin after a single exercise bout in the NO girls are in agreement with studies in adults (Brestoff et al., 2009; Kokalas et al., 2005). However, similar studies in young people do not appear to be available for comparison and few studies have directly examined the potential effect of weight status on this relationship. Only one study appears to have reported glucose and insulin concentrations the day after exercise in young people, although this study was not specifically designed to examine these health markers (MacEneaney et al., 2009). In support of the present study, 2.51 MJ (600 kcal) of exercise did not affect fasting and postprandial glucose or insulin in overweight adolescent boys; although, in contrast, insulin concentrations were not reduced in the non-overweight boys (MacEneaney et al., 2009). However, this study included a high-fat breakfast meal and only three blood samples were taken during the immediate 120 min postprandial period (MacEneaney et al., 2009); thus a direct comparison between the present study and the available literature in young people is limited. In adults, exercise reduced postprandial insulin in both overweight and non-overweight women (Mitchell et al., 2008) and in obese, but not non-obese, men (Gill et al., 2004). However, unlike the present study, all participants exercised at the same intensity and duration; the higher exercise energy expenditure in the obese men might explain why only they experienced an exercise-induced reduction in postprandial insulin concentration (Gill et al., 2004). Differences in participant characteristics, breakfast size and composition, and post-exercise energy and macronutrient intake may have also contributed to these disparate findings. In addition, differences in metabolism between young people and adults may have resulted in discrepancies between the present study and some of the adult-based literature (Riddell, 2008).

Exercise-induced reductions in insulin concentrations in the NO girls may be attributed to enhanced insulin sensitivity the morning after exercise. Studies in adults have shown that insulin sensitivity increases up to 72 h after exercise to facilitate muscle glycogen replenishment (see section 2.9.1). The major cellular mechanism controlling this increased insulin sensitivity appears to be increased GLUT-4 translocation to the plasma membrane (Thorell et al., 1999), although other mechanisms have emerged more recently (Maarbjerg et al., 2011). The exercise-induced reduction in insulin with no change in glucose indicates improved insulin sensitivity the morning after exercise in the NO girls; a lower insulin concentration was needed to control the rise in glucose. Although the reduction in HOMA-IR after exercise compared with rest was not statistically significant, it may have been meaningful enough to alter postprandial insulin. Exercise-induced reductions in postprandial insulin with no change in HOMA-IR have also been shown in adults (Burton et al., 2008; Holtz et al., 2008), whereas others have reported reduced HOMA-IR (Brestoff et al., 2009). It is possible that more sensitive measures of insulin sensitivity (e.g., glucose clamp methods) were required to detect potential differences between trials in the present study.

A bout of Fatmax exercise increased fat oxidation the next day in both the OW and NO girls, which appears to be another novel finding in young people and is consistent with studies in overweight (Burton et al., 2008) and non-overweight (Schenk and Horowitz, 2007) adults. Several cellular mechanisms may contribute to this increased fat oxidation, including increased muscle LPL activity (Kiens and Richter, 1998), reduced PDH activity (Kimber et al., 2003) and possibly, similar to GLUT-4, the translocation of specific fatty acid transporters to the plasma membrane (Koonen et al., 2004). In adults, acute exercise increased the partitioning of fatty acids toward oxidation and IMTG synthesis, reduced the accumulation of fatty acid metabolites within skeletal muscle and suppressed activation of proinflammatory pathways known to impair insulin action (Schenk and Horowitz, 2007), suggesting elevations in fat oxidation after exercise may have contributed to the reduced insulin concentrations in the NO girls. Furthermore, reducing exercise fat oxidation through the ingestion of a lipolysis inhibitor abolished the exercise-induced reduction in postprandial insulin in men (Malkova et al., 1999). It is, therefore, conceivable that the high rates of fat oxidation during Fatmax exercise can augment potential exercise-induced enhancements in insulin sensitivity. However, a study comparing the acute effect of exercise at Fatmax

and exercise eliciting low rates of fat oxidation in young people is required for a more complete understanding of this relationship. The lower insulin concentrations after exercise may have also enhanced lipolysis and contributed to the elevated fat oxidation in the NO girls (Horowitz et al., 1997). Interestingly, unlike the NO girls, the OW girls did not experience an increase in fat oxidation as a proportion of total energy expenditure. This suggests that increased post-exercise total energy expenditure partly explained the elevated fat oxidation in the OW girls; thus different mechanisms may control the increased fat oxidation observed in the two groups.

Importantly, exercise-induced reductions in insulin in the NO girls and elevations in fat oxidation in both groups occurred following just a single exercise bout and despite the maintenance of habitual diet, suggesting that these extend to a 'real world' setting where diet is not prescribed. Reduced postprandial insulin concentration indicates improved glucose control and may have implications for the prevention of insulin resistance and related chronic disease. Although insulin was not affected in the OW girls, elevations in fat oxidation may have long term implications for alleviating insulin resistance (Holloway et al., 2009; Kelley, 2002) and for weight control (DeLany et al., 2006). Future research should consider the time-course of these metabolic improvements in young people, which may persist up to 72 h post-exercise (King et al., 1995).

Since insulin concentrations were markedly higher in the OW compared with NO girls in both the present study and Chapter 6, interventions to reduce insulin concentration may be particularly relevant for this population. A number of plausible reasons could explain why exercise did not affect insulin in the OW girls. Dietary analysis indicates that post-exercise energy and/or CHO replacement may have facilitated muscle glycogen restoration and attenuated potential exercise-induced reductions in insulin in the OW girls, as previously reported in adults (Burton et al., 2008; Newsom et al., 2010). Indeed, post-intervention energy and CHO intake was higher in the OW compared with NO girls and may have resulted in positive energy/CHO balance, although energy and CHO balance was not measured directly. Interestingly, the OW girls consumed more energy and CHO regardless of whether they completed EX or CON first, which suggests that this dietary 'compensation' was not specifically exercise-induced, but may be related to body size or composition. It is also possible that

the study design did not permit changes in insulin in the OW girls. All participants expended a set amount of energy during exercise expressed in absolute terms, rather than relative to body mass, to reduce between-participant variability in exercise duration. Consequently, the OW girls expended less energy relative to body mass during the exercise bout, which may not have been sufficient to affect insulin in the heavier participants. A HGI breakfast was purposefully chosen due to the findings of Chapter 6, where it was concluded that interventions to attenuate the glucose and insulin response to this type of breakfast are required, particularly for OW girls. It is possible that the exercise was not sufficient to influence the response to a breakfast known to induce particularly exaggerated postprandial glucose and insulin responses. Finally, between-group differences in metabolism could also underpin the contrasting findings between the OW and NO girls. 'Metabolic inflexibility' has been observed in obese young people (Aucouturier et al., 2011; see section 2.1) and may have compromised the OW girls' ability to change insulin metabolism in response to acute exercise, as observed following dietary changes (Sunehag et al., 2005). It has been suggested that a single exercise bout may not be a large enough stimulus to increase insulin action in those with reduced fitness and limited cellular mechanisms that might be required to enhance insulin action after exercise (Holtz et al., 2008). Future research should address these issues in OW and NO young people by manipulating exercise energy expenditure, post-exercise energy and/or CHO intake and examining the potential role of metabolic inflexibility.

