METABOLIC RESPONSES TO FEEDING AND EXERCISE IN MEN AND WOMEN

By

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ABSTRACT

Nutrition and physical activity are the main strategies to improve metabolic health among individuals who are obese. However, nutrition (e.g. energy intake reduction) and physical activity are often considered independently rather than interactively; hence, these strategies are not always effective. The purpose of this thesis was to investigate how nutrition and physical activity interact to influence metabolic relevant factors in obesity. In a first study it was observed, in a group of obese or overweight/centrally obese men, that an acute bout of aerobic exercise performed in the overnight-fasted state substantially increased whole-body exercise fat oxidation and Type I fibre intramyocellular triacylglycerol (IMTG) utilisation as compared to exercise performed in the fed state. In a second study, it was demonstrated that, like men, obese or overweight/centrally obese women responded to overnight-fasted vs. fed state exercise by considerably increased whole-body fat oxidation during exercise. Additionally, consuming breakfast after exercise as compared to before exercise suppressed appetite sensations and decreased subsequent energy intake at a later *ad libitum* style lunch. Comparisons between the men and women provided initial evidence that the magnitude of change in substrate utilisation with pre-exercise feeding was greater in women as compared to men. In a final study, a sex-difference in the response to feeding was confirmed, with women exhibiting an earlier metabolic response to glucose feeding and quicker return to baseline than men. In conclusion, the findings from this thesis have developed new insights in the understanding of the impact of feeding on substrate utilisation during exercise and the influences of biological sex on metabolic

responses to feeding. This understanding may have practical implications on current practice, for example in terms of guiding future experimental research whereby sex-differences should be accounted in the metabolic research. The findings also can be applied in promoting healthy lifestyle behaviours and informing public health policy.

"This work is dedicated to my children; Airaa, Aathif and Afyaa whom had made me stronger, better and more fulfilled than I could have ever imagined - I love you to the moon and back"

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PUBLICATIONS AND COMMUNICATIONS

During the period of postgraduate study, the findings of some of the studies have been published as follows:

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ABBREVIATIONS

ADP	adenosine diphosphate
ATP	adenosine triphosphate
AUC	area under the curve
BCAA	branched-chain amino acids
BF	body fat
BMI	body mass index
CO_2	carbon dioxide
CoA	coenzyme A
CV	co-efficient of variation
DAG	diacylglycerol
DEE	daily energy expenditure
dH_2O	distilled water
ECG	electrocardiogram
EDTA	ethylenediamine tetra-acetate
ELISA	enzyme linked-immuno-sorbent assay
FAT	fatty acid transporters
FM	fat mass
GHQ	general health questionnaire
GIP	gastric inhibitory polypeptide
GLP-1	glucagon-like peptide-1
GLUT4	glucose transporter 4
HCl	hydrogen chloride

HFLC	high fat low carbohydrate
HSL	hormone-sensitive lipase
IMTG	intramuscular triglyceride
IPAQ-SF	International Physical Activity Questionnaire – Short Form
ISAK	International Society for the Advancement of Kinanthropometry
LBM	lean body mass
LCFA-CoA	long chain fatty acyl-CoA
MET	metabolic equivalent
MHCI	myosin heavy chain I
NaOH	sodium hydroxide
NEFA	non-esterified fatty acids
O_2	oxygen
OGTT	oral glucose tolerance test
PAL	physical activity level
PBS	phosphate-buffered saline
PDC	pyruvate dehydrogenase complex
Pi	inorganic phosphorous
Ra	rate of appearance
RER	respiratory exchange ratio
RMR	resting metabolic rate
RPE	rating of perceived exertion
rev/min	revolutions per minute
RQ	respiratory quotient
SD	standard deviation

SE	standard error
SPSS	statistical package for the social sciences
TG	triglyceride
TCA cycle	tricarboxylic acid cycle
VAS	visual analogue scale
vs	versus
VLDL	very-low-density-lipoprotein
$\dot{V}CO_2$	carbon dioxide production
$\dot{V}O_2$	oxygen consumption
$\dot{V}O_{2max}$	maximal aerobic capacity
$\dot{V}O_{2peak}$	peak aerobic capacity
W	watt
β	beta

THESIS OUTLINE

This thesis explores the metabolic responses to feeding and exercise in both men and women. The thesis begins with a General Introduction (**Chapter 1**) to the topic areas pertinent to the overall thesis followed by sections that discuss in more detail key areas in the current literature that warranted further investigation. **Chapter 2**, the first experimental chapter of the thesis, presents a randomized and crossover study that was conducted to investigate the impact of exercise performed in the overnight fasted vs. fed state on whole-body substrate utilisation, intramuscular triglyceride (IMTG) use and blood metabolic responses among overweight and obese men. The conclusions drawn from **Chapter 2** informed the design of the study described in **Chapter 3**, which presents the second experimental chapter of the thesis. This study sought to investigate metabolic responses to overnight-fasted vs. fed state exercise in overweight and obese women with addition of investigating the impact of feeding status around exercise on subsequent energy intake at an *ad libitum* lunch. The finding from **Chapter 3** established the appearance of a potential sex-difference in the metabolic response to feeding and exercise, which informed the focus of the final experimental chapter of this thesis, **Chapter 4**. This chapter was designed to investigate the impact of biological sex on metabolic responses after acute carbohydrate (glucose) feeding among men and women, pair-matched on a number of relevant criteria. The thesis ends with a General Discussion and Conclusion (**Chapter 5**) which discusses the new contributions made in light of the aims and objectives of the thesis, as well as their relevance, limitations and implications for future research.

CHAPTER 1

General Introduction

1.0 Introduction

Overweight and obesity (defined typically as body mass index [BMI] of 25-29.9 kg/m^2 and ≥30 kg/m^2, respectively) has been considered as one of the emerging public health concern of the 21st century (WHO, 2000) due to the rising prevalence in the United Kingdom and other parts of the world (Rennie and Jebb, 2005, Stevens et al., 2012). It is estimated that by the year of 2025 more than 4 million people in the UK will be diagnosed with diabetes due to rapidly rising numbers of overweight and obese individuals and the increasing ageing population (Diabetes UK 2010). Overweight and obesity has been associated with increased risk for a number of non-communicable diseases, such as type 2 diabetes mellitus, hypertension, dyslipidaemias, atherosclerosis and cardiovascular disease and also reduced life expectancy (Must et al., 1999, Grundy, 2004, Olshansky et al., 2005, Grundy, 2008, Lim et al., 2012). In addition, the fat accumulation in the body stored within adipose tissues and non-adipose tissue such as the liver and skeletal muscle seen in obesity is strongly associated with insulin resistance (Goodpaster et al., 2000), which is another major independent risk factor for type 2 diabetes and cardiovascular disease. Insulin resistance can be defined as a failure of target tissues to increase whole body glucose disposal in response to insulin. The tissue responsible, especially fat and skeletal muscle, exhibit reduced insulin-stimulated glucose uptake

and metabolism. Hence, a concerted research effort needs to be explored in order to prevent the increasing prevalence of overweight and obesity and also to reduce the insulin resistance that is induced by the accumulation of body fat.

Traditionally, regular physical activity or exercise is often recommended as a strategy in the prevention and management of obesity (Blomqvist and Saltin, 1983, Holloszy and Coyle, 1984) and its related consequences including insulin resistance (Shaw et al., 2010). Regular physical activity is widely recognized as playing a large role in the regulation of energy balance by increasing energy expenditure and stimulating lipid metabolism to manage body fat levels and possibly ectopic fat. However, exercise training is not always effective for weight loss, or insulin sensitivity improvement (King et al., 2008, Coker et al., 2009).

Nutrition or diet is often considered independently from exercise for the management of obesity (e.g. energy intake reduction) but not as something that interacts with exercise with the potential to modulate the benefits of exercise training. However, even diet alone, particularly restricting energy intake, is generally unsuccessful for the long-term; with more than 90% of obese individuals regaining lost body fat within two years after following a weight reduction diet of at least two months followed by a free-living eating pattern (Vogels et al., 2005). Hence, overnight-fasted exercise has been extensively researched among healthy active young lean adults, as reviewed recently (Vieira et al., 2016, Wallis and Gonzalez, 2019). Fat oxidation during exercise is higher in an overnight-fasted state compared to exercise in the fed state, clearly showing that diet can interact with exercise

responses that are relevant in the context of obesity, such as stimulating fat metabolism. The impact of such interactions are therefore important to investigate in obesity as they may provide insights into ways to optimise with the combination of physical activity and nutrition for improvements in metabolic health.

Additionally, research in nutritional influences on exercise metabolism has been carried out mostly in men with women often neglected and findings in men often extrapolated to women. Yet, it has been known for many years that there are sex-differences in some aspects of metabolism (Tarnopolsky, 2000) due to their anatomy and physiology differences. For instance, compared to men, women oxidise a greater proportion of fat to carbohydrate during exercise (Tarnopolsky et al., 1990, Tarnopolsky et al., 1995). This is mainly because women has greater circulating concentration of the hormone estrogen, whereby this hormone shown to enhance whole body fat oxidation during exercise (Maher et al., 2010), and regulate the transcription of genes involved with IMTAG storage which can then further modulate substrate oxidation (Fu et al., 2009). Therefore, it is important to study both men and women in order to understand if there is a need for sex-specific approaches in treatment strategy or even in exercise training.

This chapter critically appraises relevant literature related to fuel metabolism at rest and during exercise with a particular focus on the influences and interactions of diet and biological sex, in the context of overweight, obesity and metabolic health. In doing this, key knowledge gaps in the field are highlighted that underpin the need

for new research, some of which has been undertaken during the course of this doctoral research and are presented in this thesis.

1.1 Fuel metabolism at rest and during exercise

1.1.1 Rest

Carbohydrate and fat are the main sources of fuel substrate for oxidative phosphorylation and energy production (adenosine triphosphate [ATP]) (Hargreaves, 2012, Spriet, 2012). On the other hand, the quantitative contribution of protein to energy expenditure is usually less than 1% and not more than 10% even during extreme conditions such as prolonged exercise in fasted conditions (Wagenmakers et al., 1989, Wagenmakers, 1991).

Carbohydrate is stored largely in skeletal muscles and the liver in the form of glycogen, with smaller amounts circulating freely in the blood stream as blood glucose (Wasserman, 2009). The carbohydrate storage capacity is small, with the liver typically containing about 80–100g of glycogen in the post-absorptive state, whereas muscle glycogen can vary from 50 g after strenuous exercise to 900 g in a well-fed, well-trained, muscular person (Jeukendrup et al., 1998). Meanwhile, fat is stored in the form of triacylglycerol [TG] and predominantly stored in adipose tissue. A small amount is stored as IMTG, plasma non-esterified fatty acid [NEFA] bound to albumin and plasma TG circulating freely or incorporated in circulating lipoprotein particles (Jeukendrup et al., 1999, Lambert and Parks, 2012). The IMTG content

makes up only a fraction (~1% to 2%) of the total fat stores within the body (van Loon, 2004). The fat store is dynamic, which means the depot can increase in size when there is the case of positive fat balance and decrease in a negative fat balance situation.

When energy demand is increased, fuel substrates will be mobilized from their body stores and utilized mainly within skeletal muscle. For carbohydrate, within the muscle cell, glucose or glycogen is first converted to glucose-6-phosphate and then to pyruvate via glycolysis. Pyruvate then enters into mitochondria and then pyruvate dehydrogenase complex [PDC] converts pyruvate to acetyl-coenzyme A [CoA]. Meanwhile, fat stores in the form of TG are first hydrolysed to glycerol and NEFAs. NEFAs are subsequently activated to fatty acyl-CoA and transported across the mitochondrial membrane by a carnitine dependent transport system. Inside the mitochondria, fatty acyl-CoA is cleaved in a stepwise fashion to produce acetyl-CoA via β-oxidation. From this point on, fat and carbohydrate metabolism follow the same biochemical pathways. Acetyl-CoA enters the citric acid cycle [TCA-cycle] to proceed through a series of biochemical reactions coupled to the electron transport system. The electrochemical energy generated is used to couple adenosine diphosphate [ADP] and inorganic phosphorous [Pi] to form ATP (Hargreaves, 2012, Spriet, 2012). ATP is the high-energy molecule that releases energy through hydrolysis to support energy-consuming processes such as muscular contraction. However, the cellular level of ATP storage is very small and must be constantly re-synthesized in an energy consuming process; thus, the process of metabolism occurs continuously to support this.

The utilisation of fuel substrates can be measured using indirect calorimetry that quantifies volumes of gas exchange (carbon dioxide [CO_2] production [$\dot{V}CO_2$], oxygen [O_2] consumption [$\dot{V}O_2$]), which allows the respiratory quotient ([RQ] also referred to as the Respiratory Exchange Ratio [RER]) to be calculated (RQ = O_2 consumption / CO_2 production). When the RQ is 0.70, it is indicating an exclusive reliance on fat for fuel, whereas RQ of 1.00 indicates that glucose is the only energy source. For instance, the average resting RQ is 0.82; thus reflects that the human body derives more than half of its energy from fatty acids and the rest of it is from glucose (Arner et al., 1990).

At rest, the main fuel source for energy requirement for basal metabolic process is from fat oxidation, predominantly supplied through the oxidation of circulating free fatty acids derived from lipolysis of adipose tissue TG, and small contribution from other fat sources such as IMTG and lipoprotein-derived TG (van Loon et al., 2001, van Loon et al., 2003). On the contrary, carbohydrate only makes a relatively small contribution, which for utilization by glucose-requiring tissues such as the brain (Owen et al., 1967). As hepatic glycogen are limited during fasting period, most glucose production during rest is contributed from gluconeogenesis. The rate of gluconeogenesis during resting conditions has been reported to be dependent on mobilization and delivery of gluconeogenic substrate as well as the conversion efficiency within the liver (Jahoor et al., 1990, Jenssen et al., 1990).

1.1.2 Exercise

During exercise, energy requirement increases much greater than the resting state hence the metabolic rate. In order to accommodate the increase of energy demand during exercise, fuel substrates are oxidised through two pathways, which are the extracellular and intracellular substrate to supply energy (van Hall, 2015). The extracellular energy supply during exercise is mediated via the blood substrate delivery of glucose from either carbohydrate intake or liver glycogenolysis and gluconeogenesis and plasma NEFA mainly from lipolysis of TAG derived from adipose tissue, and chylomicron or very-low-density lipoproteins (VLDL). The increase in delivery of these substrates to the active muscle is mediated by an increase in blood flow (van Hall et al., 2002), including an increase in capillary recruitment (Calbet et al., 2005), and substrate concentration (Ahlborg et al., 1974). Transport of blood glucose into skeletal muscle is facilitated by glucose transporter-4 (GLUT4), whereas the long-chain NEFAs via fatty acid transporters (FAT), which also facilitates the transport of NEFA into the mitochondria.

The intracellular energy supply during exercise is mainly via a fast breakdown of glycogen to cover the rapidly many fold increase in energy demand from rest to exercise. It is well recognised that muscle glycogen represents a significant fuel source for sustained moderate- to high-intensity exercise contributing to more than 50% of total energy requirements (Tsintzas and Williams, 1998). This might be due to the ability of carbohydrate oxidation to yield greater ATP re-synthesis rate per unit of oxygen delivered to the mitochondria, when compared with fat, thereby making it a logical choice for skeletal muscle to use given the effect of improved gross efficiency (Krough and Lindhard, 1920). Furthermore, carbohydrate can support

energy production in both anaerobic and aerobic exercise, even when working at very high exercise intensities carbohydrate is still able to derive energy through oxidative phosphorylation (van Loon et al., 2001). However, the rate of glycogen breakdown decreases with exercise duration, in which the primary contribution shifts to fatty acid oxidation. The intracellular store of fat as a fuel source is derived from IMTG. During exercise, the breakdown of IMTG is completed by hormone-sensitive lipase [HSL] that is regulated by intra-muscular (contraction of muscle) and extra-muscular (adrenaline) factors (Langfort et al., 1999, Langfort et al., 2000). The increase in NEFA availability from IMTG breakdown during exercise is facilitated by a reduction in NEFA re-esterification and possibly an increase in IMTG lipolysis (van Loon et al., 2001, van Hall, 2015).

1.1.3 Interaction of carbohydrate and fat oxidation

Carbohydrate and fat are oxidised simultaneously, but there can be reciprocal shifts in the proportion of carbohydrate and fat that are oxidised once the metabolic demand and/or when steady state is established (Coyle et al., 1997, Holloway and Spriet, 2012). The interaction between carbohydrate and fatty acid oxidation during exercise is dependent on factors such as availability of substrate, both from inside (storage) and outside (blood circulation) of the muscle and exercise intensity and duration (Bergman and Brooks, 1999, Coyle et al., 2001, Romijn et al., 1993, van Loon et al., 2001).

Brooks and Mercier (1994) have described the relative contribution of fat and carbohydrate to total energy expenditure based on exercise intensity as the crossover concept. The concept explained that the contribution of fat to energy expenditure is high during rest (~60%) and low intensity exercise but declines to <30% at moderate exercise intensity (50-65% of maximal aerobic capacity [$\dot{V}O_{2max}$]) and further declines (<10% contribution) more when exercise intensity is higher than 65% $\dot{V}O_{2max}$ (Achten et al., 2002, Achten et al., 2003). In contrast, the relative contribution of carbohydrate as a primary fuel following the opposite pattern. Muscle glycogen and plasma glucose represents a significant fuel source for sustained moderate- to high-intensity exercise and the rate of glycogenolysis is most rapid during the first 5-10 minutes of exercise (Romijn et al., 1993, van Loon et al., 2001).

Meanwhile, during low-intensity exercise, e.g., 25% $\dot{V}O_{2max}$, it is assumed that plasma NEFA are almost the exclusive fat source as a fuel based on the very close matching between the rate of fat oxidation and the rate at which NEFA molecules disappear from the blood (Romijn et al., 1993). NEFA is breakdown from TAG in adipose tissue via lipolysis and the process is mostly dependent on β-adrenergic stimulation and the endocrine environment, in which adrenaline (‘epinephrine’) stimulates lipolysis and insulin inhibits lipolysis. When exercise is commenced, adrenaline concentrations increase and insulin concentrations decrease, thus the rate of lipolysis increase hence more fatty acids are released from the adipose tissue (Jeukendrup, 2003). During transition into moderate-intensity exercise, lipolysis increases approximately 3-fold (Wolfe et al., 1990). The blood flow to adipose tissue is doubled with the rate of re-esterification halved (Wolfe et al., 1990, Romijn et al.,

1993), as well the blood flow in skeletal muscle increases dramatically (van Hall et al., 2002) and therefore the delivery of fatty acid to the muscle is increased several-fold. Nevertheless, the overall contribution of plasma sources remaining constant but with a decline in plasma NEFA contribution and an increase in glucose at higher intensities (Romijn et al., 1993).

The stable isotope tracer technique is another method that widely used to quantify substrate oxidation and/or kinetics in more detail than that gained from using indirect calorimetry and measuring plasma metabolite concentrations alone. This technique involves continuous infusion of different stable isotope tracers such that the metabolism specific substrates can be 'traced' during different physiological contexts (e.g. [U-^{13}C]palmitate for plasma free fatty acid (FFA) kinetics or oxidation and [6,6-$^{2}H_2$]glucose for plasma glucose kinetics). Several isotope tracers studies in active men following an overnight fast have shown that, during moderate-intensity exercise, almost 60% of total fat oxidation is provided by plasma derived free fatty acid oxidation (Romijn et al. 1993; Sidossis et al. 1998; Coyle et al. 2001; van Loon et al. 2001). Hence, it suggests that non-plasma fat sources including IMTG and lipoprotein-TG may contribute substantially to total fat oxidation during moderate-intensity exercise. Indeed, previous work that applied both stable isotope and fluorescence microscopy methodologies concluded that IMTG stores provide an important substrate source during moderate intensity (van Loon et al., 2003). In fact, when exercise intensity increased (>75% $\dot{V}O_{2max}$), fat oxidation rate was decreased and it involved a significant decline in the oxidation rate from both plasma and non-plasma free fatty acids (van Loon et al., 2001). Collectively, plasma free fatty acid

oxidation rates substantially increased during exercise, whereas the rate of muscle- and lipoprotein-derived TG oxidation declined during the second hour of exercise. This is might be explained by the progressive increase in peripheral lipolytic rate and subsequent increase in plasma free fatty acids concentrations can suppress IMTG hydrolysis and its subsequent rate of oxidation.

At the onset of exercise, liver glycogen breakdown is also increased and its output increases in line with exercise intensity (Felig and Wahren, 1975). Glycogenolysis of liver glycogen will release glucose that will be transported to muscle to be oxidised along with glucose-1-phosphate derived from muscle glycogen. Meanwhile, when exercise intensity reaches more than 72% $\dot{V}O_{2max}$, the contribution of muscle glycogen increases almost exponentially, by supplying over half (58%) of the requirements compared to 35% at low-moderate intensities (Romijn et al., 1993, van Loon et al., 2001), whereas plasma glucose typically contributes up to about 1 g/min. In summary, carbohydrate oxidation gradually increases with exercise intensity, while fat oxidation increases from low to moderate exercise intensities and then decreases from moderate to high exercise intensities.