In conclusion, a bout of Fatmax exercise performed 16 h prior to HGI breakfast consumption reduced fasting and postprandial insulin in NO girls and elevated fat oxidation in both OW and NO girls. Importantly, these metabolic improvements were observed after just a single bout of exercise (2.09 MJ) and despite the maintenance of habitual diet. The higher post-intervention energy and CHO intake and/or a degree of 'metabolic inflexibility' may have compromised a potential exercise-induced reduction in insulin in the OW girls. Further examination of these issues in young people is warranted.

Chapter 8

General Discussion

Over the past few decades, there has been an alarming increase in the prevalence of overweight and obesity among young people in England and throughout the world (Ebbeling et al., 2002; Health Survey for England, 2009; Wang et al., 2011). Since high adiposity is associated with numerous adverse health outcomes and often tracks into adulthood, immediate action is required to alleviate this public health problem. An imbalance between energy intake and expenditure is the underlying cause of weight gain, thus exercise and dietary strategies are strongly recommended for the management of obesity and associated metabolic disorders. Exercise is often advocated to confer additional health benefits that may not manifest following dietary intervention alone. Although expending energy through the oxidation of all macronutrients during exercise could facilitate weight loss, maximising fat oxidation in particular appears to have clinical relevance and is related to insulin resistance. The most efficacious interventions and recommendations must be evidence-based. Hence, a clear understanding of exercise metabolism and factors influencing fat oxidation and insulin resistance in young people is required. However, research investigating the interplay between exercise, diet, fat oxidation and insulin resistance in young people is sparse. This is somewhat surprising, since childhood and adolescence have been identified as critical periods of weight gain and the establishment of lifestyle behaviours. The experimental studies presented within this thesis sought to extend our understanding of how manipulations in exercise and breakfast consumption might influence glucose, insulin and fat metabolism in overweight and non-overweight young people. The purpose of this chapter is to collectively discuss and reflect upon the findings from these studies.

8.1 Overweight, insulin resistance and fat oxidation in young people

The clinical importance of increasing fat oxidation and reducing insulin resistance in young people has been highlighted throughout this thesis, providing a rationale for much of the experimental work presented. Therefore, first, it should be highlighted that the experiments within this thesis have clearly shown that overweight girls are more insulin resistant and, consequently, have insulin concentrations that are substantially

elevated compared with non-overweight girls (Chapters 6 and 7), confirming prior reports (Lee et al., 2006; Sinha et al., 2002a). It is noteworthy that much of the previous literature has compared obese and non-obese participants; the results reported herein have clearly shown that even overweight young people manifest elevated HOMA-IR values and insulin concentrations.

Insulin resistance may be accompanied by low fasting fat oxidation and metabolic inflexibility in obese young people (Aucouturier et al., 2011). The OW and NO girls in Chapter 6 and 7 oxidised a similar amount of fat (per kg FFM) during fasting conditions (even when the data from the two studies were pooled, $P \geq 0.05$), postprandially and during exercise, although metabolic inflexibility was not assessed directly. Similar findings have emerged from studies that have estimated resting fat oxidation in young people (Maffeis et al., 1995; McMurray and Hosick, 2011; Zunquin et al., 2009b), although previous work indicates that obese young people have a reduced ability to oxidise fat during exercise (Lazzer et al., 2007; Zunquin et al., 2009b; see section 2.5.3). Inconsistencies between the findings reported herein and some previous studies may be due to the inclusion of overweight rather than obese participants. Moreover, the comparison of fat oxidation between obese, overweight and non-overweight groups is further complicated by the unit of measurement for fat oxidation, i.e., absolute, per kg BM, per kg FFM or % total energy expenditure. It is, therefore, not clear whether overweight or obese young people have an impaired ability to oxidise fat. However, the work presented within this thesis has shown that Fatmax is reduced in OW girls (Chapter 7). This indicated that fat oxidation begins to decline at a lower exercise intensity in these girls, which may limit fat oxidation to a narrower range of intensities and reflect a reduced capacity for fat oxidation, particularly during higher exercise intensities. Furthermore, several lines of evidence indicate that enhancing fat oxidation has implications for protecting against future weight gain (DeLany et al., 2006; Marra et al., 2004) and insulin resistance (Aucouturier et al., 2011). Consequently, others have recommended that an effort to increase the capacity for fat oxidation within skeletal muscle should be among the goals for the prevention and treatment of obesity and insulin resistance (Holloway et al., 2009; Kelley, 2002).

8.2 'Optimising' fat oxidation during exercise

Despite the potential for exercise to elevate fat oxidation, no consensus has been reached on the type of exercise that is preferable for maximising the amount of fat oxidised. Moreover, evidence in young people is extremely limited relative to that in adults. Exercise characteristics (intensity, mode, duration), individual participant characteristics (age, sex, weight status) and pre-exercise diet are among the factors influencing fat oxidation during exercise. Of these factors, exercise intensity and mode are both central and easily modifiable. Therefore, Chapters 4 and 5 sought to determine how exercise intensity and mode might be best manipulated to promote high rates of fat oxidation in children.

8.2.1 Estimation of Fatmax

There has been a recent surge in studies that have prescribed exercise training at Fatmax in young people, demonstrating subsequent improvements in various health markers (Ben Ounis et al., 2010; 2011; Elloumi et al., 2009). However, a number of methodological considerations are associated with the estimation of Fatmax, which should be investigated prior to its implementation. In trained adults, a 3 min incremental protocol was validated for the estimation of Fatmax some time ago (Achten et al., 2002). This was necessary as there are two primary issues with a 3 min incremental protocol: (1) whether a physiological steady state is attained before the onset of the sampling period; and (2) whether there is a residual effect from stage to stage that influences subsequent fat oxidation estimations. Before attempting to contribute further to existing paediatric research, the first study within this thesis examined some of these issues by comparing two protocols to estimate Fatmax in a group of prepubertal girls and boys (Chapter 4). It was demonstrated that individual Fatmax values compare well when estimated using a 3 min incremental protocol and 10 min isolated bouts, extending the results from Achten et al. (2002) in trained men to prepubertal children. Although group MFO values were similar, the large 95% LoA indicated that MFO did not compare well between the protocols on an individual level. Achten et al. (2002) also reported similar group fat oxidation values, but the statistical methods employed did not allow insight at the individual level; the results from Chapter 4 indicate that this individual analysis was required. Although these findings suggest the estimation of MFO depends on the exercise protocol employed, the 3 min incremental protocol did not systematically under- or over-estimate MFO. Therefore,

other factors may have been partly responsible for variations in fat oxidation, including diet and physical activity in the days prior to the Fatmax exercise tests (Bagger et al., 2003; Meyer et al., 2007).

It was concluded that a 3 min incremental protocol can be used to estimate Fatmax, but caution should be exercised when estimating MFO for some individuals. The 3 min protocol has several clear advantages over using prolonged exercise bouts. Most notably, the estimation of fat oxidation across a wide range of exercise intensities (10 on average) enables a more precise estimation of Fatmax. In addition, Fatmax can be measured on a single visit to the laboratory. This is important since Fatmax should be measured on an individual basis due to the large inter-individual variation in this metabolic marker; 10 min isolated bouts requiring multiple visits to the laboratory and the control of diet and exercise in the days preceding each measurement may not be feasible for many children. With these advantages in mind and the clear practical applications of estimating Fatmax, the discrepancy between the two protocols concerning MFO is not clear enough to recommend the use of prolonged isolated exercise bouts. Importantly, studies estimating Fatmax should acknowledge the issues highlighted within this study and caution should be maintained when reporting MFO values and making inter-study comparisons.

8.2.2 Exercise mode

Puberty (Riddell et al., 2008), obesity (Zunquin et al., 2009b) and training (Brandou et al., 2003) may influence exercise fat oxidation and Fatmax in young people; Chapter 5 has extended this research by demonstrating that exercise mode is another important determinant. A novel finding of Chapter 5 was that Fatmax was higher for treadmill compared with cycling exercise (59 and 51% $V\square O_{2peak}$, respectively) in pre- to early pubertal children, suggesting children must exercise at a higher exercise intensity during treadmill exercise to elicit the mode-specific MFO. A review of the adult-based literature revealed that only one study has reported higher Fatmax values for treadmill compared with cycling exercise (Chenevière et al., 2010), with others reporting no difference (Achten et al., 2003; Glass et al., 1999); inconsistent findings may be due to differences in the treadmill exercise protocols employed (see section 2.5.4). Interestingly, the Fatmax zone was wider for treadmill exercise, thus fat oxidation remained high (within 5% of MFO) over a wider range of intensities. This indicates that

walking or slow running rather than cycling increases the likelihood of exercising within the Fatmax zone. Moreover, Chapter 5 provided strong evidence that rates of fat oxidation are elevated during treadmill compared with cycling exercise. The higher fat oxidation during treadmill exercise over a range of absolute and relative exercise intensities extended recent findings in obese adolescent boys (Lafortuna et al., 2010) to non-overweight pre- to early pubertal girls and boys. Importantly, the estimation of fat oxidation over a range of intensities and comparison at both relative and absolute exercise intensities overcame the limitations associated with previous research examining the effect of exercise mode in children (Mácek et al., 1976). It is, therefore, evident that treadmill exercise is preferable for promoting fat oxidation; children oxidise more fat during treadmill exercise regardless of exercise intensity and are more able to oxidise fat at higher intensities (higher Fatmax) and over a greater range of intensities (wider Fatmax zone).

8.2.3 Determinants of Fatmax

Group Fatmax values from Chapters 4 and 5 ranged between 49 and 62% V□O_{2peak}, which is comparable with other studies in healthy prepubertal children (see Chapter 2, Table 2.1). However, there appears to be considerable inter-individual variation in Fatmax and potential determinants have emerged from the studies reported within this thesis. Chapter 5 showed, for the first time, that Fatmax was higher in boys compared with girls during treadmill exercise, whereas sex did not influence cycling Fatmax even when the sample size was increased by pooling the data from Chapters 4 and 5 (P=0.137). However, differences in V□O_{2peak}, BMI and body fat between the boys and girls may have affected between sex comparisons. Importantly, V□O_{2peak} explained 44% of the variation in Fatmax for treadmill, but only 4% for cycling exercise (Chapter 5). This strong correlation along with the higher V□O_{2peak} in the boys may have partially explained the higher treadmill Fatmax in the boys. Indeed, increased fitness and ability to oxidise fat at high intensities are common training adaptations (Bell et al., 2007; Lazzer et al., 2008). It has previously been reported that sex did not influence cycling Fatmax in a group of obese and non-obese pubertal and postpubertal young people (Lazzer et al., 2007). However, similar studies including treadmill exercise do not appear to be available. Studies with adults are also limited and have yielded inconsistent findings; cycling Fatmax was higher in healthy women compared with men (Chenevière et al., 2011), whereas no effect of sex on treadmill Fatmax was observed in

overweight adults (Bogdanis et al., 2008). However, it is not possible to directly compare findings in pre- to early-pubertal children (Chapters 4 and 5) with these adult-based studies due to the potential influence of puberty and sex hormones on fat oxidation (section 2.5.2). Finally, in Chapter 7, treadmill Fatmax was lower in the OW compared with NO girls, suggesting weight status is another important determinant of Fatmax. This compliments previous reports of reduced Fatmax values in obese pubertal boys (Zunquin et al., 2009b), while extending this finding to OW girls.

8.3 Postprandial metabolism: effect of breakfast and exercise

As discussed, brisk walking or slow running at Fatmax is a promising strategy to elevate fat oxidation in young people (Chapters 4 and 5). However, pre-exercise diet has a profound effect on substrate oxidation (Achten and Jeukendrup, 2003b; Timmons et al., 2007b). It is well established that fat oxidation is maximised in the fasted state (Riddell et al., 2000; Timmons et al., 2007a; 2007b), but this may not be a practical or desirable option for young people and the health benefits of regular breakfast consumption are well documented (Panagiotakos et al., 2008; Timlin et al., 2008). Moreover, exercise and dietary recommendations for weight control and health are commonly advocated in combination rather than independently. Consequently, Chapters 6 and 7 aimed to extend our understanding of the interaction between exercise, breakfast, postprandial metabolism and weight status in adolescent girls. This population was targeted specifically, as physical activity levels are lower in girls compared with boys (Riddoch et al., 2007) and decline rapidly during adolescence (Armstrong and Welsman, 2006) and girls are less likely to eat breakfast daily (Timlin et al., 2008). Therefore, it may be particularly worthwhile for interventions promoting physical activity and breakfast consumption to focus on this population.