Other than that, the pattern of substrate utilization also changes with exercise duration, even when the exercise intensity remains constant. It has been demonstrated that the longer duration of exercise, the higher contribution of fat as an energy substrate (Romijn et al., 1993). Generally, during low intensity exercise lasting longer than 2 hours, the substrate utilization is not significantly altered as compared to those utilized during shorter bouts of low intensity exercise. However,

when the moderate intensity exercise performed in prolonged duration, the carbohydrate oxidation is reduced in overall, mostly due to the progressive depletion of muscle glycogen. Previous work demonstrates that the prominent use of muscle glycogen is only during the first 120 minutes of moderate intensity exercise (Watt et al., 2002b). Once the glycogen stores become depleted, the maintenance of carbohydrate oxidation is met through an increased reliance on hepatic glucose output. However, blood glucose only can supply limited carbohydrate energy and is unable to support the higher demand rate of energy production; thus, the fuel source shift to fat oxidation.

As the duration of exercise with constant intensity (steady state) is extended, the contribution of fat to energy expenditure is increased that supplied by both IMTG and plasma NEFA, with the latter becoming a more important substrate as exercise continues (Turcotte et al., 1992, van Hall et al., 2002, Watt et al., 2002a, Watt et al., 2002b, van Loon et al., 2003). Meanwhile, the contribution of IMTG to energy expenditure appears to be greatest during the initial phase, before NEFA release from adipose tissue lipolysis is adequate to meet the energy requirements (Romijn et al., 1993). For example, when exercise is performed in the overnight-fasted state the contribution of IMTG to fat oxidation is reduced only after ~120 minutes of exercise period, and then plasma NEFA becomes the dominant substrate supplying around 60% of the energy requirements at 57% $\dot{V}O_{2max}$ compared to ~40% over the first 120 minutes (Watt et al., 2002b, van Loon et al., 2003). In summary, the evidences suggested that the oxidation rate of TG sources gradually declines with

exercise duration, whereas plasma free fatty acid oxidation rates increase with exercise duration.

As mentioned earlier, the choice of fuel substrate utilisation during exercise is also influences by the availability of substrate at the cellular level that can be altered via dietary intake (Wu et al., 2003, De Bock et al., 2005, Cluberton et al., 2005, Backhouse et al., 2007). The earliest study that investigated the influence of dietary intake on substrate utilisation was almost 100 years ago (Krough and Lindhard, 1920). They demonstrated that consuming a diet high in fat and low in carbohydrate reduced the RER value, which indicates that fat oxidation rate increased by consumed high fat and low carbohydrate diet. In addition, based on muscle biopsy data, consuming a high carbohydrate diet the muscle glycogen concentrations was increased (Bergström et al., 1967). Conversely, after several days of a low-carbohydrate diet, as a result, muscle glycogen store depleted and consequently, carbohydrate oxidation during exercise decreased. Indeed, previous work had showed that the rate of glycogenolysis is directly related to muscle glycogen concentration (Laurent et al., 2000).

Several studies have demonstrated that increasing the muscle glycogen content before exercise, and the availability of exogenous carbohydrate before and during dynamic exercise, increases carbohydrate oxidation and reciprocally decreases fat oxidation (Kiens and Helge, 1998), as well as promoting glucose storage, particularly in muscle (Jéquier, 1994). For instance, previous work in well-trained cyclist had demonstrated that after seven days on protocols that involved training at

70% $\dot{V}O_{2max}$ and eating a diet containing 88% carbohydrate, their muscle glycogen concentration were tremendously high (Coyle et al., 2001). In contrast to carbohydrate oxidation, fat oxidation during exercise was reduced by 27% and this was believed to be partly attributed to reduced IMTG stores as no fat source from diet. In addition, the acute effect of carbohydrate ingested in the period immediately before exercise also results in increased carbohydrate oxidation and suppressed fat oxidation by the action of insulin that stimulates suppression of whole body lipolysis (Sidossis et al., 1996, Horowitz et al., 1997, Wolfe, 1998).

In contrast to carbohydrate intake, the adaptation towards dietary manipulation for fat intake takes longer than the almost immediate adaptation to high-carbohydrate diet as shown above. Thus, prolonged alteration in normal diet is required to alter fat oxidation, particularly IMTG stores. Indeed, longer-tem of high fat low carbohydrate [HFLC] diets (>3–7 days) have been shown to increase IMTG content by 50-80% (Coyle et al., 2001, Yeo et al., 2011), whilst reduced glycogen utilization and total carbohydrate oxidation rates during moderate-intensity exercise, without altering glucose uptake (Helge et al., 2001). Conversely, when IMTG is reduced following a low-fat diet, the whole-body carbohydrate oxidation and muscle glycogen utilization are increased without altering whole-body glucose uptake (Coyle et al., 2001). Hence, adaptation to high-fat or low-fat diet over time has no influence on muscle glucose uptake during exercise, but does influence substrate storage and availability, which is evident for both glycogen and IMTG during moderate-intensity exercise (Coyle et al., 2001, Zehnder et al., 2006, Van Proeyen et al., 2011). However, it is noteworthy that when consuming low-fat diet, the diet also contain

high carbohydrate content, and therefore the diet might influence on the storage of both IMTG and glycogen, in direction of reducing IMTG content and increasing glycogen content. In fact, the opposite is also true for high-fat diet as it lead to low glycogen stores. Therefore, it is difficult to examine the pure interaction between the IMTG store and fuel substrates metabolism.

The timing of feeding or nutrient ingestion has been well established to influence the choice of substrate utilised during exercise and more recently physiological adaptations after long-term exercise training (Derave et al., 2008, Stannard et al., 2010, Van Proeyen et al., 2010, Van Proeyen et al., 2011). For instance, evidence shows that ingestion of pre-exercise carbohydrate has a very strong inhibiting effect on fat oxidation (Coyle et al., 2001, Horowitz et al., 1997, Achten and Jeukendrup, 2003). Indeed, ingestion of 50–100 g of carbohydrate in the hours before exercise reduces fat oxidation by about 30–40%. The reduction is result from the less fatty acid availability due to suppress of lipolysis (Horowitz et al., 1997) and also because of the effect of hyperinsulinemia in the muscle (Coyle et al., 1997). However, the exact mechanism on whether glucose and/or insulin reduce fat oxidation at an intramuscular level is still unknown and debatable. Nevertheless, it has also been suggested, the suppression might likely involve the sites of fat regulation including the transport of fatty acid into the muscle by the FAT, and the transport of fatty acid across the mitochondrial membrane (Jeukendrup, 2002, Spriet, 2002, Horowitz, 2003).

Previous work has also revealed that a pre-exercise carbohydrate feeding/mix meal feeding blunted some of the key skeletal muscle adaptive responses to exercise training (Bartlett et al., 2015). Nevertheless, surprisingly, research on substrate utilisation responses to acute exercise under different dietary conditions is limited in overweight and obese populations. Several studies show that obese individuals, compared with lean individuals, display a reduced fat oxidation under post-absorptive and/or postprandial conditions (Kelley and Simoneau, 1994a, Colberg et al., 1995, Kelley et al., 1999, Hulver et al., 2003) and this may be a result of reduced muscle oxidative capacity. Therefore, translating the impact of feeding timing around exercise in this population is a novel area that needs to be explored in order to establish new strategies in improving their metabolic health; hence the primary focus of this thesis.

1.2 Obesity and exercise

1.2.1 Metabolic disturbances in obesity

According to the World Health Organization [WHO], “abnormal or excessive fat accumulation that may impair health” is the definition for obesity (WHO, 2013). The accumulation of excess fat in adipose tissue due to elevated free fatty acid delivery is associated with poor fat oxidation (Kelley et al., 1999) and leads to ectopic fat storage (Kelley et al., 1999) including muscle IMTG (Goodpaster et al., 2000). Elevated concentrations of IMTG have been linked to lower the rates of fatty acid oxidation by muscle (Kelley et al., 1999) or even impaired oxidative capacity of

muscle (Kelley and Simoneau, 1994b, Simoneau et al., 1995). Additionally, accumulation of IMTG is also associated with increased risk of insulin resistance (Phillips et al., 1996a, Pan et al., 1997, Krssak et al., 1999, Boden et al., 2001). This is most likely due to increased intramuscular lipid metabolite concentrations are associated with high IMTG content, such as long chain fatty acyl-CoA [LCFA-CoA], diacylglycerols [DAG] and ceramides. These metabolites compromise the action of insulin to facilitate glucose uptake in skeletal muscle (Hulver et al., 2003, Hulver and Dohm, 2004), which could induce defects in the insulin signalling cascade, causing skeletal muscle insulin resistance. The progressive accumulation of IMTG in obese individuals should therefore form a major therapeutic target and efforts should be made to develop interventions that prevent excess IMTG accretion by stimulating their rate of oxidation. Indeed, it has been suggested that a high turnover rate of the IMTG pool (utilisation and storage) may aid in reducing the intracellular concentration of such lipid metabolites, and thus reduce lipotoxic stress and insulin resistance in skeletal muscle (Moro et al., 2008); which is thought to be of benefit for metabolic health.

Obese individuals also display blunted adipose tissue lipolytic activity (Langin et al., 2005), which is responsible for the hydrolysis of TGs and release of NEFAs (Guilherme et al., 2008). This inability to release NEFAs may contribute to the augmented adipose cell hypertrophy that is associated with obesity (Greenberg and Obin, 2006).

Poor metabolic flexibility is also often linked with obesity (Corpeleijn et al., 2009). Metabolic flexibility has been defined as the clear capacity to utilize fat and carbohydrate fuels for energy production and to transition between them based on fuel availability and energy demand (Kelley & Mandarino, 2000). However, if the system is metabolically inflexible, the capacity to switch between fuels will be impaired. For instance, a study in patients with non-insulin-dependent diabetes mellitus (NIDDM) revealed that, utilization of free tatty acid by muscle was reduced during post-absorptive conditions, while during postprandial conditions there is impaired suppression of free fatty acid uptake across the leg (Kelley & Simoneau, 1994). Previous studies among obese individual also showed that the ability to take up fatty acids in skeletal muscle during beta-adrenergic stimulation was impaired in obese individuals (Blaak et al., 1994, Colberg et al., 1995). Poor metabolic flexibility also has been linked with higher risk of type 2 diabetes incidence (Galgani el al., 2008, Corpeleijn et al., 2009).

Collectively, obesity is associated with profound disturbances in lipid metabolism, which suggests strategies to help mobilise and utilise fat as fuels, especially IMTG due to links with muscle insulin sensitivity, could be particularly beneficial in this population.

1.2.2 Exercise and obesity

Exercise is a cornerstone in obesity prevention and treatment. It seems to be beneficial for prevention of weight gain or regain following weight-loss, but exercise

alone is not always effective for improvements in insulin sensitivity or weight loss *per se* (Ross et al., 2000, Coker et al., 2009). For weight loss, the possible reason for the unsuccessful weight loss may be related to interactions with food intake as it has been suggested that a possible energy compensation may occur by either increased subsequent energy intake or decreased the energy expenditure during non-exercise periods (Thompson et al., 2014). Indeed, a study showed that not all individuals who undertake long-term exercise would lose weight under conditions of *ad libitum* feeding (Hopkins et al., 2010). Meanwhile, for insulin sensitivity, previous work has shown that a higher fat oxidation during physical activity could help to improve insulin sensitivity in skeletal muscle (Goodpaster et al., 2001, Venables and Jeukendrup, 2008, Shaw et al., 2010). In addition, higher IMTG turnover induced by exercise training has also been linked to greater insulin sensitivity (Goodpaster et al., 2001). Thus, increasing the utilisation of fat, both plasma NEFA and IMTG, as the prominent fuel substrate during exercise may help to increase insulin sensitivity. However, as described above, obese individuals tend to have impairments of fat metabolism, hence investigating a strategy to induce fat oxidation, particularly IMTG utilisation, during exercise is much needed in this population.

One strategy that may be beneficial is by manipulating exercise and feeding timing (fast vs. fed state exercise). Recently, performing exercise in different states of nutrition (i.e., feeding before or after exercise) has been extensively studied and the findings show an improvement in utilising fat oxidation during exercise and potential for improved insulin sensitivity in young healthy individuals (Derave et al., 2008, Stannard et al., 2010, Van Proeyen et al., 2010, Van Proeyen et al., 2011, Vieira et

al., 2016, Wallis and Gonzalez, 2019). For instance, a study of young healthy adults showed that 2 hour of submaximal exercise performed after an overnight fast and in the absence of carbohydrate intake during exercise increased IMTG breakdown and enhanced the post-exercise insulin response to glucose ingestion, which in turn is likely to contribute to stimulation of post-exercise muscle glycogen re-synthesis (De Bock et al., 2005). In addition, previous works showed that breakfast omission under resting conditions (i.e. overnight fast) does not result in energy intake compensation at lunch (Gonzalez et al., 2013, Chowdhury et al., 2015); this could lead to better energy balance regulation with breakfast omission if the findings translate into the exercise context. Additionally, it has been demonstrated that exercise after an overnight fast increases 24-h fat oxidation compared to exercise performed in the fed state in active young individuals (Iwayama et al., 2015, Iwayama et al., 2017). However, feeding timing is not always controlled in training studies, which could be important in determining efficacy of training. As well, most of the research was conducted in lean groups; thus, it is unclear if similar results would be obtained in populations at high risk for metabolic disease and hence a gap exists in the literature for studying obese populations conducting fasted vs. fed state exercise.

1.3 Sex-differences in metabolism

It is well known that there are sex-differences in various metabolic processes. For instance, evidence suggests that women appear to utilise more fat as an energy substrate during periods of sustained exertion than men do (Tarnopolsky, 2008,

Horton et al., 1998, Henderson et al., 2007, Henderson and Alderman, 2014). In contrast, men are more likely to use more carbohydrate (and protein) for fuel during sustained exercise bouts (Lamont et al., 2001, Lamont, 2005). In addition, some evidence also suggests an apparent sex-difference in response to nutritional modulation. There is evidence showing that men and women respond differently to a range of nutrition manipulation such as fasting (Mittendorfer et al., 2001), lipid-infusion (Høeg et al., 2011) and insulin stimulation (Kuhl et al., 2005). Indeed, a comprehensive review suggested that, women might be more flexible in response to dietary carbohydrate manipulation as compared to men (Lundsgaard and Kiens, 2014), although this has not been definitively studied. This might be because women have an approximately 30% greater insulin stimulated glucose uptake than men (Høeg et al., 2009, Høeg et al., 2011). Overall, this suggests sex-differences may exist in metabolic responses to feeding or combined feeding and exercise, but this needs to be followed up more comprehensively.

Recently, the function of nutrition feeding around exercise has been extensively researched and a recent systemic review concluded that fat oxidation during overnight-fasted exercise is higher compared to exercise in the fed state in young lean adults (Vieira et al., 2016). Indeed, a recent study among healthy young lean adult men and women showed an increase of fat oxidation when exercise performed in overnight-fasted state (Iwayama et al., 2015, Iwayama et al., 2017), however the authors did not examine sex-differences in their studies. In line, previous works in obese and overweight men (Derave et al., 2007, Farah and Gill, 2013) also showed a similar outcome. However, whether women respond similarly to fed vs. fasted

exercise in obesity is not known; therefore, work is needed to explore the metabolic responses in both men and women. Furthermore, there is an absence of well-controlled studies even simply comparing metabolic responses between men and women in response to feeding interventions such as carbohydrate provision. Part of the problem with this area of research is the need for careful control of numerous factors such as menstrual cycle status/influences, fitness, body composition, relative dose of nutrition provided, which all can affect metabolic responses (Tarnopolsky et al., 1990, Tarnopolsky, 2000, Tarnopolsky, 2008, Rattarasarn et al., 2010). Therefore, carefully controlled studies remain critical in order to identify clear biological sex-based differences in metabolic responses to feeding and/or feeding and exercise, as new knowledge in this area may reveal the potential for sex-specific recommendations around physical activity and nutrition for the maintenance of good health.

1.3.1 Influence of sex-hormone and menstrual cycle on sex differences in substrate metabolism

It has been proposed that sex hormones, estrogen and progesterone may play a significant role in determining the differences between genders (Tarnopolsky, 2008). This is because, after puberty the hormonal environment between men and women becomes markedly different, and this change responsible for many of the typical sex based differences in size, adiposity, body composition, and substrate oxidation (Comitato et al., 2015). Prior to the menopause, the circulating concentration of estrogen, even during the early follicular phase of the menstrual cycle when it is at

its lowest, is still ~2 fold higher in women than men (Stachenfeld and Taylor, 2014). With estrogen receptors found ubiquitously throughout the body including skeletal muscle (even in men) then there is potential for it to be a driving factor behind difference in metabolism during exercise (Oosthuyse and Bosch, 2012). The role of estrogen in regulation of substrate oxidation was also proven when it was exogenously administered to men and women with amenorrhoea. For example, fat oxidation increased approximately 30% during exercise at 65% $\dot{V}O_{2max}$ after 8 days of administration in men (Hamadeh et al., 2005). This occurs alongside a reduction in hepatic glucose output (Ra) and (Rd) indicative of reduced flux, but does not appear to change muscle glycogen utilisation (Carter et al., 2001, Devries et al., 2005). Estrogen administered for a few days also increases plasma NEFA concentration during exercise in either amenorrhea women or men although it does not seem to alter whole body lipolysis with similar glycerol Ra and Rd (Ruby et al., 1997). Nonetheless, the directional consistency of the findings mentioned for estrogen to favour lipid oxidation whilst reducing hepatic carbohydrate reliance is suggestive of estrogen's important role in the sex based differences seen in substrate oxidation. Meanwhile, circulating progesterone levels are also substantially different between men and women; however, the independent role of progesterone on substrate metabolism has not been adequately assessed.

These sex-hormones fluctuate markedly throughout the menstrual cycle. The cycle can be divided into three general phases based upon events in the follicles of the ovary and the resultant changes in hormonal milieu; the follicular phase, ovulation and the luteal phase. The widely divergent hormonal profiles throughout a typical

menstrual cycle, in particular that of estrogen, is often cited as the driving force behind differences in metabolism and substrate oxidation reported between the phases. Greater rates of lipid oxidation have been reported during the luteal phase (a sustained elevation of estrogen) compared to the follicular (Hackney, 1999, Zderic et al., 2001, Campbell et al., 2001) and this is associated with greater oxidation of circulating NEFAs (Zderic et al., 2001, Hackney et al., 1994). Higher carbohydrate oxidation often observed during the follicular phase is associated with a greater systemic glucose turnover when fasted or when the exercise intensity is greater than 55% $\dot{V}O_{2max}$ (Devries et al., 2006, Campbell et al., 2001, Ruby et al., 1997). The effects on muscle glycogen storage are less clear, with greater repletion seen in the luteal phase (Nicklas et al., 1989) or no differences in storage but a greater rate of utilisation seen in the follicular phase than luteal following 90mins cycling at 65% $\dot{V}O_{2max}$ during exercise (Devries et al. 2006).

As elaborated above, the influence of sex hormone on the selection of fuel during energy production is evident and therefore, it is important to control for menstrual cycle when investigating substrate metabolism in women and when comparing the findings with men.

1.4. Summary and aims of the thesis

As feeding status before exercise has been shown to have the potential to induce positive short and longer-term effects in lean individuals, it is of critical importance to understand if undertaking fasted-state exercise might also be a viable option to

optimise metabolic health outcomes in overweight and obese populations. As well, given the dearth of studies undertaken in women, it is also relevant to examine metabolic responses to feeding and exercise intervention in both sexes in order to understand the potential necessity for sex-specific strategies for health optimisation.

Therefore, the primary aim of this PhD work was to investigate the metabolic response to feeding and exercise in both men and women, with the following specific research questions:

- What are the effects of a single exercise bout performed before feeding on exercise substrate and IMTG utilisation in obese or overweight/centrally obese men?

- How does exercise performed in the overnight-fasted influences substrate metabolism during exercise in obese or overweight centrally obese women?

- How are subjective appetite responses and subsequent energy intake modulated by exercise performed in the overnight-fasted vs. fed-state in obese or overweight centrally obese women?

- Does biological sex influence metabolic responses to glucose feeding at rest in pair-matched men and women?

The hypothesises of this PhD work were:

- A single exercise bout performed before feeding can increases fat oxidation and stimulate greater IMTG utilisation during exercise as compared to exercise after feeding in obese or overweight/centrally obese men.

- A single exercise bout performed in the overnight-fasted will promote greater fat oxidation during exercise than fed-state exercise in obese or overweight centrally obese women.

- Appetite responses and subsequent energy intake across the study will be similar regardless of whether exercise is performed in the overnight-fasted or fed state in obese or overweight centrally obese women.

- Women would display an improved homeostatic response of fuel selection following glucose feeding than men.

1.5 References

ACHTEN, J., GLEESON, M. & JEUKENDRUP, A. E. 2002. Determination of the exercise intensity that elicits maximal fat oxidation. *Medicine and Science in Sports and Exercise,* 34(1)**,** 92-97.