8.3.1 Breakfast glycaemic index

There has been considerable interest in the health benefits of LGI diets in adults (Brand-Miller et al., 2009) and, to some extent, in young people (Rovner et al., 2009; Spieth et al., 2000). Similarly, the acute metabolic effects of breakfasts differing in GI have been studied in adults, but mainly active and trained men (Stevenson et al., 2006; 2009; see section 2.8.2). Chapter 6 has extended some of these findings to young people (girls) and examined the interaction with weight status.

In agreement with studies in adults (Stevenson et al., 2006; 2009) and young people (Ball et al., 2003; Ludwig et al., 1999), Chapter 6 has provided further evidence that LGI compared with HGI breakfast consumption attenuates the postprandial glucose response. However, previous work has not examined the potential interaction with weight status. Chapter 6 demonstrated, for the first time, that the higher glycaemic response following a HGI compared with LGI breakfast was more exaggerated in OW than NO girls. The results indicated a delayed blood glucose uptake up to 60 min following HGI breakfast consumption in OW girls, possibly reflecting a reduced ability to cope with the metabolic demands of this breakfast. The combination of readily absorbed glucose from the HGI (but not LGI) breakfast and higher insulin resistance (HOMA-IR) in the OW girls may have contributed to this exaggerated glycaemic response. Interestingly, the glycaemic response to HGI breakfast consumption was not different between the OW and NO girls in Chapter 7. However, it should be noted that postprandial blood glucose peaked slightly later in the OW CON condition compared with the other conditions (OW EX, NO CON and NO EX) in Chapter 7, suggesting a slightly delayed glucose uptake in the OW girls the day after rest, but not exercise. Nevertheless, differences in blood glucose between the OW and NO girls in Chapter 7 were not significant, perhaps due to these OW girls being younger (11.7 vs. 12.6 y, P=0.008) and fitter ($V\square O_{2peak}$ 41 vs. 32 $mL \cdot kg^{-1} \cdot min^{-1}$, P=0.008) than those in Chapter 6. It is plausible that the increased fitness meant that the OW girls in Chapter 7 had an increased ability to control blood glucose, since fitness appears to be a stronger predictor of insulin resistance than fatness (Allen et al., 2007). Moreover, this study did not include a LGI breakfast trial for comparison and provided a smaller breakfast containing 1.0 rather than 1.5 g CHO·kg BM^{-1}. It would, therefore, be of interest to systematically examine whether fitness and breakfast size influence the extent of the blood glucose response following HGI relative to LGI breakfast consumption in OW girls. Indeed, the GI of breakfast cereals was lower in endurance trained compared with sedentary men (Mettler et al., 2007), although this was not replicated in women (Mettler et al., 2008; see section 2.8.3).

Breakfast GI did not affect postprandial fat oxidation during rest or subsequent moderate intensity exercise. However, it is noteworthy that LGI breakfast consumption resulted in 12% higher exercise fat oxidation in both groups, a finding that may have meaningful health-related implications (Holloway et al., 2009), especially for obese

young people who may have a reduced capacity for fat oxidation during exercise (see section 2.1 and 2.5.3). Consequently, these results require confirmation with a larger independent sample. Indeed, several (Stevenson et al., 2009; Wee et al., 2005; Wu et al., 2003), but not all (Moore et al., 2010) studies in adults have reported elevated fat oxidation during exercise following LGI compared with HGI breakfasts. The results reported in Chapter 6 are, however, in accordance with the majority of studies that have shown no effect of breakfast GI on fat oxidation during rest in adults (Díaz et al., 2005; Stevenson et al., 2006; Wee et al., 2005). In a study reporting both higher resting and exercise fat oxidation following a LGI breakfast, a relatively small amount of carbohydrate (1 g CHO·kg BM^{-1}) was consumed 3 h before a 60 min walk at 50% $V\dot{O}_{2peak}$ (Stevenson et al., 2009). However, higher exercise fat oxidation following LGI breakfast consumption has been reported 45 min to 3 h (Sparks et al., 1998; Stevenson et al., 2009) following breakfasts containing 1 to 2.5 g CHO·kg BM^{-1} during exercise lasting 60 or 30 min at 50-71% $V\dot{O}_{2peak}$ (Stevenson et al., 2009; Wee et al., 2005). Resting fat oxidation is typically similar between HGI and LGI breakfasts containing ≥2 g CHO·kg BM^{-1} (Stevenson et al., 2005; Wu et al., 2003). Although the majority of studies have used a 3 h postprandial period (Wu et al., 2003; Stevenson et al., 2009), a 10 h postprandial period also resulted in similar resting fat oxidation when obese women consumed a HGI or LGI breakfast and lunch (Díaz et al., 2005). It is, therefore, difficult to ascertain which factors contribute specifically to the higher fat oxidation following LGI breakfasts in some adult studies. It is possible that the 1.5 g CHO·kg BM^{-1} breakfast, 120 min postprandial period and 30 min exercise duration at 50% $V\dot{O}_{2peak}$ used in Chapter 6 was a sub-optimal combination to induce differences in fat oxidation between HGI and LGI. Furthermore, the glycaemic response does not necessarily predict the insulinaemic response (Pi Sunyer, 2002; see section 2.7.1) and the similar insulin concentrations between HGI and LGI might have underpinned the similar fat oxidation (Horowitz et al., 1997). Therefore, studies examining the mechanistic basis for these findings in young people are welcomed.

8.3.2 Acute exercise at Fatmax

The exacerbated blood glucose response to HGI, but not LGI, breakfast consumption in the OW girls was somewhat 'unexpected' (Chapter 6). This finding, coupled with the higher insulin resistance (HOMA-IR) in the OW girls, prompted a need for interventions to improve blood glucose control in this population, particularly following

HGI breakfast consumption. The author was aware that exercise training has been used as an effective tool to increase insulin sensitivity and fat oxidation in young people (Ben Ounis et al., 2009; Shaibi et al., 2008). However, it is also well documented that just a single exercise bout can induce similar metabolic improvements in adults (Burton et al., 2008; King et al., 1995). This suggested that acute exercise could be recommended to improve insulin and fat metabolism in young people, if these findings were mirrored in this population. Documenting these so-called 'instant' health benefits of exercise that are associated with each individual bout in addition to the well known long term benefits may increase the attractiveness of exercise for young people and are more relevant to those who rarely participate in exercise. Therefore, a combination of the previous findings within this thesis culminated in a study to examine the acute effect of a bout of Fatmax treadmill exercise performed ~16 h before HGI breakfast consumption on postprandial glucose, insulin and fat oxidation in overweight and non-overweight girls.

It was reported, for the first time, that a bout of Fatmax exercise reduced fasting and postprandial insulin concentrations in NO girls, with no change in glucose. Thus, the insulin-lowering effects of acute exercise reported previously in adults (Brestoff et al., 2009; Horowitz et al., 2005; Kokalas et al., 2005) also apply to NO girls. However, this improvement in metabolism depended on the weight status of the participant, as exercise did not affect insulin concentrations in the OW girls. Differences in exercise-induced changes in insulin between the groups may have been partly attributed to the lower energy expenditure per kg BM (all girls expended the same absolute amount of energy), the higher habitual post-intervention energy and CHO intake (Burton et al., 2008; Newsom et al., 2010) or a degree of 'metabolic inflexibility' (Aucouturier et al., 2011) in the OW girls. These issues require further investigation, since interventions to alleviate insulin resistance should be targeted at this population in particular. More encouragingly, however, Fatmax exercise enhanced fat oxidation the next morning in both the OW and NO girls, which appears to be another novel finding in young people and is consistent with studies in adults (Burton et al., 2008; Votruba et al., 2002). This suggests that there is great potential for attaining a state of negative fat balance up to 16 h after exercise. Furthermore, these exercise-induced metabolic benefits occurred following just a single exercise bout and despite the maintenance of habitual diet,

suggesting that they extend to a 'real world' setting where diet is not prescribed or manipulated.

8.4 Practical implications

The discussed research findings could have important practical implications for guiding interventions for weight management and metabolic health, for public health recommendations and could, ultimately have ramifications for the health of the paediatric population. The recommendations discussed within this section would be useful for practitioners and researchers and have been simplified to enable a greater understanding and their use in young people and for their parents directly.

Regarding exercise prescription, brisk walking or slow running (5.2 to 7.6 $km \cdot h^{-1}$) provides an effective means of promoting high rates of fat oxidation and, from a purely metabolic perspective, is preferable over cycling. It is, therefore, recommended that interventions aimed at promoting high rates of fat oxidation use treadmill rather than cycling exercise. However, a combination of these two modes of exercise could be more attractive to young people wishing to experience more variety. Importantly, interventions incorporating Fatmax exercise should estimate Fatmax on an individual basis prior to implementation, which can be achieved using a single 3 min incremental exercise protocol. When it is not possible to determine Fatmax specifically, prescribing exercise around 50 to 60% $V\dot{O}_{2peak}$ should promote high rates of fat oxidation (within the Fatmax zone) in most children. In the more practical sense, walking or jogging exercise at a heart rate of 75% of the age-predicted maximum that feels 'light to somewhat hard' (corresponding to an RPE of 12) can be recommended for young people wishing to exercise within their Fatmax zone. If cycling, a slightly lower intensity of 67% of the age-predicted maximum heart rate would be preferential to increase the likelihood of achieving the exercise mode-specific maximal rate of fat oxidation. Engagement in exercise at this intensity should be achievable for overweight or obese individuals who might lack confidence and physical competence for higher intensity exercise. Indeed, low to moderate intensity exercise is associated with a more comfortable level of exertion, increased likelihood of exercising over long periods of time, increased adherence and reduced likelihood of musculoskeletal injury, particularly when weight-bearing activity is recommended (Albright et al., 2000; Lazzer et al., 2011; Perri et al., 2002). Although Fatmax was higher for treadmill exercise, it still

occurred at a moderate intensity. Therefore, treadmill exercise at Fatmax should be feasible for overweight and obese young people, particularly since Fatmax is normally lower in this population (Chapter 7). These issues appear to be relevant since policies to make physical activity easier, safer, and more attractive have been advocated for young people (Frieden et al., 2010).

Regular breakfast consumption is currently recommended for a range of benefits related to health and cognitive function in young people (Lien, 2007; Song et al., 2006; Sandercock et al., 2010). Although LGI breakfasts improve postprandial blood glucose control in both OW and NO girls, this thesis has provided evidence of an added effect for OW girls; emphasis may, therefore, be put on the importance of LGI breakfasts for this population in particular. Although GI has a negligible effect on fat oxidation in the hours following breakfast consumption, LGI breakfast consumption does promote slightly higher rates of fat oxidation during subsequent exercise and may be preferred for this reason. Consequently, campaigns endorsing breakfast consumption should specifically promote the consumption of LGI breakfasts, and actively discourage the consumption of HGI breakfasts as part of a healthy diet for young people. Since the majority of cereals marketed to children are HGI (Schwartz et al., 2008), immediate and continued action is required. Importantly, ratings of palatability indicate LGI breakfast promotion for OW girls should be feasible (Chapter 6). Young people and their parents should be made aware that girls wishing to improve their glycaemic control and increase fat oxidation should consume LGI breakfasts; examples include porridge, muesli, brown bread, all bran, fruit, and, generally, unprocessed food products that contain ‘wholegrain’ and fibre. Moreover, OW girls in particular should avoid HGI foods, which are highly processed and contain refined grains; examples include Cornflakes, Coco pops and white bread. Importantly, parents should encourage the consumption of LGI foods and make these foods available within the home.

Exercise practitioners can now promote the metabolic benefits of acute exercise in young people, in addition to the long term benefits that many people are already aware of. Acute treadmill exercise at Fatmax appears to have a more dramatic effect than LGI breakfast consumption on reducing postprandial insulin responses in non-overweight girls and should be recommended for this purpose. Regardless of weight status, it is evident that acute exercise has a more profound effect than LGI breakfast consumption

on augmenting fat oxidation. It may also be prudent to recommend that steps should be taken to avoid dietary compensation in the post-exercise period. Importantly, these implications can be applied directly to girls (overweight and non-overweight), a population that should be targeted due to their low physical activity levels and reduced likelihood of consuming breakfast. Young people and their parents should be informed of these acute exercise-induced health benefits; lifestyle and health campaigns such as 'Change4Life' may serve as an effective means to relay these messages to the general public.