ACHTEN, J. & JEUKENDRUP, A. E. 2003. Effects of pre-exercise ingestion of carbohydrate on glycaemic and insulinaemic responses during subsequent exercise at differing intensities. *European Journal of Applied Physiology,* 88(4-5)**,** 466-471.

ACHTEN, J., VENABLES, M. C. & JEUKENDRUP, A. E. 2003. Fat oxidation rates are higher during running compared with cycling over a wide range of intensities. *Metabolism: Clinical and Experimental,* 52(6)**,** 747-752.

ADAMS, J. M., PRATIPANAWATR, T., BERRIA, R., WANG, E., DEFRONZO, R. A., SULLARDS, M. C. & MANDARINO, L. J. 2004. Ceramide content is increased in skeletal muscle from obese insulin-resistant humans. *Diabetes,* 53, 25-31.

AHLBORG, G., FELIG, P., HAGENFELDT, L., HENDLER, R. & WAHREN, J. 1974. Substrate turnover during prolonged exercise in man. Splanchnic and leg metabolism of glucose, free fatty acids, and amino acids. *The Journal of Clinical Investigation,* 53(4)**,** 1080-1090.

ARNER, P., KRIEGHOLM, E., ENGFELDT, P. & BOLINDER, J. 1990. Adrenergic regulation of lipolysis in situ at rest and during exercise. *The Journal of Clinical Investigation,* 85(3)**,** 893-898.

BACKHOUSE, S. H., WILLIAMS, C., STEVENSON, E. & NUTE, M. 2007. Effects of the glycemic index of breakfast on metabolic responses to brisk walking in females. *European Journal of Clinical Nutrition,* 61(5)**,** 590-596.

BARTLETT, J. D., HAWLEY, J. A. & MORTON, J. P. 2015. Carbohydrate availability and exercise training adaptation: too much of a good thing? *European Journal of Sport Science,* 15(1)**,** 3-12.

BERGMAN, B. C. & BROOKS, G. A. 1999. Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men. *Journal of applied physiology,* 86**,** 479-487.

BERGSTRÖM, J., HERMANSEN, L., HULTMAN, E. & SALTIN, B. 1967. Diet, muscle glycogen and physical performance. *Acta Physiologica Scandinavica,* 71**,** 140-150.

BLAAK, E. E., VAN AGGEL-LEIJSSEN, D. P., WAGENMAKERS, A. J., SARIS, W. H. & VAN BAAK, M. A. 2000. Impaired oxidation of plasma-derived fatty acids

in type 2 diabetic subjects during moderate-intensity exercise. *Diabetes,* 49, 2102-2107.

BLAAK, E. E., VAN BAAK, M. A., KEMERINK, G. J., PAKBIERS, M. T., HEIDENDAL, G. A. & SARIS, W. H. 1994. Beta-adrenergic stimulation of energy expenditure and forearm skeletal muscle metabolism in lean and obese men. *The American Journal of Physiology,* 267**,** E306-15.

BLOMQVIST, C. G. & SALTIN, B. 1983. Cardiovascular adaptations to physical training. *Annual Review of Physiology,* 45**,** 169-189.

BODEN, G., LEBED, B., SCHATZ, M., HOMKO, C. & LEMIEUX, S. 2001. Effects of acute changes of plasma free fatty acids on intramyocellular fat content and insulin resistance in healthy subjects. *Diabetes,* 50, 1612-1617.

BROOKS, G. A. & MERCIER, J. 1994. Balance of carbohydrate and lipid utilization during exercise: the "crossover" concept. *Journal of Applied Physiology,* 76**,** 2253-2261.

CALBET, J. A., HOLMBERG, H. C., ROSDAHL, H., VAN HALL, G., JENSEN-URSTAD, M. & SALTIN, B. 2005. Why do arms extract less oxygen than legs during exercise? *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology,* 289**,** R1448-58.

CAMPBELL, S. E., ANGUS, D. J. & FEBBRAIO, M. A. 2001. Glucose kinetics and exercise performance during phases of the menstrual cycle: effect of glucose ingestion. *American Journal of Physiology: Endocrinology and Metabolism,* 281: E817-E825.

CARTER, S., MCKENZIE, S., MOURTZAKIS, M., MAHONEY, D. J. & TARNOPOLSKY, M. A. 2001. Short-term 17beta-estradiol decreases glucose R(a) but not whole body metabolism during endurance exercise. *Journal of Applied Physiology,* 90, 139-146.

CHOWDHURY, E. A., RICHARDSON, J. D., TSINTZAS, K., THOMPSON, D. & BETTS, J. A. 2015. Carbohydrate-rich breakfast attenuates glycaemic, insulinaemic and ghrelin response to ad libitum lunch relative to morning fasting in lean adults. *The British Journal of Nutrition,* 114**,** 98-107.

CLUBERTON, L. J., MCGEE, S. L., MURPHY, R. M. & HARGREAVES, M. 2005. Effect of carbohydrate ingestion on exercise-induced alterations in metabolic gene expression. *Journal of Applied Physiology,* 99**,** 1359-1363.

COKER, R. H., WILLIAMS, R. H., YEO, S. E., KORTEBEIN, P. M., BODENNER, D. L., KERN, P. A. & EVANS, W. J. 2009. The impact of exercise training compared to caloric restriction on hepatic and peripheral insulin resistance in obesity. *The Journal of Clinical Endocrinology and Metabolism,* 94**,** 4258-4266.

COLBERG, S. R., SIMONEAU, J. A., THAETE, F. L. & KELLEY, D. E. 1995. Skeletal muscle utilization of free fatty acids in women with visceral obesity. *The Journal of Clinical Investigation,* 95**,** 1846-1853.

COMITATO, R., SABA, A., TURRINI, A., ARGANINI, C. & VIRGILI, F. 2015. Sex hormones and macronutrient metabolism. *Critical Reviews in Food Science and Nutrition,* 55, 227-241.

CORPELEIJN, E., SARIS, W. & BLAAK, E. 2009. Metabolic flexibility in the development of insulin resistance and type 2 diabetes; effects of lifestyle. *Obesity Reviews,* 10**,** 16.

COYLE, E. F., JEUKENDRUP, A. E., OSETO, M. C., HODGKINSON, B. J. & ZDERIC, T. W. 2001. Low-fat diet alters intramuscular substrates and reduces lipolysis and fat oxidation during exercise. *American Journal of Physiology. Endocrinology and Metabolism,* 280**,** E391-8.

COYLE, E. F., JEUKENDRUP, A. E., WAGENMAKERS, A. & SARIS, W. 1997. Fatty acid oxidation is directly regulated by carbohydrate metabolism during exercise. *American Journal of Physiology, Endocrinology and Metabolism - Clinical and Experimental,* 273**,** E268-E275.

DE BOCK, K., RICHTER, E. A., RUSSELL, A. P., EIJNDE, B. O., DERAVE, W., RAMAEKERS, M., KONINCKX, E., LÉGER, B., VERHAEGHE, J. & HESPEL, P. 2005. Exercise in the fasted state facilitates fibre type-specific intramyocellular lipid breakdown and stimulates glycogen resynthesis in humans. *The Journal of Physiology,* 564**,** 649-660.

DERAVE, K. D. B., EIJNDE, B., HESSELINK, M., KONINCKX, E., ROSE, A., SCHRAUWEN, P., BONEN, A., RICHTER, E. & HESPE, P. 2008. Effect of training in the fasted state on metabolic responses during exercise with carbohydrate intake. *Journal of Applied Physiology,* 10**,** 11.

DERAVE, W., MERTENS, A., MULS, E., PARDAENS, K. & HESPEL, P. 2007. Effects of post-absorptive and postprandial exercise on glucoregulation in metabolic syndrome. *Obesity,* 15**,** 704-711.

DEVRIES, M. C., HAMADEH, M. J., GRAHAM, T. E. & TARNOPOLSKY, M. A. 2005. 17beta-estradiol supplementation decreases glucose rate of appearance and disappearance with no effect on glycogen utilization during moderate intensity exercise in men. *The Journal of Clinical Endocrinology and Metabolism,* 90, 6218-6225.

DEVRIES, M. C., HAMADEH, M. J., PHILLIPS, S. M. & TARNOPOLSKY, M. A. 2006. Menstrual cycle phase and sex influence muscle glycogen utilization and glucose turnover during moderate-intensity endurance exercise. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology,* 291: R1120-R1128.

Diabetes UK 2010. Diabetes in the UK 2010: Key statistics on diabetes. London: Diabetes UK.

ELLIS, B. A., POYNTEN, A., LOWY, A. J., FURLER, S. M., CHISHOLM, D. J., KRAEGEN, E. W. & COONEY, G. J. 2000. Long-chain acyl-CoA esters as indicators of lipid metabolism and insulin sensitivity in rat and human muscle. *American Journal of Physiology, Endocrinology and Metabolism,* 279, E554-E560.

FARAH, N. & GILL, J. 2013. Effects of exercise before or after meal ingestion on fat balance and postprandial metabolism in overweight men. *British Journal of Nutrition,* 109**,** 2297-2307.

FELIG, P. & WAHREN, J. 1975. Fuel homeostasis in exercise. *The New England Journal of Medicine,* 293**,** 1078-1084.

FROBERG, K. & PEDERSEN, P. 1984. Sex differences in endurance capacity and metabolic response to prolonged, heavy exercise. *European Journal of Applied Physiology and Occupational Physiology,* 52**,** 446-450.

FU, M.-H. H., MAHER, A. C., HAMADEH, M. J., YE, C. & TARNOPOLSKY, M. A. 2009. Exercise, sex, menstrual cycle phase, and 17beta-estradiol influence metabolism-related genes in human skeletal muscle. *Physiological Genomics,* 40, 34-47.

GALGANI, J. E., HEILBRONN, L. K., AZUMA, K., KELLEY, D. E., ALBU, J. B., PI-SUNYER, X., SMITH, S. R. & RAVUSSIN, E. 2008 Metabolic flexibility in response to glucose is not impaired in people type 2 diabetes after controlling for glucose disposal rate. *Diabetes*, 57, 841-845.

GONZALEZ, J. T., VEASEY, R. C., RUMBOLD, P. L. S. & STEVENSON, E. J. 2013. Breakfast and exercise contingently affect postprandial metabolism and energy balance in physically active males. *The British Journal of Nutrition,* 110**,** 721-732.

GOODPASTER, B. H., HE, J., WATKINS, S. & KELLEY, D. E. 2001. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *The Journal of Clinical Endocrinology and Metabolism,* 86**,** 5755-5761.

GOODPASTER, B. H., THERIAULT, R., WATKINS, S. C. & KELLEY, D. E. 2000. Intramuscular lipid content is increased in obesity and decreased by weight loss. *Metabolism - Clinical and Experimental,* 49**,** 467-472.

GREENBERG, A. S. & OBIN, M. S. 2006. Obesity and the role of adipose tissue in inflammation and metabolism. *The American Journal of Clinical Nutrition,* 83**,** 461S-465S.

GRUNDY, S. M. 2004. Obesity, metabolic syndrome, and cardiovascular disease. *The Journal of Clinical Endocrinology and Metabolism,* 89**,** 2595-2600.

GRUNDY, S. M. 2008. Metabolic syndrome pandemic. *Arteriosclerosis, Thrombosis, and Vascular Biology,* 28**,** 629-636.

GUILHERME, A., VIRBASIUS, J. V., PURI, V. & CZECH, M. P. 2008. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nature Reviews. Molecular Cell Biology,* 9**,** 367-377.

HACKNEY, A. C. 1999. Influence of estrogen on muscle glycogen utilization during exercise. *Acta Physiologica Scandinavica,* 167: 273-274.

HACKNEY, A. C., MCCRACKEN-COMPTON, M. A. & AINSWORTH, B. 1994. Substrate responses to submaximal exercise in the midfollicular and midluteal phases of the menstrual cycle. *International Journal of Sport Nutrition,* 4: 299-308.

HAMADEH, M. J., DEVRIES, M. C. & TARNOPOLSKY, M. A. 2005. Estrogen supplementation reduces whole body leucine and carbohydrate oxidation and increases lipid oxidation in men during endurance exercise. *The Journal of Clinical Endocrinology and Metabolism,* 90, 3592-3599.

HARGREAVES, M. 2012. The metabolic systems: carbohydrate metabolism. *In:* FARRELL, P., JOYNER, M. & CAIOZZO, V. (eds.) *Advanced exercise physiology.* 2 ed. Philadelphia: Lippincott, Williams and Wilkins.

HELGE, J. W., WATT, P. W., RICHTER, E. A., RENNIE, M. J. & KIENS, B. 2001. Fat utilization during exercise: adaptation to a fat-rich diet increases utilization of plasma fatty acids and very low density lipoprotein-triacylglycerol in humans. *The Journal of Physiology,* 537**,** 1009-1020.

HENDERSON, G. C. & ALDERMAN, B. L. 2014. Determinants of resting lipid oxidation in response to a prior bout of endurance exercise. *Journal of Applied Physiology,* 116**,** 95-103.

HENDERSON, G. C., FATTOR, J. A., HORNING, M. A., FAGHIHNIA, N., JOHNSON, M. L., MAU, T. L., LUKE-ZEITOUN, M. & BROOKS, G. A. 2007. Lipolysis and fatty acid metabolism in men and women during the post-exercise recovery period. *The Journal of Physiology,* 584**,** 963-981.

HØEG, L., ROEPSTORFF, C., THIELE, M., RICHTER, E. A., WOJTASZEWSKI, J. F. P. & KIENS, B. 2009. Higher intramuscular triacylglycerol in women does not impair insulin sensitivity and proximal insulin signalling. *Journal of Applied Physiology,* 107**,** 824-831.

HØEG, L. D., SJØBERG, K. A., JEPPESEN, J., JENSEN, T. E., FRØSIG, C., BIRK, J. B., BISIANI, B., HISCOCK, N., PILEGAARD, H., WOJTASZEWSKI, J. F. P., RICHTER, E. A. & KIENS, B. 2011. Lipid-induced insulin resistance affects women less than men and is not accompanied by inflammation or impaired proximal insulin signalling. *Diabetes,* 60**,** 64-73.

HOLLOSZY, J. O. & COYLE, E. F. 1984. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology,* 56**,** 831-838.

HOLLOWAY, G. & SPRIET, L. 2012. The metabolic systems: interaction of lipid and carbohydrate metabolism. *In:* FARRELL, P., JOYNER, M. & CAIOZZO, V. (eds.) *Advanced exercise physiology.* 2nd ed. Philadelphia: Lippincott, Williams and Wilkins.

HOPKINS, M., KING, N. A. & BLUNDELL, J. E. 2010. Acute and long-term effects of exercise on appetite control: is there any benefit for weight control? *Current Opinion in Clinical Nutrition and Metabolic Care,* 13**,** 635-640.

HOROWITZ, J. F. 2003. Fatty acid mobilization from adipose tissue during exercise. *Trends in Endocrinology and Metabolism: TEM,* 14**,** 386-392.

HOROWITZ, J. F., MORA-RODRIQUEZ, R., BYERLEY, L. O. & COYLE, E. F. 1997. Lipolytic suppression following carbohydrate ingestion limits fat oxidation during exercise.pdf. *American Journal of Physiology, Endocrinology and Metabolism,* 273**,** E768–E775.

HORTON, T. J., PAGLIASSOTTI, M. J., HOBBS, K. & HILL, J. O. 1998. Fuel metabolism in men and women during and after long-duration exercise. *Journal of Applied Physiology,* 85**,** 1823-1832.

HULVER, M. W., BERGGREN, J. R., CORTRIGHT, R. N., DUDEK, R. W., THOMPSON, R. P., PORIES, W. J., MACDONALD, K. G., CLINE, G. W., SHULMAN, G. I., DOHM, G. L. & HOUMARD, J. A. 2003. Skeletal muscle lipid metabolism with obesity. *American Journal of Physiology, Endocrinology and Metabolism,* 284**,** E741-7.

HULVER, M. W. & DOHM, G. L. 2004. The molecular mechanism linking muscle fat accumulation to insulin resistance. *Proceedings of the Nutrition Society,* 63**,** 375-380.

ITANI, S. I., RUDERMAN, N. B., SCHMIEDER, F. & BODEN, G. 2002. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerols, Protein Kinase C, and IκB-α. *Diabetes,* 51, 2005-2011.

IWAYAMA, K., KAWABUCHI, R., NABEKURA, Y., KURIHARA, R., PARK, I., KOBAYASHI, M., OGATA, H., KAYABA, M., OMI, N., SATOH, M. & TOKUYAMA, K. 2017. Exercise before breakfast increases 24-h fat oxidation in female subjects. *PloS One,* 12**,** e0180472.

IWAYAMA, K., KURIHARA, R., NABEKURA, Y., KAWABUCHI, R., PARK, I., KOBAYASHI, M., OGATA, H., KAYABA, M., SATOH, M. & TOKUYAMA, K. 2015. Exercise Increases 24-h Fat Oxidation Only When It Is Performed Before Breakfast. *EBioMedicine,* 2**,** 2003-2009.

JAHOOR, F., PETERS, E. J. & WOLFE, R. R. 1990. The relationship between gluconeogenic substrate supply and glucose production in humans. *The American Journal of Physiology,* 258**,** E288-96.

JENSSEN, T., NURJHAN, N., CONSOLI, A. & GERICH, J. E. 1990. Failure of substrate-induced gluconeogenesis to increase overall glucose appearance in normal humans. Demonstration of hepatic autoregulation without a change in plasma glucose concentration. *The Journal of Clinical Investigation,* 86**,** 489-497.

JÉQUIER, E. 1994. Carbohydrates as a source of energy. *The American Journal of Clinical Nutrition,* 59**,** 682S-685S.

JEUKENDRUP, A., RABEN, A., GIJSEN, A., STEGEN, J., BROUNS, F., SARIS, W. & WAGENMAKERS, A. 1999. Glucose kinetics during prolonged exercise in highly trained human subjects: effect of glucose ingestion. *Journal of Physiology,* 515(2)**,** 579—589.

JEUKENDRUP, A., SARIS, W. & WAGENMAKERS, A. 1998. Fat metabolism during exercise: a review. Part I: fatty acid mobilization and muscle metabolism. *International Journal of Sports Medicine,* 19**,** 1-16.

JEUKENDRUP, A. E. 2002. Regulation of Fat Metabolism in Skeletal Muscle. *Annals of the New York Academy of Sciences,* 967**,** 217-235.

JEUKENDRUP, A. E. 2003. Modulation of carbohydrate and fat utilization by diet, exercise and environment. *Biochemical Society Transactions,* 31**,** 1270-1273.

KAHN, B. B. & FLIER, J. S. 2000. Obesity and insulin resistance. *The Journal of Clinical Investigation,* 106, 473-481.

KELLEY, D., GOODPASTER, B., WING, R. & SIMONEAU, J. 1999. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *American Journal of Physiology, Endocrinology and Metabolism* 277**,** 12.

KELLEY, D. E. & SIMONEAU, J. A. 1994. Impaired free fatty acid utilization by skeletal muscle in non-insulin-dependent diabetes mellitus. *The Journal of Clinical Investigation,* 94**,** 2349-2356.

KIENS, B. & HELGE, J. W. 1998. Effect of high-fat diets on exercise performance. *The Proceedings of the Nutrition Society,* 57**,** 73-75.

KIM, Y.-B., SHULMAN, G. I. & KAHN, B. B. 2002. Fatty Acid Infusion Selectively Impairs Insulin Action on Akt1 and Protein Kinase C λ/ζ but Not on Glycogen Synthase Kinase-3. *Journal of Biological Chemistry,* 277, 32915-32922.

KING, N. A., HOPKINS, M., CAUDWELL, P., STUBBS, R. J. & BLUNDELL, J. E. 2008. Individual variability following 12 weeks of supervised exercise:

identification and characterization of compensation for exercise-induced weight loss. *International Journal of Obesity,* 32**,** 177-184.

KROUGH, A. & LINDHARD, J. 1920. The relative value of fat and carbohydrate as sources of muscular energy. *Biochemical Journal,* 14(3-4)**,** 290–363.

KRSSAK, M., PETERSEN, K. F., DRESNER, A., DIPIETRO, L., VOGEL, S. M., ROTHMAN, D. L., SHULMAN, G. I. & RODEN, M. 1999. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans; a 1H NMR spectroscopy study. *Diabetologia,* 42.

KUHL, J., HILDING, A., OSTENSON, C. G., GRILL, V., EFENDIC, S. & BÅVENHOLM, P. 2005. Characterisation of subjects with early abnormalities of glucose tolerance in the Stockholm Diabetes Prevention Programme: the impact of sex and type 2 diabetes heredity. *Diabetologia,* 48**,** 35-40.

LAMBERT, J. E. & PARKS, E. J. 2012. Postprandial metabolism of meal triglyceride in humans. *Biochimica et Biophysica Acta,* 1821**,** 721-726.

LAMONT, L. S. 2005. Gender differences in amino acid use during endurance exercise. *Nutrition Reviews,* 63**,** 419-422.