Overall, walking or slow running (Fatmax treadmill exercise) and LGI breakfast consumption may be best advocated in combination for maximising fat oxidation and improving postprandial blood glucose control in young people. These two simple lifestyle-related strategies may provide an effective, safe and attractive means for preventing and treating obesity, insulin resistance and related disorders. In support of this concept, one week of combined LGI diet and exercise induced favourable changes in lipid repartitioning within the muscle and increased insulin sensitivity in adults (Haus et al., 2011); thus the practical implications suggested from the findings within this thesis appear to be effective, at least in adults, and are relevant to current health-focused research. It is acknowledged that there is still much work to be done to alleviate obesity and associated metabolic disturbances in young people and the recommendations discussed within this section by no means provide a solution to this complex multi-factorial problem. Indeed, recommendations pertaining to exercise and diet may be effective, but changing long term individual behaviour is difficult (Frieden et al., 2010).

8.5 Recommendations for future research

The various themes and findings within this thesis have stimulated several new interesting research questions that, if addressed, would be invaluable in extending this research. Specific directions for future research that have emerged from the individual studies have been discussed throughout Chapters 4 to 7 and some are highlighted within this section.

Firstly, in a general context, it may be prudent to replicate some of the studies on a larger scale to increase the ability to extrapolate the findings to the wider population, as a number of the findings are novel and based on sample sizes of 20 to 30 participants.

This issue particularly pertains to between group comparisons, where the sub-group sample sizes were smaller. In this respect, there is clearly much work to be done regarding the effect of breakfast GI on metabolism in young people, including clarifying the effect of GI on fat oxidation and the impact of weight status. Similarly, studies employing more direct measures of fat oxidation (e.g., stable isotopes) and body composition (e.g., dual-energy X-ray absorptiometry) would also provide valuable information to extend and clarify some of the findings.

Regarding exercise prescription, this thesis has clearly broadened our understanding of the factors influencing fat oxidation during exercise in young people. However, there is scope for future studies comparing a wider variety of exercise modes, such as swimming, and examining the effect of exercise duration to reach consensus on the type, intensity, and duration of exercise required for maximising fat oxidation. This research should be conducted in both overweight and non-overweight children due to differences in Fatmax between these populations. Although there is evidence that training at Fatmax compared with other exercise intensities is preferable for augmenting fat oxidation and insulin sensitivity in obese men (Venables and Jeukendrup, 2008), research comparing the metabolic benefits of acute exercise or training at Fatmax with other exercise intensities in young people is not available or is fraught with methodological limitations (see section 2.5.5). This research is crucial in defining the merits of exercising at Fatmax specifically.

There is much scope to further research the interaction between GI, blood glucose and weight status. A combination of the findings from Chapters 6 and 7 suggest that it would be interesting to examine whether fitness and breakfast size influence the blood glucose response to HGI breakfast consumption in OW girls. Building on the issues raised in Chapter 7 and owing to the importance of the interplay between exercise and diet in weight management, the role of dietary ‘compensation’ in potentially attenuating exercise-induced improvements in metabolism should be addressed in young people. This can be achieved by prescribing diet to either maintain or abolish the exercise-induced energy or CHO deficit. Importantly, a systematic examination of whether exercise induces dietary compensation in overweight and non-overweight young people would have meaningful implications from a metabolic viewpoint and for weight management. Finally, studies seeking to provide a mechanistic understanding of the

observed findings would be welcomed, although the invasive measures of many of the methods employed for this purpose may preclude such studies in young people. In particular, it would be interesting to examine the acute effect of Fatmax exercise on, not only fat oxidation and insulin resistance, but also IMCL content and fatty acid metabolites within skeletal muscle in young people; nuclear magnetic resonance spectroscopy may provide a useful tool for such investigations.

The work within this thesis has provided a meaningful contribution to the existing literature and novel insights that will be useful in guiding future work. It is hoped that the directions for future research will serve as an important basis from which to extend and explore the findings in more detail, broaden our understanding of exercise metabolism and, ultimately, impact upon the long term future health of young people.

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HEALTH SCREEN QUESTIONNAIRE FOR STUDY VOLUNTEERS

Name/Number .....…….

- 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 his/her 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 thinks that she may have one, please do not allow her to 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 s/he is:

(a)	on medication, prescribed or otherwise.....	Yes		No	
(b)	attending her/his 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 her/him to:

(a)	consult her/his 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/coordination	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 or dairy products ..... 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, please describe briefly if you wish (e.g., to confirm problem was/is short-lived, insignificant or well controlled.)

5. Additional questions for female participants

(a) are your daughter's periods normal/regular? ..... Yes ☐ No ☐ Not started ☐

(b) is your daughter on "the pill"? ..... Yes ☐ No ☐ Not known ☐

(c) could your daughter be pregnant? ..... Yes ☐ No ☐ Not known ☐

6. Please provide your contact details

Name: ……

Telephone Numbers: ……

Work ☐ Home ☐ Mobile ☐

Relationship to participant:……..

Daughter's full name:……

7. Is your daughter currently involved in any other research studies at the University?

Yes ☐ No ☐

If yes, please provide details of the study

PUBIC HAIR DEVELOPMENT - MATURITY RATINGS

THE DRAWINGS 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 DRAWING AND READ THE SENTENCES UNDER THE DRAWINGS. THEN CHOOSE THE DRAWING CLOSEST TO YOUR STAGE OF HAIR DEVELOPMENT AND MARK IT 1. THEN CHOOSE THE DRAWING THAT IS NEXT CLOSEST AND MARK IT 2.

1. DRAWING A

THERE IS NO PUBIC HAIR.

2. DRAWING B

THERE IS A LITTLE LONG, LIGHTLY COLORED HAIR. THIS HAIR MAY BE STRAIGHT OR A LITTLE CURLY.

3. DRAWING C

THE HAIR IS DARKER IN THIS STAGE. IT IS COARSER AND MORE CURLED. IT HAS SPREAD OUT AND THINLY COLVERS A LARGER AREA.

4. DRAWING D

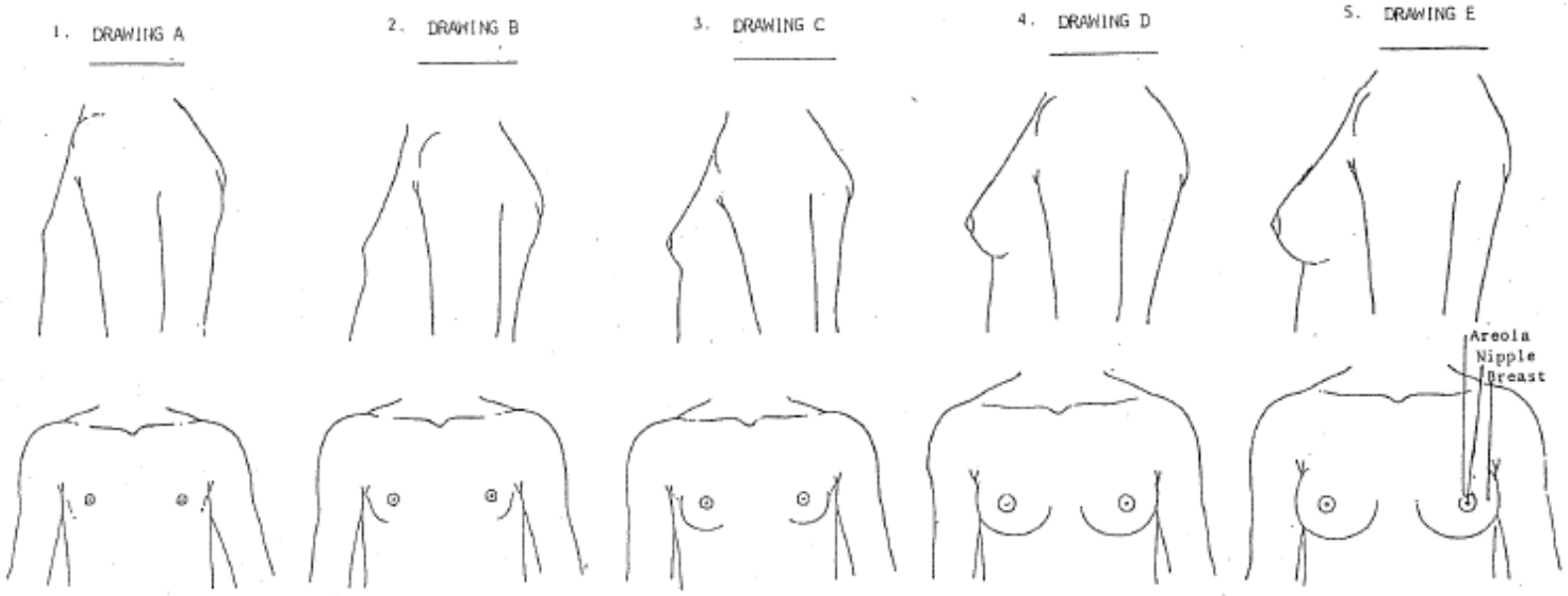
THE HAIR IS NOW AS DARK, CURLY, AND COARSE AS THAT OF AN ADULT FEMALE. HOWEVER, THE AREA THAT THE HAIR COVERS IS NOT AS LARGE AS THAT OF AN ADULT FEMALE. THE HAIR HAS NOT SPREAD OUT TO THE THIGHS.

5. DRAWING E

THE HAIR NOW IS LIKE THAT OF AN ADULT FEMALE. IT ALSO COVERS THE SAME AREA AS THAT OF THE ADULT FEMALE. THE HAIR USUALLY FORMS A TRIANGULAR (▽) PATTERN AS IT SPREADS OUT TO THE THIGHS.

BREAST DEVELOPMENT - MATURITY RATINGS

THE DRAWINGS ON THIS PAGE SHOW DIFFERENT STAGES OF DEVELOPMENT OF THE BREASTS. A FEMALE PASSES THROUGH EACH OF THE FIVE STAGES SHOWN BY THESE SETS OF DRAWINGS. PLEASE LOOK AT EACH SET OF DRAWINGS AND READ THE SENTENCES UNDER THE DRAWING. THEN CHOOSE THE SET OF DRAWINGS CLOSEST TO YOUR STAGE OF BREAST DEVELOPMENT AND MARK IT 1. THEN CHOOSE THE DRAWING THAT IS THE NEXT CLOSEST AND MARK IT 2.



THE NIPPLE IS RAISED A LITTLE IN THIS STAGE. THE REST OF THE BREAST IS STILL FLAT.

THIS IS THE BREAST BUD STAGE. IN THIS STAGE THE NIPPLE IS RAISED MORE THAN IN STAGE 1. THE BREAST IS A SMALL MOUND. THE AREOLA IS LARGER THAN IN STAGE 1.

THE AREOLA AND THE BREAST ARE BOTH LARGER THAN IN STAGE 2. THE AREOLA DOES NOT STICK OUT AWAY FROM THE BREAST.

THE AREOLA AND THE NIPPLE MAKE UP A MOUND THAT STICKS UP ABOVE THE SHAPE OF THE BREAST. (NOTE: THIS STAGE MAY NOT HAPPEN AT ALL FOR SOME GIRLS. SOME GIRLS DEVELOP FROM STAGE 3 TO STAGE 5, WITH NO STAGE 4.)

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.

GENITAL DEVELOPMENT – TESTES, SCROTUM, AND PENIS (MATURITY RATINGS)

THE DRAWINGS OF THIS PAGE SHOW DIFFERENT STAGES OF DEVELOPMENT OF THE TESTES, SCROTUM, AND PENIS. A BOY PASSES THROUGH EACH OF THE FIVE STAGES SHOWN BY THESE DRAWINGS. PLEASE LOOK AT EACH OF THE DRAWINGS AND READ THE SENTENCES UNDER THE DRAWING, THEN CHOOSE THE DRAWING CLOSEST TO YOUR STAGE OF DEVELOPMENT. MARK A "1" ON THE LINE ABOVE THAT DRAWING. THEN CHOOSE THE DRAWING THAT IS NEXT CLOSEST TO YOUR STAGE OF DEVELOPMENT AND MARK IT "2". IN CHOOSING THE RIGHT PICTURE, LOOK ONLY AT THE STAGE OF DEVELOPMENT, NOT AT PUBIC HAIR.

1. DRAWING A

THE TESTES, SCROTUM, AND PENIS ARE ABOUT THE SAME SIZE AND SHAPE AS THEY WERE WHEN YOU WERE A CHILD.

2. DRAWING B

THE TESTES AND SCROTUM HAVE GOTTEN A LITTLE LARGER, THE SKIN OF THE SCROTUM HAS CHANGED, THE SCROTUM, THE SACK HOLDING THE TESTES, HAS LOWERED A BIT. THE PENIS HAS GOTTEN ONLY A LITTLE LARGER.

3. DRAWING C

THE PENIS HAS GROWN MAINLY IN LENGTH. THE TESTES AND SCROTUM HAVE GROWN AND DROPPED LOWER THAN IN STAGE 2.

4. DRAWING D

THE PENIS HAS GROWN EVEN LARGER, IT IS WIDER. THE GLANS (THE HEAD OF THE PENIS) IS BIGGER, THE SCROTUM IS DARKER THAN BEFORE. IT IS BIGGER BECAUSE THE TESTES HAVE GOTTEN BIGGER.