LAMONT, L. S., MCCULLOUGH, A. J. & KALHAN, S. C. 2001. Gender differences in leucine, but not lysine, kinetics. *Journal of Applied Physiology,* 91**,** 357-362.

LANGFORT, J., PLOUG, T., IHLEMANN, J., HOLM, C. & GALBO, H. 2000. Stimulation of hormone-sensitive lipase activity by contractions in rat skeletal muscle. *BioChemical Journal,* 351, 207-214.

LANGFORT, J., PLOUG, T., IHLEMANN, J., SALDO, M., HOLM, C. & GALBO, H. 1999. Expression of hormone-sensitive lipase and its regulation by adrenaline in skeletal muscle. *Biochemical Journal,* 340, 459-465.

LANGIN, D., DICKER, A., TAVERNIER, G., HOFFSTEDT, J., MAIRAL, A., RYDÉN, M., ARNER, E., SICARD, A., JENKINS, C. M., VIGUERIE, N., VAN HARMELEN, V., GROSS, R. W., HOLM, C. & ARNER, P. 2005. Adipocyte lipases and defect of lipolysis in human obesity. *Diabetes,* 54**,** 3190-3197.

LAURENT, D., HUNDAL, R. S., DRESNER, A., PRICE, T. B., VOGEL, S. M., PETERSEN, K. F. & SHULMAN, G. I. 2000. Mechanism of muscle glycogen autoregulation in humans. *American Journal of Physiology, Endocrinology and Metabolism,* 278**,** E663-8.

LIM, S. S., VOS, T., FLAXMAN, A. D., DANAEI, G., SHIBUYA, K., ADAIR-ROHANI, H., AMANN, M., ANDERSON, H. R., ANDREWS, K. G., ARYEE, M., et al. 2012. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet,* 380**,** 2224-2260.

LUNDSGAARD, A.-M. & KIENS, B. 2014. Gender differences in skeletal muscle substrate metabolism - molecular mechanisms and insulin sensitivity. *Frontiers in Endocrinology,* 5**,** 1-6.

MAHER, A. C., AKHTAR, M. & TARNOPOLSKY, M. A. 2010. Men supplemented with 17beta-estradiol have increased beta-oxidation capacity in skeletal muscle. *Physiological Genomics,* 42, 342-347.

MARTIN, W. H., DALSKY, G. P., HURLEY, B. F., MATTHEWS, D. E., BIER, D. M., HAGBERG, J. M., ROGERS, M. A., KING, D. S. & HOLLOSZY, J. O. 1993. Effect of endurance training on plasma free fatty acid turnover and oxidation during exercise. *The American Journal of Physiology,* 265**,** E708-E714.

MITTENDORFER, B., HOROWITZ, J. F. & KLEIN, S. 2001. Gender differences in lipid and glucose kinetics during short-term fasting. *American Journal of Physiology, Endocrinology and Metabolism,* 281**,** E1333-E1339.

MORO, C., BAJPEYI, S. & SMITH, S. R. 2008. Determinants of intramyocellular triglyceride turnover: implications for insulin sensitivity. *American Journal of Physiology, Endocrinology and Metabolism,* 294**,** E203-13.

MUST, A., SPADANO, J., COAKLEY, E. H., FIELD, A. E., COLDITZ, G. & DIETZ, W. H. 1999. The Disease Burden Associated With Overweight and Obesity. *The Journal of the American Medical Association,* 282**,** 1523-1529.

NICKLAS, B. J., HACKNEY, A. C. & SHARP, R. L. 1989. The menstrual cycle and exercise: performance, muscle glycogen, and substrate responses. *International Journal of Sports Medicine,* 10: 264-269.

OLSHANSKY, S. J., PASSARO, D. J., HERSHOW, R. C., LAYDEN, J., CARNES, B. A., BRODY, J., HAYFLICK, L., BUTLER, R. N., ALLISON, D. B. & LUDWIG, D. S. 2005. A Potential Decline in Life Expectancy in the United States in the 21st Century. *The New England Journal of Medicine,* 352**,** 1138-1145.

OOSTHUYSE, T. & BOSCH, A. N. 2012. Estrogen's regulation of fat metabolism during exercise and gender specific effects. *Current Opinion in Pharmacology,* 12, 363-371.

OWEN, O. E., MORGAN, A. P., KEMP, H. G., SULLIVAN, J. M., HERRERA, M. G. & CAHILL, G. F. 1967. Brain metabolism during fasting. *The Journal of Clinical Investigation,* 46**,** 1589-1595.

PAN, D., LILLIOJA, S., KRIKETOS, A., MILNER, M., BAUR, L., BOGARDUS, C., JENKINS, A. & STORLIEN, L. 1997. Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes,* 46**,** 983-988.

PHILLIPS, D. I., CADDY, S., ILIC, V., FIELDING, B. A., FRAYN, K. N., BORTHWICK, A. C. & TAYLOR, R. 1996a. Intramuscular triglyceride and

muscle insulin sensitivity: evidence for a relationship in non-diabetic subjects. *Metabolism: Clinical and Experimental,* 45**,** 947-950.

PHILLIPS, S. M., GREEN, H. J., TARNOPOLSKY, M. A., HEIGENHAUSER, G. F., HILL, R. E. & GRANT, S. M. 1996b. Effects of training duration on substrate turnover and oxidation during exercise. *Journal of Applied Physiology,* 81**,** 2182-2191.

RATTARASARN, C., LEELAWATTANA, R. & SOONTHORNPUN, S. 2010. Contribution of skeletal muscle mass on sex differences in 2-hour plasma glucose levels after oral glucose load in Thai subjects with normal glucose tolerance. *Metabolism: Clinical and Experimental,* 59**,** 172-176.

RENNIE, K. L. & JEBB, S. A. 2005. Prevalence of obesity in Great Britain. *Obesity Review,* 6**,** 11-12.

ROMIJN, J. A., COYLE, E. F., SIDOSSIS, L. S., GASTALDELLI, A., HOROWITZ, J. F., ENDERT, E. & WOLFE, R. R. 1993. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *American Journal of Physiology,* 265**,** E380-E391.

ROSS, R., DAGNONE, D., JONES, P. J. H., SMITH, H., PADDAGS, A., HUDSON, R. & JANSSEN, I. 2000. The effects of losing weight through diet or exercise programs on obesity. *Annals of Internal Medicine,* 133**,** 92-92.

RUBY, B. C., ROBERGS, R. A., WATERS, D. L., BURGE, M., MERMIER, C. & STOLARCZYK, L. 1997. Effects of estradiol on substrate turnover during exercise in amenorrheic females. *Medicine and Science in Sports and Exercise,* 29, 1160-1169.

SAVAGE, D. B., PETERSEN, K. F. & SHULMAN, G. I. 2007. Disordered lipid metabolism and the pathogenesis of insulin resistance. *Physiological Reviews,* 87, 507-520.

SCHMITZ-PEIFFER, C. 2000. Signalling aspects of insulin resistance in skeletal muscle. *Cellular Signalling,* 12, 583-594.

SCHRAUWEN-HINDERLING, V. B., SCHRAUWEN, P., HESSELINK, M. K., VAN ENGELSHOVEN, J. M., NICOLAY, K., SARIS, W. H., KESSELS, A. G. & KOOI, M. E. 2003. The increase in intramyocellular lipid content is a very early response to training. *The Journal of Clinical Endocrinology and Metabolism,* 88**,** 1610-1616.

SHAW, C. S., CLARK, J. & WAGENMAKERS, A. J. M. 2010. The Effect of Exercise and Nutrition on Intramuscular Fat Metabolism and Insulin Sensitivity. *Annual Review of Nutrition,* 30**,** 13-34.

SIDOSSIS, L. S., STUART, C. A., SHULMAN, G. I., LOPASCHUK, G. D. & WOLFE, R. R. 1996. Glucose plus insulin regulate fat oxidation by controlling the rate

of fatty acid entry into the mitochondria. *The Journal of Clinical Investigation,* 98**,** 2244-2250.

SIMONEAU, J. A., COLBERG, S. R., THAETE, F. L. & KELLEY, D. E. 1995. Skeletal muscle glycolytic and oxidative enzyme capacities are determinants of insulin sensitivity and muscle composition in obese women. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology,* 9**,** 273-278.

SPRIET, L. 2012. The metabolic systems: lipid metabolism. *In:* FARRELL, P., JOYNER, M. & CAIOZZO, V. (eds.) *Advanced Exercise Physiology.* Philadelphia: Lippincott, Williams and Wilkins.

SPRIET, L. L. 2002. Regulation of skeletal muscle fat oxidation during exercise in humans. *Medicine and Science in Sports and Exercise,* 34**,** 1477-1484.

STACHENFELD, N. S. & TAYLOR, H. S. 2014. Challenges and methodology for testing young healthy women in physiological studies. *American Journal of Physiology, Endocrinology and Metabolism,* 306, E849-E853.

STANNARD, S. R., BUCKLEY, A. J., EDGE, J. A. & THOMPSON, M. W. 2010. Adaptations to skeletal muscle with endurance exercise training in the acutely fed versus overnight-fasted state. *Journal of Science and Medicine in Sport,* 13**,** 465-469.

STEVENS, G. A., SINGH, G. M., LU, Y., DANAEI, G., LIN, J. K., FINUCANE, M. M., BAHALIM, A. N., MCINTIRE, R. K., GUTIERREZ, H. R., COWAN, M., PACIOREK, C. J., FARZADFAR, F., RILEY, L., EZZATI, M. & GLOBAL BURDEN OF METABOLIC RISK FACTORS OF CHRONIC DISEASES COLLABORATING, G. 2012. National, regional, and global trends in adult overweight and obesity prevalence. *Population Health Metrics,* 10**,** 22-22.

TARNOPOLSKY, L. J., MACDOUGALL, J. D., ATKINSON, S. A., TARNOPOLSKY, M. A. & SUTTON, J. R. 1990. Gender differences in substrate for endurance exercise. *Journal of Applied Physiology,* 68**,** 302-308.

TARNOPOLSKY, M. A. 2000. Gender differences in metabolism; nutrition and supplements. *Journal of Science and Medicine in Sport,* 3**,** 287-298.

TARNOPOLSKY, M. A. 2008. Sex differences in exercise metabolism and the role of 17-beta estradiol. *Medicine and Science in Sports and Exercise,* 40**,** 648-654.

TARNOPOLSKY, M. A., ATKINSON, S. A., PHILLIPS, S. M. & MACDOUGALL, J. D. 1995. Carbohydrate loading and metabolism during exercise in men and women. *Journal of Applied Physiology,* 78, 1360-1368.

THOMPSON, D., PEACOCK, O. J. & BETTS, J. A. 2014. Substitution and compensation Erode the energy deficit from exercise interventions. *Medicine and Science in Sports and Exercise,* 46**,** 423.

TURCOTTE, L. P., RICHTER, E. A. & KIENS, B. 1992. Increased plasma FFA uptake and oxidation during prolonged exercise in trained vs. untrained humans. *The American Journal of Physiology,* 262**,** E791-9.

VAN HALL, G. 2015. The physiological regulation of skeletal muscle fatty acid supply and oxidation during moderate-intensity exercise. *Sports Medicine,* 45 Suppl 1**,** S23-32.

VAN HALL, G., SACCHETTI, M., RÅDEGRAN, G. & SALTIN, B. 2002. Human skeletal muscle fatty acid and glycerol metabolism during rest, exercise and recovery. *The Journal of Physiology,* 543**,** 1047-1058.

VAN LOON, L. J. C. 2004. Use of intramuscular triacylglycerol as a substrate source during exercise in humans. *Journal of Applied Physiology,* 97**,** 1170-1187.

VAN LOON, L. J. C., GREENHAFF, P. L., CONSTANTIN-TEODOSIU, D., SARIS, W. H. M. & WAGENMAKERS, A. J. M. 2001. The effects of increasing exercise intensity on muscle fuel utilisation in humans. *The Journal of Physiology,* 536**,** 295-304.

VAN LOON, L. J. C., KOOPMAN, R., STEGEN, J. H. C. H., WAGENMAKERS, A. J. M., KEIZER, H. A. & SARIS, W. H. M. 2003. Intramyocellular lipids form an important substrate source during moderate intensity exercise in endurance-trained males in a fasted state. *Journal of Physiology,* 553.2, 611-625.

VAN PROEYEN, K., SZLUFCIK, K., NIELENS, H., PELGRIM, K., DELDICQUE, L., HESSELINK, M., VAN VELDHOVEN, P. P. & HESPEL, P. 2010. Training in the fasted state improves glucose tolerance during fat-rich diet. *The Journal of Physiology,* 588**,** 4289-4302.

VAN PROEYEN, K., SZLUFCIK, K., NIELENS, H., RAMAEKERS, M. & HESPEL, P. 2011. Beneficial metabolic adaptations due to endurance exercise training in the fasted state. *Journal of Applied Physiology,* 110**,** 236-245.

VENABLES, M. C. & JEUKENDRUP, A. E. 2008. Endurance training and obesity: effect on substrate metabolism and insulin sensitivity. *Medicine and Science in Sports and Exercise,* 40**,** 495-502.

VENABLES, M. C. & JEUKENDRUP, A. E. 2009. Physical inactivity and obesity: links with insulin resistance and type 2 diabetes mellitus. *Diabetes/Metabolism Research and Reviews,* 25 Suppl 1, S18-S23.

VIEIRA, A. F., COSTA, R. R., MACEDO, R. C. O., COCONCELLI, L. & KRUEL, L. F. M. 2016. Effects of aerobic exercise performed in fasted v. fed state on fat and carbohydrate metabolism in adults: a systematic review and meta-analysis. *The British Journal of Nutrition,* 116**,** 1153-1164.

VOGELS, N., DIEPVENS, K. & WESTERTERP-PLANTENGA, M. 2005. Predictors of long-term weight maintenance. *Obesity Research,* 13**,** 2162-2168.

WAGENMAKERS, A., BROOKES, J., COAKLEY, J., REILLY, T. & EDWARDS, R. 1989. Exercise-induced activation of the branched-chain 2-oxo acid dehydrogenase in human muscle. *European Journal of Applied Physiology,* 59**,** 9.

WAGENMAKERS, A. J. M. 1991. Carbohydrate supplementation glycogen depletion and amino acid metabolism during exercise. *American Journal of Physiology,* 260**,** E883-E890.

WALLIS, G. A. & GONZALEZ, J. T. 2019. Is exercise best served on an empty stomach? *The Proceedings of the Nutrition Society,* 78**,** 110-117.

WASSERMAN, D. 2009. Four grams of glucose. *American Journal of Physiology, Endocrinology and Metabolism,* 296(1)**,** E11–E21.

WATT, M. J., HEIGENHAUSER, G. J. & SPRIET, L. L. 2002a. Intramuscular triacylglycerol utilization in human skeletal muscle during exercise; is there a controversy. *Journal of Applied Physiology,* 93(4)**,** 1185-1195.

WATT, M. J., HEIGENHAUSER, G. J. F., DYCK, D. J. & SPRIET, L. L. 2002b. Intramuscular triacylglycerol, glycogen and acetyl group metabolism during 4 h of moderate exercise in man. *The Journal of Physiology,* 541**,** 969-978.

WHO 2000. Obesity preventing and managing the global epidemic. Report of a WHO consultation. World Health Tech Rep Ser. 894: i-xii. *World Health Organization,* 9 5.

WHO 2013. Obesity and overweight. *World Health Organization*.

WOLFE, R. R. 1998. Metabolic interactions between glucose and fatty acids in humans. *The American Journal of Clinical Nutrition,* 67**,** 519S-526S.

WOLFE, R. R., KLEIN, S., CARRARO, F. & WEBER, J. M. 1990. Role of triglyceride-fatty acid cycle in controlling fat metabolism in humans during and after exercise. *The American Journal of Physiology,* 258**,** E382-9.

WU, C.-L., NICHOLAS, C., WILLIAMS, C., TOOK, A. & HARDY, L. 2003. The influence of high-carbohydrate meals with different glycaemic indices on substrate utilisation during subsequent exercise. *The British Journal of Nutrition,* 90**,** 1049-1056.

YEO, W. K., CAREY, A. L., BURKE, L., SPRIET, L. L. & HAWLEY, J. A. 2011. Fat adaptation in well-trained athletes: effects on cell metabolism. *Applied Physiology, Nutrition and Metabolism,* 36**,** 12-22.

ZDERIC, T. W., COGGAN, A. R. & RUBY, B. C. 2001. Glucose kinetics and substrate oxidation during exercise in the follicular and luteal phases. *Journal of Applied Physiology,* 90: 447-453.

ZEHNDER, M., CHRIST, E. R., ITH, M., ACHESON, K. J., POUTEAU, E., KREIS, R., TREPP, R., DIEM, P., BOESCH, C. & DÉCOMBAZ, J. 2006.

Intramyocellular lipid stores increased markedly in athletes after 1.5 days lipid supplementation and are utilized during exercise in proportion to their content. *European Journal of Applied Physiology,* 98**,** 341-354.

CHAPTER 2

Influence of Fed vs. Overnight-Fasted State Exercise on Whole-body and Skeletal Muscle Substrate Utilisation in Obese or Overweight/Centrally Obese Men

In this chapter, there were several individuals involved during the data collection and analysis other than author of this thesis:

1. *Nurul Fadhilah Abdullah (author) conducted data collection, sample analysis for substrate utilisation, blood samples, IMTG content for eight participants and performed all statistical analysis.*
2. *Dr Helen Bradley provided oversight on sample analysis for IMTG content and conducted muscle glycogen analysis.*
3. *Dr Scott Robinson contributed in data collection and sample analysis (breath and blood samples only) for four participants.*
4. *Dr Gareth Wallis performed the muscle biopsy procedure.*
5. *Dr Konstantinos Manolopoulos gave medical oversight including inspection of ECG traces.*

2.1 Summary

An elevated storage of fat within muscle (IMTG) is common in obese individuals and is associated with insulin resistance. Exercise performed in the overnight-fasted versus fed-state has been shown to augment IMTG utilisation in lean individuals; however this has not yet been studied in obese populations. This study aimed to investigate the effects of a single exercise bout performed either before or after breakfast on exercise substrate and IMTG utilisation in obese or overweight/centrally obese men. Using a randomized, crossover design, twelve obese or overweight/centrally obese (BMI 30.2 ± 3.5 kg/m^2; waist circumference 105.7 ± 11.6 cm) but otherwise healthy young (29.7 ± 9.9 years) men performed 1-h of constant load bicycle exercise (65% $\dot{V}O_{2peak}$) followed by 3-h of controlled recovery. On one occasion, exercise was performed after an overnight fast (Fasted) and a standardised breakfast (25% daily energy intake; 65% carbohydrate, 20% fat and 15% protein) was consumed immediately after exercise, and on the other occasion they consumed the breakfast 90 minutes before exercise (Fed). Indirect calorimetry was used during exercise to measure whole body substrate utilisation and venous blood was collected routinely before, during and after exercise to assess the plasma hormone and metabolite response (n=12). Skeletal muscle biopsies were collected immediately before and after exercise for determination of fibre-type specific IMTG content (fluorescence microscopy) and mixed muscle glycogen concentration (n=8). During exercise, fat oxidation (0.31 ± 0.09 vs. 0.22 ± 0.07 g/min respectively, P=0.014) and concentration of plasma glycerol (P<0.001), NEFA (P<0.001) were significantly higher in Fasted vs. Fed. There was a significant diet-

time interaction for Type I fibre IMTG content (P<0.05). Exercise performed in fasted state significantly reduced Type I fibre IMTG content (13.78 ± 4.06% to 10.34 ± 3.41% area covered by lipid staining, P=0.008, post-hoc P=0.015), but no significant change was seen in the fed state (9.78 ± 4.81% to 11.22 ± 4.86% area covered by lipid staining, P=0.725). There was no significant change in Type II fibre IMTG content from pre- to post-exercise in either trial. Muscle glycogen use during exercise was similar in both trials (~60% reduction, P<0.001). This study showed that in obese or overweight/centrally obese men, an acute bout of aerobic exercise performed in the overnight-fasted state augmented whole-body exercise fat oxidation and promoted Type I fibre IMTG utilisation as compared to exercise performed in the fed state. The potential significance of these observations for metabolic health now needs to be studied with long-term training studies in overweight and obese populations.

2.2 Introduction

Overweight and obesity are an established health problem worldwide with prevalence increasing (Baskin et al., 2005, Rennie and Jebb, 2005, Gallus et al., 2015). Importantly, obesity has been related to insulin resistance, a precursor to cardiovascular disease (Klein et al., 2004) and type 2 diabetes (WHO, 2000). Overweight and obesity has been defined as excessive fat accumulation in the body that is stored largely in subcutaneous and deep visceral adipose tissue. In addition, overweight and obesity are associated with ectopic fat storage, that is, storage of fat in sites such as the liver and in skeletal muscle as IMTG (Goodpaster et al., 2000). Even though IMTG content makes up for only small portions of the total fat stores within the body (van Loon, 2004), elevation of IMTG content has been associated with insulin resistance (Goodpaster et al., 1997, Krssak et al., 1999). It seems most likely that increased intramuscular lipid metabolite concentrations associated with high IMTG content, such as LCFA-CoA, DAGs and ceramides, compromise the action of insulin to facilitate glucose uptake in skeletal muscle (Hulver and Dohm, 2004). Thus, it has been suggested that a high turnover rate of the IMTG pool (utilisation and storage) may aid in reducing the intracellular concentration of such lipid metabolites, and thus reduce lipotoxic stress and insulin resistance in skeletal muscle (Moro et al., 2008).

One strategy that may increase IMTG turnover and reduce accumulation of insulin resistance inducing lipid metabolites is exercise (Shaw et al., 2010). For example, endurance trained athletes typically have higher IMTG concentrations yet also high

muscle oxidative capacity and insulin sensitivity (Goodpaster et al., 2001, Russell, 2004). It is thought that the regular utilisation (oxidation) and re-synthesis of IMTG serves to maintain high levels of insulin sensitivity in these individuals. Indeed, prolonged moderate intensity aerobic exercise (2 h cycling at 60% $\dot{V}O_2max$) performed in the fasted state promotes the utilisation of IMTG in lean young men, particularly in Type 1 muscle fibres (van Loon et al., 2003). Further, De Bock and colleagues demonstrated in young lean men that 2-hour of submaximal cycling exercise (~75% $\dot{V}O_{2max}$) performed in the overnight-fasted state induced a high fat oxidation rate and breakdown of IMTG in Type 1 skeletal muscle fibres (De Bock et al., 2005). In contrast, they found that fat oxidation was reduced and IMTG breakdown completely blunted when exercise was performed in the fed state. Thus, the utilisation of IMTG during exercise appears to depend on whether the individual undertakes the exercise bout in the fed or fasted state, at least in lean individuals.

Regular exercise is recommended as a treatment strategy for overweight and obesity. However, in the absence of significant weight loss, exercise training appears to have minimal or modest impact on insulin sensitivity (Ross et al., 2000). Whether this is related to the utilisation of IMTG during the exercise bouts in these training studies is unknown. Interestingly, a short-term (6 weeks) exercise training study in lean men showed training in fasted state, which presumably augmented IMTG turnover, protected participants from high-fat diet induced impairments in oral glucose tolerance that were observed when participants performed exercise training bouts in the fed state (Van Proeyen et al., 2010). Given that exercise-training studies in obese that did not induce significant weight loss have generally not considered

control for timing of recent nutrition around exercise, it could be that the nutritional status was not optimal to promote fat oxidation and utilisation of IMTG during each bout. Thus, the benefits of exercise training for insulin sensitivity obese populations in the absence of clear weight loss might have been compromised.

The current study investigated the effects of a single exercise bout performed either before or after feeding on exercise substrate and IMTG utilisation, muscle glycogen use and plasma and hormone metabolite in obese or overweight/centrally obese men. It was hypothesized that, an acute bout of aerobic exercise performed in the overnight-fasted can increase fat oxidation and stimulate greater IMTG utilisation during exercise as compared to fed-state exercise.

2.3 Methods

2.3.1 Participants

The present randomized, crossover study was carried-out in Birmingham, UK. Several recruitment approaches were used, including advertisement in the university online portal, poster and newspaper advertisement. Participants were selected on the basis that they were apparently healthy men, low active and they were obese (white European men, BMI 30-34.9 kg/m^2 or Asian BMI 27.5-35 kg/m^2) or overweight/centrally obese (BMI, white European 25-29.9 kg/m^2 or Asian 23-27.4 kg/m^2 with a high waist circumference, white European ≥ 94 cm [37 inches], Asian >90 cm [35 inches]). This population was selected as they were identified as at risk of cardiovascular disease and type II diabetes based on the anthropometry (NICE, 2014). A total of 12 individuals undertook the study. For muscle analysis, only eight participants were included because the muscle samples of the other four participants were destroyed due to freezer failure. The present study was approved by the National Research Ethics Committee West Midlands - The Black Country (Ref: 15/WM/0128). Written informed consent was also obtained prior to the study from participants. The participant characteristics are presented in **Table 2.1**.

Table 2.1 General characteristics of participants

	n = 12	*n* = 8 (muscle analysis group)
Age (years)	29.7 ± 9.9	28.9 ± 10.1
Weight (kg)	95.1 ± 13. 6	90.0 ± 13.2
Height (m)	1.77 ± 0.06	1.75 ± 0.06
BMI (kg/m^2)	30.2 ± 3.5	29.2 ± 3.1
Waist circumference (cm)	105.7 ± 11.6	101.3 ± 10.2
Hip circumference (cm)	110.9 ± 6.5	109.4 ± 7.1
Systolic blood pressure (mmHg)	129 ± 6	132 ± 8
Diastolic blood pressure (mmHg)	79 ± 6	78 ± 7
$\dot{V}O_{2peak}$ (ml/kg/min)	29.1 ± 5.3	28.9 ± 5.9
Resting metabolic rate (kcal/d)	1557 ± 323	1458 ± 308
Estimated energy expenditure (kcal/d)	2365 ± 494	2204 ± 462
Fasting plasma glucose (mmol/L)	5.2 ± 0.5	5.3 ± 0.3
2-h plasma glucose (OGTT) (mmol/L)	5.1 ± 1.0	4.7 ± 0.9
Ethnicity, *n*		
White	6	2
Bangladeshi	1	1
Chinese	2	2
Other Asian	3	3

Mean ± SD

2.3.2 Study design

The study involved four laboratory visits. The first visit involved provision of informed consent followed by completion of a General Health Questionnaire [GHQ] and determination of BMI, waist circumference, blood pressure, electrocardiogram [ECG] and an oral glucose tolerance test [OGTT]. Visit 2 involved assessment of resting metabolic rate [RMR] and a peak aerobic capacity test [$\dot{V}O_{2peak}$], as well as familiarisation with the appropriate exercise intensity to be used in the experimental trials (Visits 3 and 4).

Visits 3 and 4 were the main experimental visits. These two visits were separated by one to two weeks. Two days before these visits, participants were provided with a standardized food package based on individual energy requirements. These visits lasted for ~8 hours, and included exercise performed in overnight fasted or fed state (randomized order), with indirect calorimetry during exercise, skeletal muscle biopsy samples collected before and after exercise and venous blood samples were obtained before, during and in recovery from exercise.

2.3.3 Experimental procedures

Visit 1 (Screening): This visit was used for consenting and screening based on inclusion criteria which included initially being: healthy as assessed by successful completion of a GHQ; aged between18 and 49 years; Class I obese BMI (white European men, BMI 30-34.9 kg/m^2 or Asian BMI 27.5-35 kg/m^2) or overweight (white

European, BMI 25-29.9 kg/m^2 or Asian, BMI 23-27.4 kg/m^2) with a high waist circumference (white European ≥ 94 cm [37 inches], Asian > 90 cm [35 inches]) (NICE, 2014); low active; stable weight (± 2 kg) for more than 3 months before enrolment. On the screening day, participants came to the laboratory in the morning in an overnight fasted state. On arrival, an investigator gave a verbal explanation about the purpose of the study and procedures involved to the participants before they signed informed consent. They then completed a GHQ (**Appendix 2**) to determine their health history. Body mass was measured using an electronic weighing scale (OHaus Champ II Scales, USA) and height was measured using a stadiometer (Stadiometer, SECA, UK) to the nearest 0.1 kg and 0.1 cm respectively. BMI was calculated as weight (kg) divided by height squared (m^2). Meanwhile, waist and hip circumferences were measured to the nearest 0.1 cm using a measuring tape. Resting blood pressure was determined using an automatic blood pressure monitor (Omron M6, Netherlands).

If participants successfully completed the Screening Form and met the eligibility requirements with respect to BMI, waist circumference and blood pressure (<140/90 mmHg), they were asked to undertake a 12-lead ECG examination (Oxycon Pro, Jaeger, Wurzburg, Germany) to check for potential cardiac abnormalities. Following this, an OGTT was performed to ensure none of the participants was undiagnosed diabetic. It involved comparing the concentrations of glucose in the plasma before and after drinking a glucose drink (containing 75 g glucose). For this, 5 ml blood was drawn via venepuncture at pre OGTT (fasting) and 2 hour after ingestion of glucose. The diagnostic criteria for diabetes is fasting plasma glucose ≥7 mmol/L or 2 hours

plasma glucose ≥11.1 mmol/L and for impaired glucose tolerance are fasting plasma glucose <7.0 mmol/L and 2-hour plasma glucose ≥7.8 and <11.1mmol/L (WHO and IDF, 2006).

Visit 2 (Baseline Assessments): This visit was conducted at least a week before the first main experiment trial (Visit 3). Upon arrival to the laboratory, participants rested for 20 minutes in the supine position in a dimmed and quiet room under a ventilated hood that connected to an indirect calorimeter (GEM Nutrition, Cheshire, UK) for RMR determination (see Section 2.3.6 for calculation). They were instructed to refrain from any movement during this period. Then, they were asked to perform an incremental exercise test on a stationary bicycle (Lode Sport Excalibur, Groningen, Netherlands) to determine their level of aerobic fitness ($\dot{V}O_{2peak}$) and also the workload required to elicit 65 % $\dot{V}O_{2peak}$ for use in later experimental trials. Participants started cycling at 35 watt [W] and were asked to maintain a pedal rate of between 60-70 revolutions per min [rev/min] with the power increased by 35 W every 3 minutes until they reached voluntary exhaustion (i.e. they stop on their own accord or the pedal rate dropped below 50 rev/min despite verbal encouragement from the investigator).

Their heart rate was monitored continuously throughout the testing using a heart rate monitor (Polar A300, Finland) and was recorded at the final 10 seconds of each exercise stage, as well as their self-rated perceived exertion using the Borg RPE scale (Borg, 1982). Breath-by-breath measurements of $\dot{V}O_2$ and $\dot{V}CO_2$ during the exercise were collected using a computerised gas analysis system (Oxycon Pro,

Jeager, Wurzburg, Germany). The data obtained was used to determine $\dot{V}O_{2peak}$ that was taken as the highest value of $\dot{V}O_2$ attained during a 10-second period on this test. After an approximately 30 minute rest period, participants were asked to perform a 30-minute moderate intensity (65% $\dot{V}O_{2peak}$) exercise bout to familiarise themselves with the exercise bout to be employed during subsequently experimental trials.

Visits 3 and 4 (Experimental trial; ***Figure 2.1****):* These two visits were separated by one to two weeks and for the 48 hours prior to each visit participants were provided with a standardized weight maintaining diet (50% carbohydrate, 35% fat, 15% protein) based on estimated individual energy requirements (see Section 2.3.6 for calculation). In addition, they were asked not to perform strenuous physical activity and abstain from alcohol and caffeine consumption during this period.

On the day of the experimental trials, participants were asked to drink a glass of water upon waking up to ensure there were adequately hydrated and they reported to the laboratory in the morning (approximately 7.30 am) after an approximately 12-hour overnight fast. Participants were instructed on the time and duration of their evening meal based on the estimated time of arrival at the laboratory. Upon arrival, a cannula was placed in a forearm vein for repeated blood sampling during the experimental visit. On one visit (Fed), immediately after a baseline blood sample was taken a standardised breakfast was consumed within 15 minutes (25% daily energy intake; 65% carbohydrate, 20% fat and 15% protein) based on estimated individual energy requirements. Further blood samples were at 45 and 90 minutes

after breakfast after which exercise was undertaken for one hour at moderate intensity (65% $\dot{V}O_{2peak}$).

During the exercise test, water was provided *ad libitum*. Heart rate was monitored continuously during exercise and recorded every 10 minutes (Polar A300, Finland), as well as self-rated perceived exertion (Borg, 1982). Breath-by-breath measurements were collected using indirect calorimetry at 25-30 and 55-60 minute of the exercise bouts. Blood samples were obtained before exercise, every 30 minutes during exercise and thereafter during a 3-h post exercise period at 1-hour intervals. Skeletal muscle biopsy samples were obtained immediately pre- and post-exercise and at 3 hours after exercise cessation. On the other visit (Fasted), participants underwent the same procedure with the difference of feeding timing, with breakfast consumed immediately after the post-exercise muscle biopsy was collected.

The environmental condition of the laboratory during the exercise bout was recorded and there was no significant difference between the two visits with humidity at 44 ± 8 % and 45 ± 9 %; temperature at 22.8 ± 1.1 °C and 22.8 ± 1.1 °C for fasted and fed conditions, respectively.

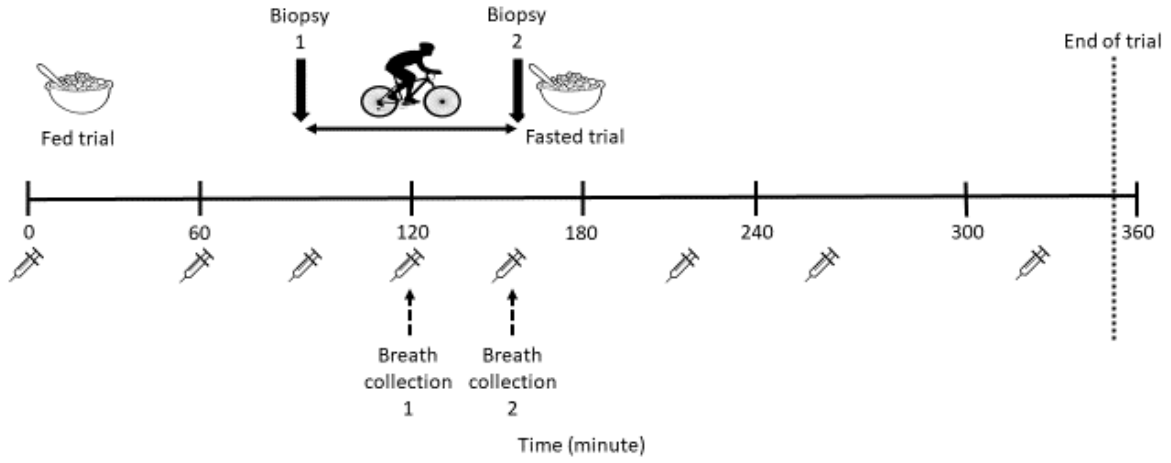

Figure 2.1 A schematic representation of the Experimental Trials.

2.3.4 Blood sampling and analysis

Venous blood samples (~10 ml) were obtained from an antecubital forearm vein at various time points as explained above. Approximately 6 ml blood samples were transferred into vacutainer containing ethylenediaminetetraacetic-acid [EDTA] and immediately centrifuged at 1361 g for 15 minutes at 4 ºC. The remaining sample was allowed to clot in a plain vacutainer, and the serum was separated after centrifugation. Aliquots of plasma or serum were stored at -80ºC until analysed. Plasma samples were analysed for glucose (Glucose Oxidase, Instrumentation Laboratories, Cheshire, UK), glycerol (GLY, Randox, London, UK), NEFA (NEFA, Randox, London, UK) and TG (TG kit, Instrumentation Laboratory, Warrington, UK) using an ILAB 650 Clinical Chemistry Analyser (Instrumentation Laboratory, Warrington, UK). The average intra-assay coefficient of variation for glucose was

1.5 %, glycerol was 2.8 %, NEFA was 3.3 % and TG was 1.3 %, while the inter-assay coefficient of variation for glucose and glycerol were 5.9 %, NEFA was 3.1 % and TG was 3.5 % based on analysis of 20 duplicate plasma samples ran across two assays.

Serum insulin concentration was measured by using a human insulin Enzyme Linked-Immuno-Sorbent Assay [ELISA] kit (Invitrogen, Paisley, UK) and a Biotek ELx800 analyser (Biotek Instruments, Vermont, USA). The average intra-assay coefficient of variation was 3.6 % based on 96 duplicate samples with the inter-assay coefficient of variation 7.3 % based on 10 samples ran across two assays.

2.3.5 Muscle sampling and analysis

Muscle biopsy collection: Skeletal muscle biopsy samples were obtained from the vastus lateralis for each participant using the suction-modified percutaneous needle biopsy technique (Bergstrom, 1975). Anaesthetic (1% lidocaine; Braun, Melsungen, Germany) was injected locally in skin, soft tissue below, and to the muscle fascia in the middle region of the vastus lateralis. Thereafter, a small incision (~6 mm) using a surgical scalpel (Swann-Morton, Sheffield, UK) was made through the skin and the fascia at ~15 cm above the patella. A Bergstrom needle was inserted to a depth of ~2–3 cm below the entry of the fascia, and a muscle sample (typically 50-100 mg wet weight) was obtained. Post-exercise muscle was collected from the opposite leg immediately after exercise. For IMTG content analysis, ~15-20mg of muscle was embedded in Tissue-Tek OCT (Sigma Aldrich, Dorset, UK) on cork disc, and rapidly

frozen in liquid nitrogen cooled isopentane, then transferred into an aluminium cryotube and stored at -80°C until assayed. The remaining muscle was transferred into microtubes in duplicate and immediately frozen in liquid nitrogen for glycogen and/or gene expression analysis. Pre- and post-exercise muscle was used herein, while 3-h post-exercise muscle in combination with pre-exercise muscle was used for gene expression analysis but this data was not part of present thesis.

IMTG content analysis: The muscle mounted in Tissue-Tek was cut into 5 µm thick transverse sections using a cryostat at −25°C (Bright 5040, Bright instrument Company limited, Huntingdon, England), then collected on an uncoated, pre-cleaned glass slide. Each slide contained four muscle samples for one participant (pre- and post-exercise muscle of visit 3 and 4) to decrease variation in staining intensity between muscle sections. As much as ten slides were prepared for each participant.

Then, cryosections were fixed in 3.7% formaldehyde for 1 hour. Slides were then rinsed three times with distilled water (dH_2O) for 30 seconds and treated for 5 minutes with 0.5% Triton-X100 in phosphate-buffered saline (PBS). Following this, the slides were washed three times for 5 min in PBS and incubated for 2 hours at room temperature with anti-dysthrophin (mouse IgG2b, Sigma D8168; 1:50 dilution in NGS) in 5% normal goat serum (Thermo Fisher 50-062Z; 1:1 dilution in PBS) and anti-myosin heavy chain I antibody (MHCI; mouse IgM, DSHB A4, 480; 1:50 dilution in NGS). This step enables visualisation of individual cell membranes and to determine muscle fibre-type (I or II), respectively. Incubation was followed by three

times 5 minutes washes in PBS, after which the appropriate conjugated antibodies which are GAM IgM 633 for MHCI (Thermo Fisher A21046; 1:150 dilution in PBS) and GAM IgG2b 594 for dystrophin (Thermo Fisher A21145; 1:200 dilution in PBS) were applied on the muscle sections and incubated for 30 minutes at room temperature. Then, it followed by another three times 5 minutes washes in PBS. Thereafter, muscle sections were incubated in Bodipy 493/503 solution (Thermo Fisher D3922; 1:50 dilution in PBS) for 20 minutes at room temperature in the dark room following two times 3 minutes washes in PBS. After the final wash, the stained sections were embedded with mowiol and covered with a cover slip to protect the muscle sections and preserve the fluorescence signal. Mowiol medium was made up from 6 g glycerol (Sigma Aldrich G5150), 2.4 g mowiol 4-88 (Fluka 81381) and 0.026 g 1,4-diazobicyclo- [2,2,2]-octane (DABCO) (Fluka 33490) which dissolved in 18 ml 0.2M Tris-buffer (pH 8.5) (Sigma Aldrich T5030). Slides were left to dry overnight at room temperature before analysed by confocal microscope. The staining was done in duplicate slides for each participant.

Images were obtained on a Leica confocal microscope (DMIRE2, Leica Microsystems) using 40x oil objective. An argon laser 488 nm was used to excite Bodipy-493/503, while a helium-neon [HeNe] 594 nm and 633 nm laser line were used to excite Alexa Fluor 594 (dystrophin) and Alexa Fluor 633 (MHCI), respectively. Images were scanned in projection of four lines saved in 1024x1025 pixels format. Imaging conditions such as gain, offset and laser power were kept constant for all muscle sections of each participant when acquiring images to be compared.

Quantification of lipid droplets was performed using Image J software. A spot threshold was set to detect IMTG content (bodipy spot stained; green) within the intracellular regions (dystrophin stained; red), and individual muscle fibres were manually delineated (major histocompatibility complex I [MHCI] stained; blue) as shown in **Figure 2.2**. Thresholds were kept constant between all images of each participant. IMTG content was measured as percent area of bodipy staining of the total fibre area ([bodipy stained area [um^2] / area of muscle [um^2]*100).

Glycogen analysis: For the determination of muscle glycogen concentration, 10-15mg of frozen muscle (-80 ºC) was powdered using a pestle and mortar pre-cooled on dry ice. The powdered muscles were then transferred into a glass tube pre-cooled on dry ice. Thereafter, the samples were hydrolysed by adding a 500 µl of 2M hydrogen chloride [HCL] and incubated for 2 h at 95 ºC. After cooling to room temperature, a 500µl 2M sodium hydroxide [NaOH] was added to the muscle samples. Samples were then centrifuged, and the supernatant was analysed for glucose concentration (Glucose Oxidase, Instrumentation Laboratories, Cheshire, UK) by using an ILAB 650 Clinical Chemistry Analyser (Instrumentation Laboratory, Warrington, UK).