5. DRAWING E

PENIS
SCROTUM
GLANS (HEAD)
TESTES

THE PENIS, SCROTUM, AND TESTES ARE THE SIZE AND SHAPE OF THAT OF AN ADULT MALE.

PUBIC HAIR DEVELOPMENT (MATURITY RATINGS)

THE DRAWINGS 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 DRAWING AND READ THE SENTENCES UNDER THE DRAWING. THEN CHOOSE THE DRAWING CLOSEST TO YOUR STAGE OF YOUR HAIR DEVELOPMENT. MARK A 1 ON THE LINE ABOVE THAT DRAWING. THEN CHOOSE THE DRAWING THAT IS NEXT CLOSEST TO YOUR STAGE OF HAIR DEVELOPMENT AND MARK IT A 2. IN CHOOSING THE RIGHT PICTURE, LOOK ONLY AT THE PUBIC HAIR, AND NOT AT THE SIZE OF THE TESTES, SCROTUM, AND PENIS.

1. DRAWING A

THERE IS NO PUBIC HAIR AT ALL.

2. DRAWING B

THERE IS A LITTLE SOFT, LONG, LIGHTLY COLORED HAIR, MOST OF THE HAIR IS AT THE BASE OF THE PENIS. THIS HAIR MAY BE STRAIGHT OR A LITTLE CURLY.

3. DRAWING C

THE HAIR IS DARKER IN THIS STAGE. IT IS COARSER AND MORE CURLED. IT HAS SPREAD OUT AND THINLY COVERS A SOMEWHAT LARGER AREA.

4. DRAWING D

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.

5. DRAWING E

THE HAIR HAS SPREAD OUT TO THE THIGHS. THE HAIR IS NOW LIKE THAT OF AN ADULT MALE. IT COVERS THE SAME AREA AS THAT OF AN ADULT MALE.

Visual Analogue Scale

Please indicate how hungry you are now by circling a relevant number

Not Hungry Fairly Hungry Hungry Very Hungry

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Place a mark on the horizontal lines below after considering the following questions:

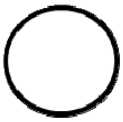
How hungry do you feel?

I am not hungry at all

I have never been more hungry

How full do you feel?

Not at all full



Totally full

How much do you think you could eat?

A lot

How much did you like your breakfast?

Not at all

A lot

Temperature (°C)	Humidity (%)

Time of day (hours)	Description of type of food or drink and cooking method (e.g. boiled potatoes, canned sweetcorn)	Amount/ Portion (e.g. 20 g, 1 packet, 1 small portion)
07.00 – 08.00		
08.00 – 09.00		
09.00 – 10.00		
10.00 – 11.00		
11.00 – 12.00		
12.00 – 13.00		
13.00 – 14.00		
14.00 – 15.00		
15.00 – 16.00		
16.00 – 17.00		
17.00 – 18.00		
18.00 – 19.00		
19.00 – 20.00		
20.00 – 21.00		

*Please fill in the form the day before your 1st morning visit and eat/ drink what you have written above (at similar times the day) before your 2nd morning visit. Thank you!

FOOD DIARY

Please record everything you eat and drink for **1 day before and on the day of your 2nd visit to the University (i.e., 2 full days)**. You can then copy the information that you record in an effort to eat and drink identical amounts of the same food and drink the days before and on your subsequent visits to the University – please see your study schedule.

INSTRUCTIONS FOR USING THE FOOD DIARY AND SCALES

- **Everything** that you eat and drink over the course of the day should be weighed and the weight and type of food or drink recorded.
- Record **each food item** (e.g., bread, carrots) on a **separate line**.
- To weigh food...switch scales on and check the display is working

1. Put empty plate (or whatever container you are eating/drinking from) on the scales then press ZERO on the scales (the display should read zero)
2. Add the first food item to the plate (e.g., potatoes) and record the weight of the food
3. ZERO the scales again
4. Add next food item (e.g., peas) to the plate
5. ZERO the scales again... repeat until all separate food items are weighed and recorded
6. If you leave any food, try to estimate how much you leave and write the proportion in your diary (e.g., left ¼ of peas, ½ of apple, etc..).

- Do not forget to weigh and record second helpings and snacks eaten between meals.
- **Leftovers** (e.g., apple cores) should be weighed and recorded in the leftovers column.
- **Eating Out** – Most people eat foods away from home each day, please do not forget to record these. Take your diary and scales with you wherever it is possible. If this is too inconvenient just record the type of food eaten with an estimated weight (**guess** the weight/how much you ate) – but please write in your diary when a weight has been **estimated rather than weighed**.
- Most snack foods will have the weight of the food **on the packet** so they do not need weighing if you eat the whole item (e.g., packet of crisps) – you just need to remember to write the weight down from the packet.

We understand that accurately recording your food and drink intake requires time and effort and there may be occasions where weighing all food/drink is too difficult. However, please avoid just missing things out or simply making it up! If you are unable to weigh the food/drink that you consume, please indicate this on the food diary and express the amount in a different way (e.g. 1 slice of bread instead of 30 g of bread). This information is important for understanding our results from the study. Thank you!

DAY 1 – day before visit 2

The times of day in the table below are just there to help prompt you – it does not matter if you need to use some of the space that does not correspond to when you actually ate or drank something.

Time of day (hours)	**Brand name** of food/drink (e.g. Heinz, Kelloggs)	**Detailed** description of food/drink and cooking method (e.g. boiled potatoes, canned sweetcorn, bacon fried in butter/olive oil)	**Weight** served (grams)	Did you leave any? Weight of leftovers (grams)
06.00 – 9.00				
9.00 – 12.00				
12.00 – 15.00				

15.00 – 18.00				
18.00 – 21.00				

DAY 2 – day of visit 2

The times of day in the table below are just there to help prompt you – it does not matter if you need to use some of the space that does not correspond to when you actually ate or drank something.

Time of day (hours)	**Brand name** of food/drink (e.g. Heinz, Kelloggs)	**Detailed** description of food/drink and cooking method (e.g. boiled potatoes, canned sweetcorn, bacon fried in butter/olive oil)	**Weight** served (grams)	Did you leave any? Weight of leftovers (grams)
06.00 – 9.00				
9.00 – 12.00				
12.00 – 15.00				

VISIT 2 AFTER SCHOOL TODAY!

You do not need to record your food/drink the evening after visit 2, as there is no morning trial after visit 2 (there are only morning trials for visits 3 and 4).

Please record what you eat and drink after visit 3 below...this is the most important part of the food diary!

18.00 – 21.00				
		*		