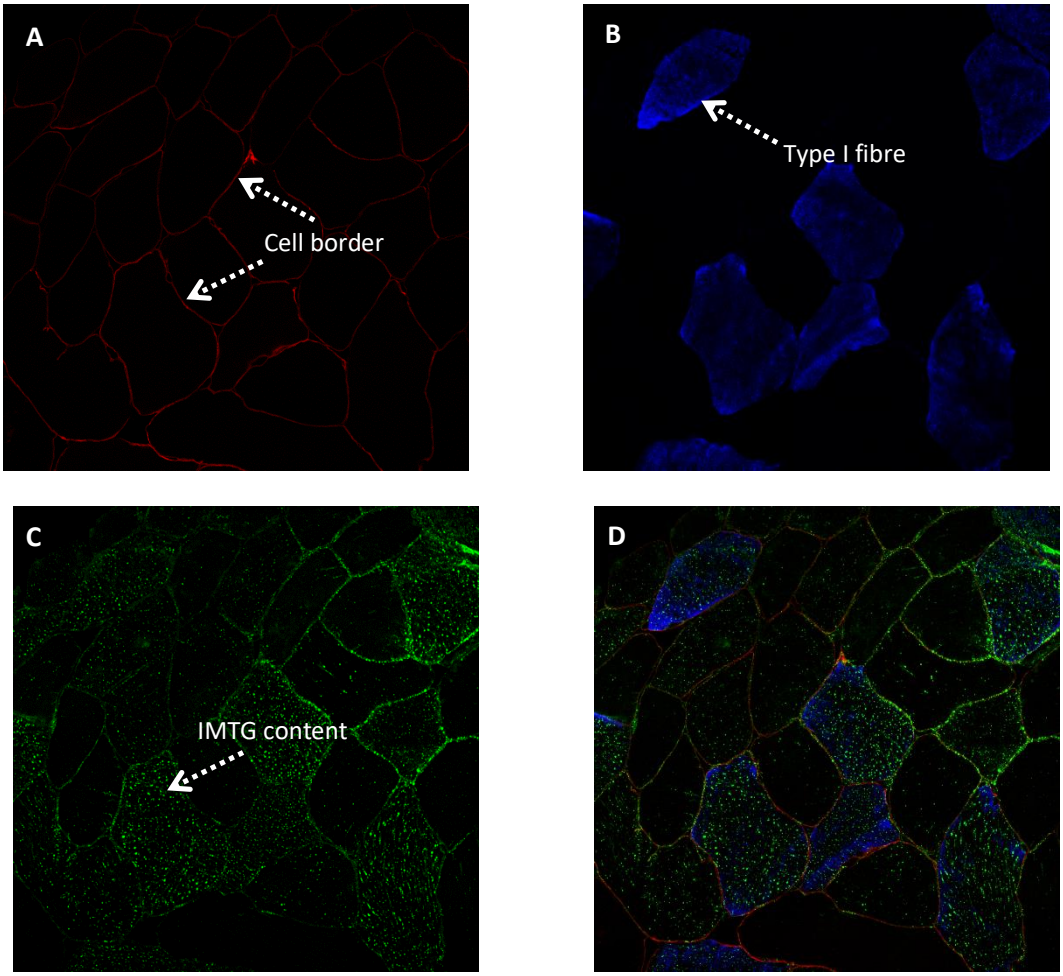

Figure 2.2 Images captured using confocal microscope (A) Dystrophin stained (red; cell border), (B) MHCI stained (blue; type I fibre), (C) Bodipy stained (green; IMTG content), (D) Overlaid image of dystrophin, MHCI and bodipy stained.

2.3.6 Calculations

During the preliminary testing, indirect calorimetry was used to collect the resting expired breath in order to measure the rate of $\dot{V}O_2$ and $\dot{V}CO_2$ for RMR determination, which was calculated using the equation of Weir (1949). Daily energy expenditure [DEE] was then estimated by multiplying RMR by a population specific activity factor (1.53; FAO, 2004).

$$RMR = 3.9*\dot{V}O_2 + 1.1*\dot{V}CO_2$$

$$DEE = RMR*1.53$$

For the experimental trials (Visit 3 and 4), the $\dot{V}O_2$ and $\dot{V}CO_2$ were used to quantify RER by diving $\dot{V}CO_2$ over $\dot{V}O_2$, while whole body carbohydrate and fat oxidation was calculated using equations from Frayn (1983) , with the assumption that the urinary nitrogen excretion rate was negligible during exercise.

$$\text{Carbohydrate oxidation (g/min)} = 4.55*\dot{V}CO_2 - 3.21*\dot{V}O_2$$

$$\text{Fat oxidation (g/min)} = 1.67*\dot{V}O_2 - 1.67*\dot{V}CO_2$$

For plasma and serum concentration, an area under the curve [AUC] was calculated using the trapezoid method:

$$AUC = AUC_{0\text{-}1} + AUC_{1\text{-}2} + \ldots + AUC_{x\text{-}last}$$

$$= ((C_0 + C_1)/2)*(T_1 - T_0) + ((C_1 + C_2)/2)*(T_2 - T_1) + \ldots + ((C_x + C_{last})/2)*(T_{last} - T_x)$$

Where;

C = plasma concentration

T = time

2.3.7 Statistical analysis

Data were analysed using Statistical Package for the Social Sciences [SPSS] software package (version 22, SPSS Inc. Chicago, IL) and the post-hoc test was performed manually in excel. Normality of the data was checked by Shapiro Wilk tests. Homogeneity of variances was confirmed by Mauchley's test of sphericity and a Greenhouse-Geisser correction was applied to the degrees of freedom if the sphericity assumption was violated. A paired-sample t-test was used to compare differences between trials in heart rate, rating of perceived exertion [RPE], $\dot{V}O_2$, $\dot{V}CO_2$, RER, fat oxidation (g/min and % energy from fat oxidation), carbohydrate oxidation (g/min and % energy from carbohydrate oxidation) and AUC for blood metabolites and insulin. A two-way ANOVA with repeated measures (trial x time) was used to compare differences in blood metabolites and insulin between trials and over time. Further, a paired-sample t-test was used to test the difference of each time point from baseline during each trial separately and the difference between trials for each time point. Where a significant effect (i.e., $P<0.05$) was indicated for these variables, the Holm-Bonferroni stepwise correction was made for location of variance post-hoc (Holm, 1979). A one-way within measures ANCOVA was used to compared the IMTG content and muscle glycogen utilisation between trials by adjusting the pre-exercise value. Normally distributed data are presented as means ± standard deviation unless otherwise stated. The 95% confidence intervals (CI) for mean absolute pairwise differences between trials were calculated using the t-distribution and degrees of freedom (n – 1). Absolute standardised effect sizes (ES)

were calculated for within-measures comparisons to supplement important findings as follows:

$$\text{ES} = \frac{\text{Mean v2} - \text{Mean v1}}{CON\ SD} \text{ (Cumming and Finch, 2001)}$$

In the absence of a clinical anchor, an ES of 0.2 was considered the minimum important difference for all outcome measures, 0.5 moderate and 0.8 large (Cohen, 1988). Statistical significance was accepted as $P<0.05$.

2.4 Result

2.4.1 Physiological and perceptual response to exercise

The exercise intensity attained was similar between experimental trials (66 ± 2 vs. 66 ± 2 % $\dot{V}O_{2peak}$ for Fasted and Fed, respectively). In addition, the RPE was also similar in both experimental trials (14 ± 2 vs. 14 ± 2 for Fasted and Fed, respectively) which qualitatively corresponded to a perception of somewhat hard to hard. The mean heart rate was significantly higher in Fasted than Fed trials (141 ± 15 vs. 133 ± 16 beat/min, respectively; *P*=0.041). There was no significant difference in $\dot{V}O_2$ during exercise between Fasted and Fed conditions (1802 ± 422 and 1810 ± 437 ml/min, respectively; *P*=0.686). In contrast, $\dot{V}CO_2$ was significantly higher during exercise in the Fed state as compared to Fasted (1676 ± 417 vs 1615 ± 387 ml/min for Fed and Fasted, respectively; *P*=0.021).

2.4.2 Substrate utilisation during exercise

Table 2.2 shows the substrate utilisation during exercise for the full set of 12 participants. There were significant differences observed for RER, fat and carbohydrate oxidation. RER was significantly lower during Fasted than Fed conditions (95% CI -0.05 to 0.01, ES = 1.56, *P*=0.012). Accordingly, the calculated fat oxidation rate, expressed as gram per minute, was significantly higher during Fasted exercise (95% CI 0.02 to 0.15, ES = 1.12, *P*=0.014). In contrast, the carbohydrate oxidation rate was significantly lower in Fasted exercise when

compared with Fed exercise (95% CI -0.42 to -0.08, ES = 0.53, *P*=0.009). When comparing among eight participants who had muscle biopsies, the differences between trials were not statistically significant but these differences were directionally consistent with the *n* = 12 (RER: 95% CI 0.01 to 0.06, ES = 1.18, *P*=0.099; fat oxidation rate: 95% CI -0.01 to 0.15, ES = 0.88, *P*=0.078; carbohydrate oxidation rate: 95% CI -0.39 to 0.02, ES = 0.35, *P*=0.067).

Table 2.2 Gas exchange and substrate oxidation during exercise

	n = 12		*n* = 8	
	Fasted state	Fed state	Fasted state	Fed state
RER	0.89 ± 0.03	0.93 ± 0.02*	0.89 ± 0.03	0.92 ± 0.02
Fat oxidation (g/min)	0.31 ± 0.09	0.22 ± 0.07*	0.31 ± 0.10	0.23 ± 0.08
Carbohydrate oxidation (g/min)	1.57 ± 0.44	1.82 ± 0.51**	1.47 ± 0.52	1.65 ± 0.52

Mean ± SD. Significant difference from fasted state at *P<0.05, **P<0.01

Consistently, as presented in **Figure 2.3**, percentage of energy from fat was higher during Fasted exercise as compared to Fed exercise (31.58 ± 8.7% vs. 22.23 ± 6.11%, respectively, 95% CI 1.92 to 1.58, ES = 1.24, *P*=0.018). In contrast percentage of energy from carbohydrate was higher during exercise in Fed than Fasted trials (68.52 ± 8.71% vs. 77.77 ± 6.11%, respectively, 95% CI -16.58 to -1.91, ES = 1.63, *P*=0.018). When comparing among eight participants, the differences were in similar direction but not statistically significant (*P*=0.105). Additionally, the result showed that the carbohydrate was the main source used as

a fuel during exercise in both trials with mean percentage of carbohydrate contribution to energy expenditure were 69 ± 9% during Fasted and 78 ± 6% during Fed exercise.

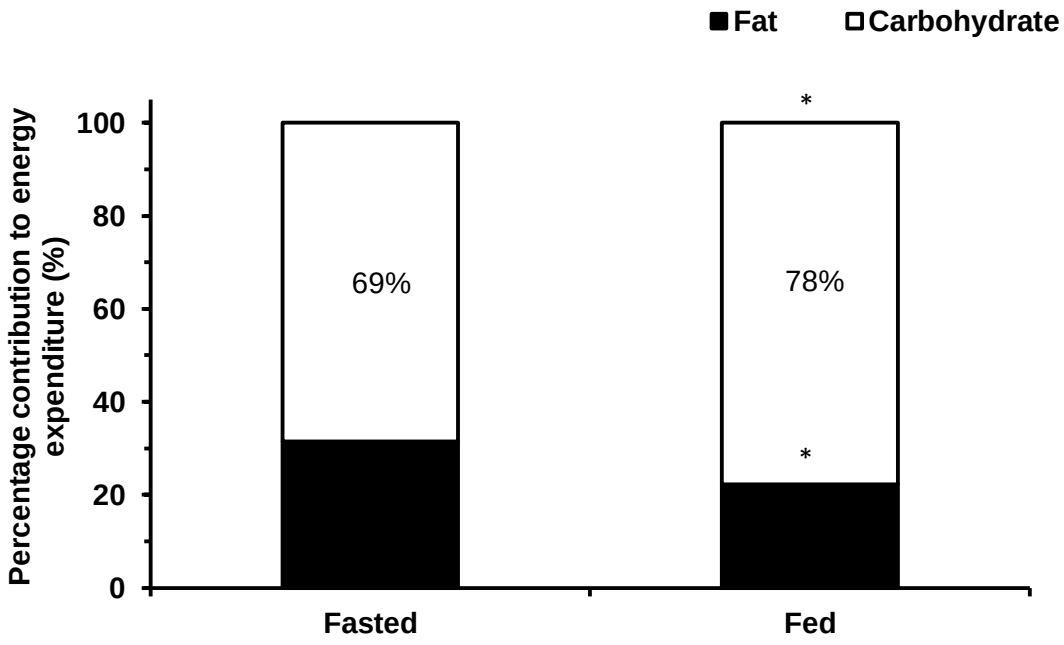

Figure 2.3 Percentage contributions to energy expenditure during exercise (n = 12). Data are means ± SEM. *Significant difference from fasted state at P<0.05.

2.4.3 Blood metabolites and hormone responses

At baseline, plasma glucose, glycerol, NEFA, triglyceride and serum insulin concentrations were not statistically different from one trial to another. There were however significant trial x time interactions for plasma glucose (*P*<0.001), glycerol (*P*<0.001), NEFA (*P*<0.001), triglyceride (*P*=0.017) and serum insulin concentrations (*P*=0.003).

During the Fasted trial, plasma glucose concentration was stable in the period before and during exercise, with no significant difference from baseline. However, the plasma glucose concentration increased significantly after exercise following meal ingestion (P=0.001), and had returned to almost baseline value after 3-hour of exercise finished (**Figure 2.4**). In contrast, in the Fed trial glucose concentration significantly increased following ingestion of the pre-exercise meal (P=0.019), returned to baseline values after 30 minutes of exercise and remained stable until the end of the trial. Plasma glucose concentration was significantly higher in the Fed trial at 45-minute post-meal before exercise than the Fasted trial (P=0.021), while the glucose concentration was significantly higher in the Fasted trial as compared to the Fed trial at 1-hour (P<0.001) and 2-hour (P=0.030) post exercise. AUC for glucose was 13.6% higher in the Fasted trial as compared to Fed (6.70 ± 1.09 mmol•L^{-1} vs. 5.90 ± 0.90 mmol•L^{-1}, respectively, 95% CI 0.40 to 1.19, ES = 0.80, P=0.001).

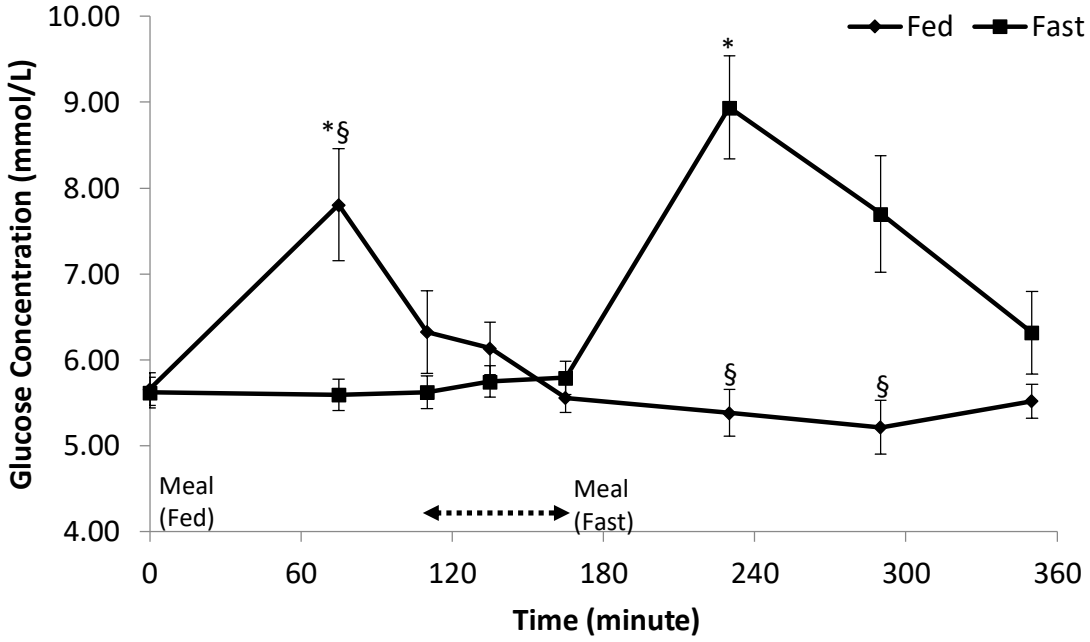

Figure 2.4 Changes in glucose concentrations in response to feeding and exercise (n = 12). Data are means ± SEM. Arrow represents the exercise period. *Significant difference from baseline. §Significant difference from Fasted.

A similar pattern to glucose concentration was also shown in serum insulin concentration (**Figure 2.5**), with the concentration stable before and during exercise but markedly increased following the meal ingestion after exercise (*P*=0.006) during the Fasted trial and remain elevated for the duration of the trial but this was not statistically different from baseline at 2 and 3 h post-exercise. In contrast, in the Fed trial, insulin concentration increased following ingestion of food before exercise and remained elevated at the onset of exercise (*P*=0.018) but returned to baseline values after the exercise bout was completed and then remained stable until the end of trial. Insulin concentration was significantly higher in the Fed vs. Fasted trial at 45-minute and 90-minute after pre-exercise meal (*P*=0.019 and *P*=0.012, respectively), in contrast insulin concentration was higher in the Fasted vs. Fed trial

at 1-hour post-exercise (45-minute post-exercise meal; *P*=0.005). AUC for insulin was 52.6% higher in the Fasted than Fed trial (491.79 ± 349.10 pmol•L^{-1} vs. 322.40 ± 220.05 pmol•L^{-1}, respectively, 95% CI 57.70 to 281.09, ES = 0.58, *P*=0.007).

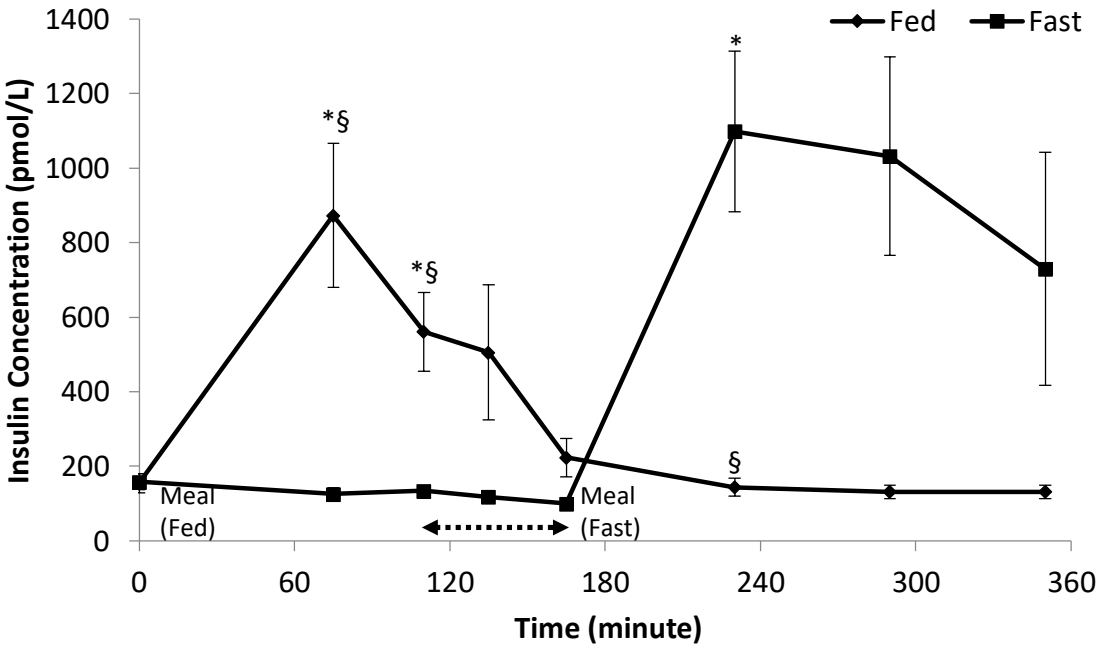

Figure 2.5 Changes in insulin concentrations in response to feeding and exercise (n = 12). Data are means ± SEM. Arrow represents the exercise period. *Significant difference from baseline. §Significant difference from Fasted.

During the Fasted trial, plasma glycerol concentration, as shown in **Figure 2.6,** was significantly decreased immediately before exercise (*P*=0.035) but significantly and markedly increased during exercise (*P*=0.003) and at the end of exercise bout (*P*<0.001) as compared to baseline. The concentration was then markedly decreased after the post-exercise meal and became significantly lower than baseline at 2-hour post exercise and remained so until the end of trial (*P*=0.001). In contrast, in the Fed trial glycerol concentration was significantly decreased after the

pre-exercise meal (*P*=0.001) but slightly increased during exercise and the concentration kept increasing reaching statistical significance from baseline by the end of trial (*P*=0.007). Plasma glycerol concentration was significantly higher in the Fasted than Fed trial at the onset of exercise (*P*=0.006) and during exercise (*P*=0.004), but significantly lower in the Fasted than Fed trial at post-exercise (2-hour, *P*=0.006; 3-hour, *P*<0.001). AUC for glycerol was 23.6% higher in Fasted than Fed trial (56.60 ± 19.94 μmol•L^{-1} vs. 45.80 ± 16.98 μmol•L^{-1}, respectively, 95% CI 0.09 to 20.76, ES = 0.58, *P*=0.036).

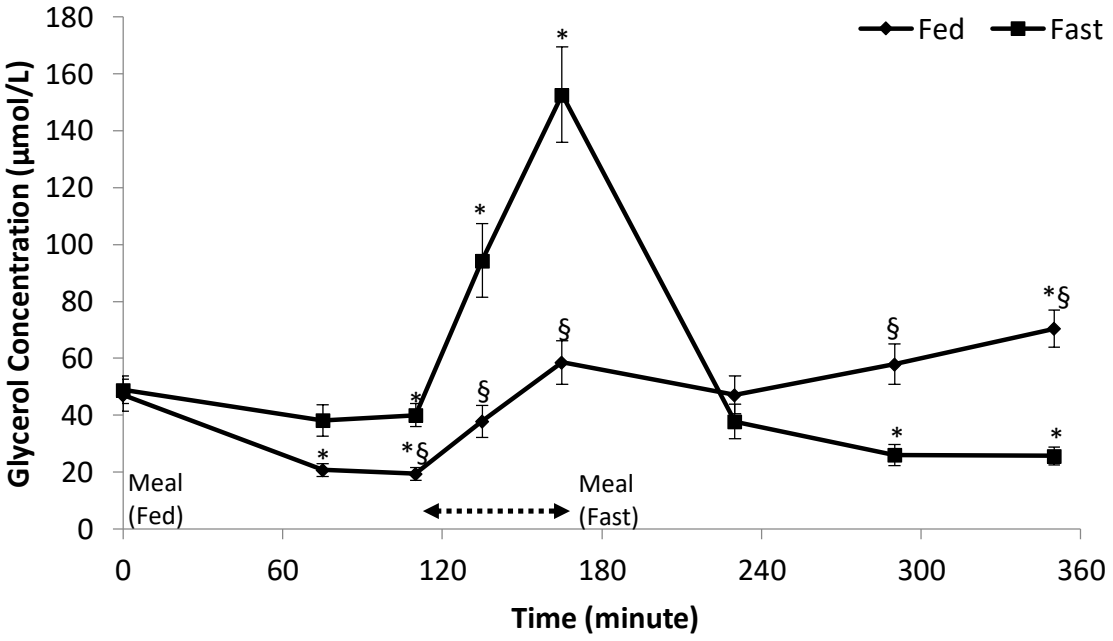

Figure 2.6 Changes in glycerol concentrations in response to feeding and exercise (n = 12). Data are means ± SEM. Arrow represents the exercise period. *Significant difference from baseline. §Significant difference from Fasted.

In the Fasted trial, plasma NEFA concentration was stable before exercise, but significantly decreased after 30-minute of exercise (*P*=0.024), and returned to

baseline values at the end of exercise (**Figure 2.7**). The post-exercise meal induced a decline in NEFA concentration which became significantly lower than baseline from 2-hour post-exercise and remained so until the end of trial (P<0.001). In contrast, in the Fed trial the pre-exercise meal significantly reduced NEFA concentration below baseline value before exercise (45-minute post-meal, P=0.004; 90-minute post-meal, P<0.001), and it stayed low during exercise (30-minute, P<0.001; 60-minute, P=0.001). However, it then started increasing above baseline after exercise reaching statistical significance at the end of the trial (P=0.009). NEFA concentration was higher in the Fasted trial during pre-exercise (P=0.030) and exercise (P=0.001) but lower during post-exercise as compared to the Fed trial (P=0.014). There was no significant difference of NEFA AUC between Fasted and Fed trial (0.46 ± 0.19 mmol•L^{-1} vs. 0.44 ± 0.19 mmol•L^{1}, respectively, 95% CI -0.10 to 0.12, ES = 0.11, P = 0.818).

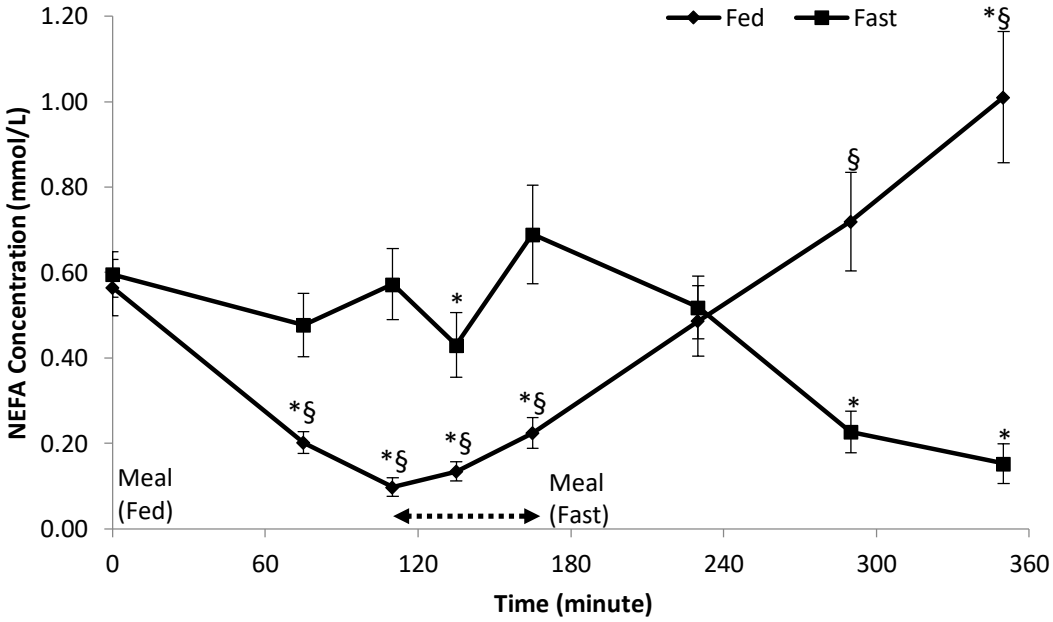

Figure 2.7 Changes in NEFA concentrations in response to feeding and exercise (n = 12). Data are means ± SEM. Arrow represents the exercise period. *Significant difference from baseline. §Significant difference from Fasted.

Plasma TG concentration at baseline was non-significantly higher in the Fasted than Fed trials (1.49 ± 1.04 mmol•L^{-1} vs. 1.34 ± 0.51 mmol•L^{-1}, respectively, 95% CI -0.29 to 0.58 ES = 0.18, *P*=0.473) as shown in **Figure 2.8**. In both trials, plasma TG concentration appeared to increase above baseline during exercise but this was only statistically significant in Fasted trial (30-minute: *P*=0.006; 60-minute: *P*=0.025). No significant difference between trials was observed at any points. AUC for TG was 4.6% lower in Fasted state than Fed trial albeit it is not significantly difference (1.52 ± 1.03 mmol•L^{-1} vs. 1.59 ± 0.74 mmol•L^{-1}, respectively, 95% CI -0.46 to 0.32, ES = 0.08, *P* = 0.700).

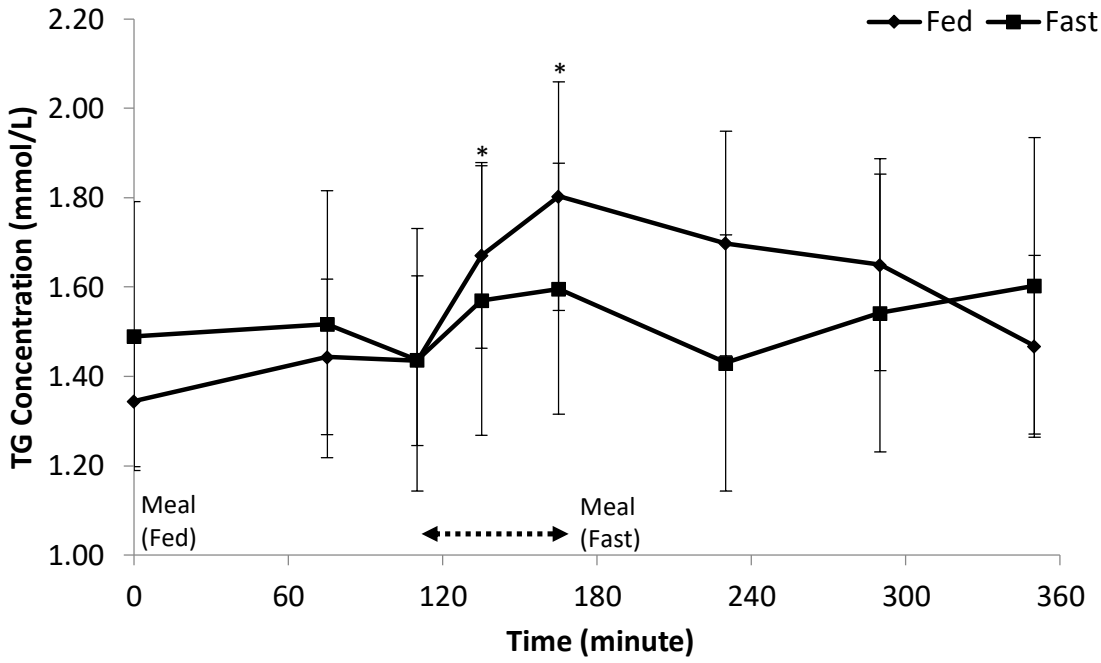

Figure 2.8 Changes in TG concentrations in response to feeding and exercise (*n* = 12). Data are means ± SEM. Arrow represents the exercise period. *Significant difference from baseline.

2.4.4 IMTG content in muscle

A repeated measures ANOVA showed that there was a significant trial x time interaction for IMTG content in both Type I (*P*=0.024) and Type II muscle fibre (*P*=0.038). Type I fibre IMTG content pre-exercise appeared higher in the Fasted as compared to the Fed trial (13.78 ± 4.06 % vs. 9.78 ± 4.81 % fibre area covered by lipid staining, respectively, 95% CI 0.45 to 7.45, ES = 0.90, *P*=0.032) but was not statistically significant after post-hoc correction (*P*=0.064) (**Figure 2.9**). Exercise performed in Fasted state significantly reduced Type I fibre IMTG content from 13.78 ± 4.06 % to 10.34 ± 3.41 % fibre area covered by lipid staining (95% CI 1.24 to 5.64, ES = 0.92, *P*=0.008) and this remained significant even after post-hoc analysis was

performed (*P*=0.015). However, no significant change was seen in the fed state (9.8 ± 4.8 % to 11.2 ± 4.9 % fibre area covered by lipid staining, 95% CI -4.94 to 2.06, ES = 0.29, *P*=0.363). No significant difference between trials existed for post-exercise Type I fibre IMTG content. When pre-exercise IMTG values were included as a covariate in a one-way within measures ANCOVA, the trial effect became insignificant (P=0.077, ES = 0.221). However, it should be noted that, the pre-exercise IMTG values is not a 'true' baseline IMTG value as the intervention had already started. Hence, unadjusted repeated measures ANOVA data and covariate controlled within measures ANCOVA outcomes are reported for transparency.

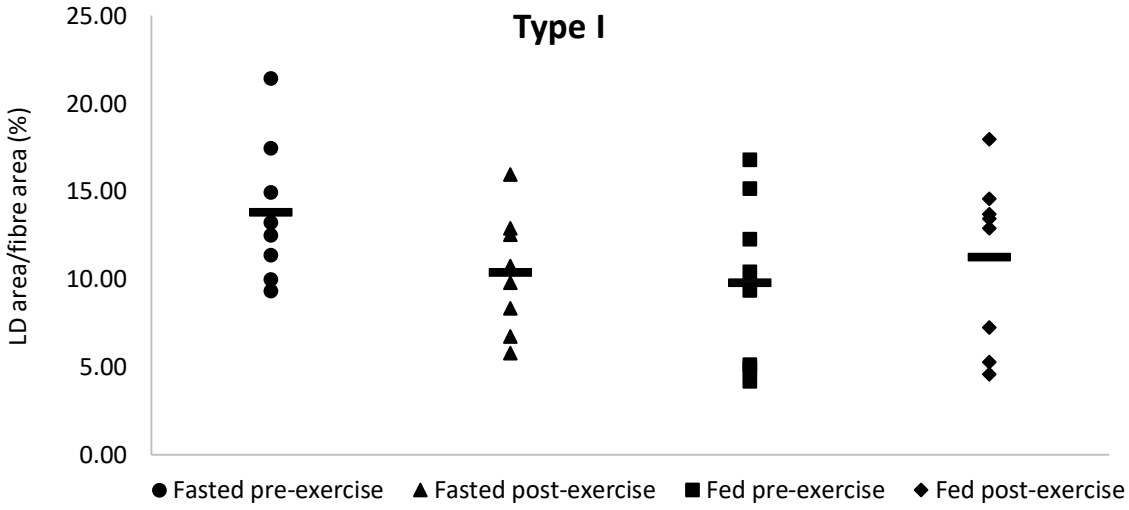

Figure 2.9 IMTG content in Type I muscle fibres in response to exercise (*n* = 8).

Similarly, pre-exercise IMTG content in Type II fibres appeared higher in the Fasted than Fed trial, but this was not statistically significant after post-hoc analysis (7.77 ± 5.13 % vs. 4.29 ± 2.46 % fibre area covered by lipid staining, respectively, 95% CI 0.39 to 6.57, ES = 0.87, post-hoc *P*=0.064) (**Figure 2.10**). Exercise performed in the Fed state appeared to increase Type II IMTG content but this was not significant

after post-hoc analysis (4.29 ± 2.46 % to 6.12 ± 4.09 % area covered by lipid staining, respectively, 95% CI -3.44 to -0.22, ES = 0.54, post-hoc *P*=0.062). No significant change was seen in the fasted state (7.77 ± 5.13 % to 5.88 ± 3.23 % covered by lipid staining, respectively, 95% CI -0.19 to 3.96, ES = 0.45, post-hoc *P*=0.068). No significant difference between trials existed for post-exercise Type II fibre IMTG content. Nonetheless, a one-way within measures ANOVA showed that the trial effect to be significant after adjusting for IMTG content pre-exercise (P=0.025, ES = 0.330), providing some evidence of a difference in the IMTG content response in Type 2 fibres.

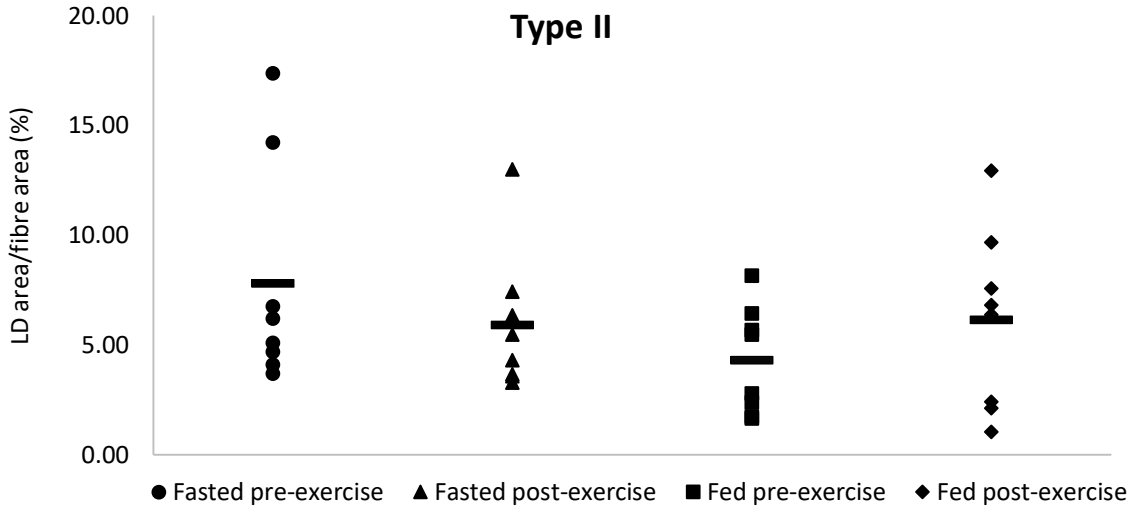

Figure 2.10 IMTG content in Type II muscle fibres in response to exercise (*n* = 8).

2.4.5 Muscle glycogen concentration

There was a significant main effect of time for muscle glycogen concentration (*P*<0.001). Muscle glycogen use was similar in both trials, which reduction of 62 ±

12% and 60 ± 18% of basal in Fasted and Fed state respectively as depicted in **Figure 2.11**

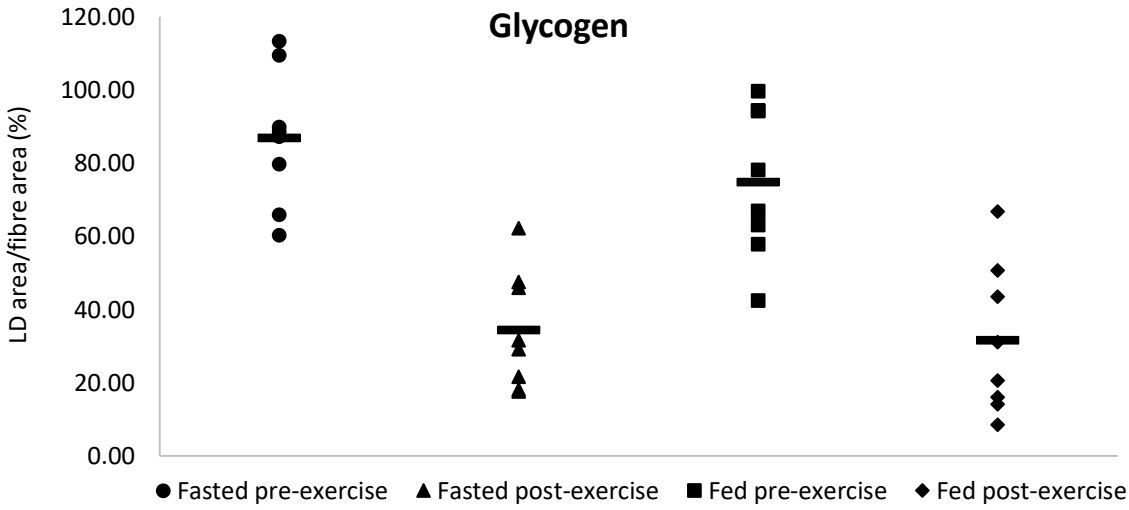

Figure 2.11 Muscle glycogen concentrations in response to exercise (*n*=8). Data are means ± SEM.

2.5 Discussion

This study was aimed to investigate the effects of a single exercise bout performed either before or after feeding on exercise substrate and IMTG utilisation, muscle glycogen use and blood metabolic responses in obese or overweight/centrally obese men. The main findings from this study were that fasted state exercise as compared to fed state exercise resulted in higher fat oxidation, higher circulating markers of lipolysis (glycerol and NEFA) and evidence of Type 1 fibre specific IMTG utilisation during exercise.

A higher fat oxidation and lower carbohydrate oxidation during exercise when performed in fasted state versus fed was observed in the present study. This finding is consistent with earlier works reporting that exercise performed during overnight-fasted state resulted in a greater total amount of fat oxidised compared with exercise performed after a meal in overweight and obese people (Derave et al., 2007, Farah and Gill, 2013). Indeed, a recent systematic review and meta-analysis showed total fat oxidation during exercise was increased by ~3.53 g on average when aerobic exercise was performed in the fasted state as compared with fed state (Vieira et al., 2016). Further, in the present study the greater contribution of fat towards exercise energy expenditure observed in the fasted condition was paralleled with a greater circulating concentration of plasma glycerol and NEFA during exercise, compared with fed, with insulin being higher, albeit not significant, at both measurement time-points during exercise in the fed condition. This finding aligns with De Bock and colleagues who reported markedly higher NEFA and glycerol concentrations

throughout fasted exercise compared with fed in lean man (De Bock et al., 2005). Indeed, a previous study showed that ingestion of meal (particularly containing carbohydrate) before exercise resulting in elevation in a 10-30 μU/ml plasma insulin concentration and consequently reduced fat oxidation primarily by suppressing lipolysis during exercise (Horowitz et al., 1997). Additionally, the observations in the present study are consistent with an effect of the carbohydrate in the pre-exercise meal on reducing lipid oxidation and accelerating carbohydrate oxidation (likely blood glucose oxidation, as muscle glycogen use was similar between conditions) in the fed trial (Wu et al., 2003, Bennard and Doucet, 2006).

It is well recognised that fat balance (fat intake [+ synthesis] – oxidation) ultimately governs whether body fat is gained or lost and therefore increasing fat oxidation during exercise could be an influential means of creating a negative fat balance. Interestingly, previous studies have showed the influence of fasted- versus fed-exercise on daily fat utilisation in lean individuals (Iwayama et al., 2015). They demonstrated that, under energy-balanced conditions, 60 minutes of fasted exercise (50% $\dot{V}O_{2max}$) was superior to fed exercise at increasing daily fat utilisation (independent of energy balance) and this could be due to transient carbohydrate deficits. Nevertheless, in this respect, it is acknowledged as a limitation of the present study that (due to reasons of equipment availability) substrate utilisation around the exercise bout i.e., before and after exercise, was not assessed. Thus, future research is needed in order to establish whether this effect (i.e., increased *daily* fat oxidation resulting from overnight-fasted exercise) persists in people living with obesity.

In previous studies, carbohydrate feeding has been linked to reduced utilisation of IMTG during exercise (Coyle et al., 1997) and more specifically pre-exercise feeding blunts IMTG particularly in Type 1 fibres (De Bock et al., 2005). However, the effect of feeding status on IMTG use had not until now been studied in people with obesity. In fact, in previous studies where IMTG use during exercise *per se* has been studied in obese (Nellemann et al., 2014, Ipavec-Levasseur et al., 2015) and overweight populations (Larson-Meyer et al., 2006) no net use of IMTG has been observed. This might be as they did not measure fibre specific use as this is often cited as the reason why even in lean people some studies do not see evidence of net IMTG use during exercise (van Loon et al., 2003). The point is that the resolution provided by single fibre analysis allows detection of utilisation in a more specific manner. By using fibre specific analysis, the data from this present study suggests for the first time that IMTG is used by obese people during exercise, but this is restricted to Type I fibre use and only when exercise is performed in the overnight fasted state. The use of IMTG observed in this study is consistent with previous studies among lean populations (van Loon et al., 2003, De Bock et al., 2005, Shepherd et al., 2012). That there was net use of IMTG Type 1 fibre after exercise in the fasted state suggests that this could be an effective approach to stimulate IMTG turnover in the obese population.

It is important to recognise that the pre-exercise Type I fibre IMTG content was higher, albeit not statistically significant, in the fasted versus the fed state condition, and as such the utilisation of IMTG during exercise in the fasted state did not result

in significantly lower post-exercise IMTG content than when exercise was performed in the fed state. It is unclear why IMTG contents were higher, albeit not statistically higher, at pre-exercise in the fasted condition, but one plausible explanation may be continued IMTG synthesis due to high plasma fatty acid availability (and slightly high basal insulin) during the period before exercise (overnight-fasting), which may be negated in the fed-state condition with feeding induced suppression of adipose tissue lipolysis (Watt et al., 2004, Chow et al., 2017). However, this seems an unlikely explanation to fully account for the differences observed in such a short-time period (~90 minutes). Another factor to be consider is the possible non-compliance with diets and that a biopsy on arrival would have been better to see if baseline IMTG were truly different. Finally, despite evidence showing a reduction in type I fibre IMTG in overnight-fasted-state exercise, the IMTG contents did not reduce to lower levels than those observed at the end of the fed-state exercise. The significance of this finding is unclear, for example, whether the potential benefits of IMTG utilisation requires reduction to a certain absolute level or whether stimulation of net utilisation *per se* is sufficient. This would require further study.

As an equivalent meal to the pre-exercise feeding was provided post-exercise in the fasted exercise condition, it provided an opportunity to assess the metabolite and hormone responses over the entire period of the trial, under broadly similar states of energy balance. The current study showed that AUC glucose and insulin appeared higher in fasted trial as compared to fed trial, but overall no difference were found in AUC NEFA and triglyceride. The AUC of plasma glucose was 13.6% greater in fasted trial than fed trial, which appears to be driven by augmented

response post-exercise meal in fasted trial that might be due to enhanced glucose delivery, possibly resulting from adaptations in splanchnic tissues following exercise (Rose et al., 2001). In addition, AUC of insulin was 52.6% higher in fasted condition as shown previously (De Bock et al., 2005), which might be driven by the increase glucose delivery or the higher pre-feeding NEFA – which has been shown to potentiate insulin release (McGarry and Dobbins, 1999, Deeney et al., 2000, Yaney et al., 2000). Although glucose tolerance and insulin sensitivity were not assessed directly in this study, a greater rise in glucose and insulin following feeding in fasted trial was observed. Indeed, others have demonstrated a greater glycaemic response to a standardised breakfast when exercise was performed in the fasted as compared with fed state (Derave et al., 2007). The authors postulated that the blunted response in fed might be attributed to an exercise-induced transient suppression of the meal-induced elevation in glucose, which is subsequently used as a substrate to fuel muscle contraction. However, regardless the AUC glucose and insulin was higher when exercise was performed in the fasted state, at this stage it is not possible to say if this is physiological or has the potential to be pathological.

In conclusion, the current study showed that an acute bout of aerobic exercise performed in the overnight-fasted state, as compared to fed state, augmented whole-body exercise fat oxidation and appeared to promote Type I fibre IMTG utilisation in obese or overweight, centrally obese men. Further research should now investigate if overnight-fasted exercise, which increases the oxidation of fat as fuel during exercise including IMTG utilisation, can optimise the effectiveness of exercise training to improve metabolic health in obese populations.

2.6 References

BASKIN, M. L., ARD, J., FRANKLIN, F. & ALLISON, D. B. 2005. Prevalence of obesity in the United States. *Obesiy Reviews,* 6**,** 5-7.

BENNARD, P. & DOUCET, E. R. 2006. Acute effects of exercise timing and breakfast meal glycemic index on exercise-induced fat oxidation. *Applied Physiology Nutrition and Metabolism,* 31**,** 502-511.

BERGSTROM, J. 1975. Percutaneous needle biopsy of skeletal muscle in physiological and clinical research. *Scandinavian Journal of Clinical and Laboratory Investigation,* 35**,** 609-616.

BORG, G. 1982. Ratings of perceived exertion and heart rates during short-term cycle exercise and their use in a new cycling strength test. *International Journal of Sports Medicine,* 3**,** 153-158.

CHOW, L. S., MASHEK, D. G., WANG, Q., SHEPHERD, S. O., GOODPASTER, B. H. & DUBÉ, J. J. 2017. Effect of acute physiological free fatty acid elevation in the context of hyperinsulinemia on fiber type-specific IMCL accumulation. *Journal of Applied Physiology,* 123**,** 71-78.

COHEN, J. 1988. Statistical power analysis for the behavioural sciences. 2nd ed. Hillsdale (NJ): *Lawrence Erlbaum Associates*, 22–25.

COYLE, E. F., JEUKENDRUP, A. E., WAGENMAKERS, A. & SARIS, W. 1997. Fatty acid oxidation is directly regulated by carbohydrate metabolism during exercise. *American Journal of Physiology, Endocrinology and Metabolism - Clinical and Experimental,* 273**,** E268-E275.

CUMMING, G. & Finch, S. 2001. A primer on the understanding, use, and calculation of confidence intervals that are based on central and noncentral distributions. *Educational and Psychological Measurement,* 61(4), 532–574.

DE BOCK, K., RICHTER, E. A., RUSSELL, A. P., EIJNDE, B. O., DERAVE, W., RAMAEKERS, M., KONINCKX, E., LÉGER, B., VERHAEGHE, J. & HESPEL, P. 2005. Exercise in the fasted state facilitates fibre type-specific intramyocellular lipid breakdown and stimulates glycogen resynthesis in humans. *The Journal of Physiology,* 564**,** 649-660.

DEENEY, J. T., GROMADA, J., HØY, M., OLSEN, H. L., RHODES, C. J., PRENTKI, M., BERGGREN, P. O. & CORKEY, B. E. 2000. Acute stimulation with long chain acyl-CoA enhances exocytosis in insulin-secreting cells (HIT T-15 and NMRI beta-cells). *The Journal of Biological Chemistry,* 275**,** 9363-9368.

DERAVE, W., MERTENS, A., MULS, E., PARDAENS, K. & HESPEL, P. 2007. Effects of post-absorptive and postprandial exercise on glucoregulation in metabolic syndrome. *Obesity,* 15**,** 704-711.

FARAH, N. & GILL, J. 2013. Effects of exercise before or after meal ingestion on fat balance and postprandial metabolism in overweight men. *British Journal of Nutrition,* 109**,** 2297-2307.

FAO. 2004. Human energy requirements. Report of a Joint FAO/WHO/UNU Expert Consultation, Rome, 17-24 October 2001.

FRAYN, K. N. 1983. Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology,* 55**,** 628-634.

GALLUS, S., LUGO, A., MURISIC, B., BOSETTI, C., BOFFETTA, P. & LA VECCHIA, C. 2015. Overweight and obesity in 16 European countries. *European Journal of Nutrition,* 54**,** 679-689.

GOODPASTER, B. H., HE, J., WATKINS, S. & KELLEY, D. E. 2001. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *The Journal of Clinical Endocrinology and Metabolism,* 86**,** 5755-5761.

GOODPASTER, B. H., THAETE, F. L., JEAN-AIME, S. & KELLEY, D. E. 1997. Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. *Diabetes,* 46**,** 1579-1585.

GOODPASTER, B. H., THERIAULT, R., WATKINS, S. C. & KELLEY, D. E. 2000. Intramuscular lipid content is increased in obesity and decreased by weight loss. *Metabolism - Clinical and Experimental,* 49**,** 467-472.

HOLM, S. 1979. A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics,* 6**,** 65-70.

HOROWITZ, J. F., MORA-RODRIQUEZ, R., BYERLEY, L. O. & COYLE, E. F. 1997. Lipolytic suppression following carbohydrate ingestion limits fat oxidation during exercise.pdf. *American Journal of Physiology, Endocrinology and Metabolism,* 273**,** E768–E775.

HULVER, M. W. & DOHM, G. L. 2004. The molecular mechanism linking muscle fat accumulation to insulin resistance. *Proceedings of the Nutrition Society,* 63**,** 375-380.

IPAVEC-LEVASSEUR, S., CROCI, I., CHOQUETTE, S., BYRNE, N. M., COWIN, G., O'MOORE-SULLIVAN, T. M., PRINS, J. B. & HICKMAN, I. J. 2015. Effect of 1-h moderate-intensity aerobic exercise on intramyocellular lipids in obese men before and after a lifestyle intervention. *Applied Physiology, Nutrition, and Metabolism,* 40**,** 1262-1268.

IWAYAMA, K., KURIHARA, R., NABEKURA, Y., KAWABUCHI, R., PARK, I., KOBAYASHI, M., OGATA, H., KAYABA, M., SATOH, M. & TOKUYAMA, K. 2015. Exercise Increases 24-h Fat Oxidation Only When It Is Performed Before Breakfast. *EBioMedicine,* 2**,** 2003-2009.

KLEIN, S., BURKE, L. E., BRAY, G. A., BLAIR, S., ALLISON, D. B., PI-SUNYER, X., HONG, Y. & ECKEL, R. H. 2004. Clinical Implications of Obesity With

Specific Focus on Cardiovascular Disease. *A Statement for Professionals From the American Heart Association Council on Nutrition, Physical Activity, and Metabolism: Endorsed by the American College of Cardiology Foundation,* 110**,** 2952-2967.

KRSSAK, M., PETERSEN, K. F., DRESNER, A., DIPIETRO, L., VOGEL, S. M., ROTHMAN, D. L., SHULMAN, G. I. & RODEN, M. 1999. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans; a 1H NMR spectroscopy study. *Diabetologia,* 42, 113-116.

LARSON-MEYER, D. E., SMITH, S. R., HEILBRONN, L. K., KELLEY, D. E., RAVUSSIN, E., NEWCOMER, B. R. & LOOK, A. A. R. G. 2006. Muscle-associated triglyceride measured by computed tomography and magnetic resonance spectroscopy. *Obesity,* 14**,** 73-87.

MCGARRY, J. D. & DOBBINS, R. L. 1999. Fatty acids, lipotoxicity and insulin secretion. *Diabetologia,* 42**,** 128-138.

MORO, C., BAJPEYI, S. & SMITH, S. R. 2008. Determinants of intramyocellular triglyceride turnover: implications for insulin sensitivity. *American Journal of Physiology, Endocrinology and Metabolism,* 294**,** E203-E213.

NELLEMANN, B., SONDERGAARD, E., JENSEN, J., PEDERSEN, S. B., JESSEN, N., JORGENSEN, J. O. & NIELSEN, S. 2014. Kinetics and utilization of lipid sources during acute exercise and acipimox. *American Journal of Physiology, Endocrinology and Metabolism,* 307**,** E199-E208.

NICE 2014. Obesity - identification, assessment and management. https://www.nice.org.uk/guidance/cg189/chapter/1-ecommendations#identification-and-classification-of-overweight-and-obesity. *National Institute for Health and Care Excellence*.

RENNIE, K. L. & JEBB, S. A. 2005. Prevalence of obesity in Great Britain. *Obesity Review,* 6**,** 11-12.

ROSE, A. J., HOWLETT, K., KING, D. S. & HARGREAVES, M. 2001. Effect of prior exercise on glucose metabolism in trained men. *American Journal of Physiology, Endocrinology and Metabolism,* 281**,** E766-E771.

ROSS, R., DAGNONE, D., JONES, P. J. H., SMITH, H., PADDAGS, A., HUDSON, R. & JANSSEN, I. 2000. THe effects of losing weight through diet or exercise programs on obesity. *Annals of Internal Medicine,* 133**,** 92-92.

RUSSELL, A. P. 2004. Lipotoxicity: the obese and endurance-trained paradox. *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity,* 28 Suppl 4**,** S66-S71.

SHAW, C. S., CLARK, J. & WAGENMAKERS, A. J. M. 2010. The Effect of Exercise and Nutrition on Intramuscular Fat Metabolism and Insulin Sensitivity. *Annual Review of Nutrition,* 30**,** 13-34.

SHEPHERD, S. O., COCKS, M., TIPTON, K. D., RANASINGHE, A. M., BARKER, T. A., BURNISTON, J. G., WAGENMAKERS, A. J. M. & SHAW, C. S. 2012.

Preferential utilization of perilipin 2-associated intramuscular triglycerides during 1 h of moderate-intensity endurance-type exercise. *Experimental Physiology,* 97**,** 970-980.

VAN LOON, L. J. C. 2004. Use of intramuscular triacylglycerol as a substrate source during exercise in humans. *Journal of Applied Physiology,* 97**,** 1170-1187.

VAN LOON, L. J. C., KOOPMAN, R., STEGEN, J. H. C. H., WAGENMAKERS, A. J. M., KEIZER, H. A. & SARIS, W. H. M. 2003. Intramyocellular lipids form an important substrate source during moderate intensity exercise in endurance-trained males in a fasted state. *The Journal of Physiology,* 553**,** 611-625.

VAN PROEYEN, K., SZLUFCIK, K., NIELENS, H., PELGRIM, K., DELDICQUE, L., HESSELINK, M., VAN VELDHOVEN, P. P. & HESPEL, P. 2010. Training in the fasted state improves glucose tolerance during fat-rich diet. *The Journal of Physiology,* 588**,** 4289-4302.

VIEIRA, A. F., COSTA, R. R., MACEDO, R. C. O., COCONCELLI, L. & KRUEL, L. F. M. 2016. Effects of aerobic exercise performed in fasted v. fed state on fat and carbohydrate metabolism in adults: a systematic review and meta-analysis. *The British journal of Nutrition,* 116**,** 1153-1164.

WATT, M. J., KRUSTRUP, P., SECHER, N. H., SALTIN, B., PEDERSEN, B. K. & FEBBRAIO, M. A. 2004. Glucose ingestion blunts hormone-sensitive lipase activity in contracting human skeletal muscle. *American Journal of Physiology, Endocrinology and Metabolism,* 286**,** E144-E150.

WEIR, J. B. D. B. 1949. New methods for calculating metabolic rate with special reference to protein metabolism. *The Journal of Physiology,* 109**,** 1-9.

WHO 2000. Obesity preventing and managing the global epidemic. Report of a WHO consultation. World Health Tech Rep Ser. 894:i-xii. *World Health Organization,* 9 5.

WHO & IDF 2006. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. *World Health Organization and International Diabetes Federation*.

WU, C.-L., NICHOLAS, C., WILLIAMS, C., TOOK, A. & HARDY, L. 2003. The influence of high-carbohydrate meals with different glycaemic indices on substrate utilisation during subsequent exercise. *The British Journal of Nutrition,* 90**,** 1049-1056.

YANEY, G. C., KORCHAK, H. M. & CORKEY, B. E. 2000. Long-chain acyl CoA regulation of protein kinase C and fatty acid potentiation of glucose-stimulated insulin secretion in clonal beta-cells. *Endocrinology,* 141**,** 1989-1998.

CHAPTER 3

Influence of Fed vs. Overnight-Fasted State Exercise on Substrate Utilisation during Exercise in Obese or Overweight/Centrally Obese Women

In this chapter, there were several individuals involved during the data collection and analysis other than author of this thesis:

1. *Nurul Fadhilah Abdullah (author) conducted all data collection, sample analysis for substrate utilisation, blood samples and performed all statistical analysis.*
2. *Sewa Abdullah contributed in blood sample analysis for TG and glycerol of four participants.*
3. *Dr Konstantinos Manolopoulos gave medical oversight including inspection of ECG traces.*
4. *For sex-hormone analysis (estrogen and progesterone), they were outsourced to Randox Clinical Laboratory Services, Co Antrim, UK.*

3.1 Summary

Increasing fat oxidation during exercise may help improve insulin sensitivity and reduce body fat. Exercise performed in the overnight-fasted state results in higher fat oxidation than exercise in the fed state in lean and obese men, but this has not been studied in obese women. The purpose of this study was to determine how exercise performed in the overnight-fasted versus fed state impacts exercise fat oxidation, appetite and subsequent energy intake at an *ad libitum* lunch in obese or overweight centrally obese women. Using a randomized, crossover design, twelve obese or overweight centrally obese (BMI, 30.1 ± 3.8 kg/m^2; waist circumference, 94.4 ± 8.7 cm) but otherwise healthy young (26.3 ± 7.0 years) women performed 1-h of constant load bicycle exercise (65% $\dot{V}O_{2max}$) followed by 3-h of controlled recovery. On one occasion, exercise was performed after an overnight fast (Fasted) and a standardised breakfast (25% daily energy intake; 65% carbohydrate, 20% fat and 15% protein) was consumed immediately after exercise, and on the other occasion they consumed the same meal 90 minutes before exercise (Fed). Indirect calorimetry was used during exercise to measure whole body substrate utilisation and venous blood was collected before, during and after exercise to assess the plasma hormone and metabolite response. Self-reported appetite sensations were recorded throughout the trials using visual analogues scales [VAS], and energy intake at an *ad libitum* lunch was determined at the end of the recovery period. During exercise, fat oxidation (0.29 ± 0.07 vs. 0.17 ± 0.07 g/min respectively, P<0.001) and concentrations of plasma glycerol (P<0.001) and NEFA (P<0.001) were significantly higher during exercise in the Fasted vs. Fed trial. Self-rated (VAS)

hunger (P<0.001) and prospective food consumption (P=0.024) were significantly higher in Fed vs. Fasted state before lunch time and total energy intake at the *ad libitum* lunch was significantly higher in Fed vs. Fasted trial (897 ± 241 vs. 767 ± 211 kcal respectively, P<0.001). This study showed that in obese or overweight/centrally obese women, an acute bout of aerobic exercise performed in the overnight-fasted state augmented exercise fat oxidation and suppressed appetite and energy intake in the short-term post-exercise period as compared to exercise performed in the fed state. This suggests that performing regular aerobic exercise in the overnight-fasted state could be a viable strategy to explore for the management of body mass and composition in obese or overweight/centrally obese women.

3.2 Introduction

The ability to oxidise fat as a fuel effectively appears to play an important role in aetiology of obesity (Zurlo et al., 1990, Seidell et al., 1992, Colberg et al., 1996, Kim et al., 2000, Kelley et al., 2001). Additionally, persistent impairments in daily fat utilisation has been associated with re-gain of body mass following a period of weight loss in obese individuals (Hainer et al., 2000), suggesting that these defects could be primary to the obese state. Thus, a better understanding of the factors regulating fat oxidation is important for the development of interventions allowing effective treatment of obesity.

Exercise has been shown to acutely increase fat oxidation during the exercise bout itself but the extent to which fat oxidation is increased by exercise appears to be strongly dependent on nutritional state (Jeukendrup, 2002). Indeed, in **Chapter 2** it was demonstrated that exercise performed during overnight-fasted versus fed state resulted in greater fat oxidation during exercise in overweight and obese men, which was consistent with previous studies in obese and overweight men (Derave et al., 2007, Farah and Gill, 2013). Interestingly, previous studies have shown that there are sex differences in substrate utilization during exercise, whereby women rely more on fat oxidation than men (Horton et al., 1998, Henderson et al., 2007, Henderson and Alderman, 2014). However, whether exercise in the overnight-fasted versus fed-state elicits similar metabolic differences in obese women as observed in lean and obese men is unknown. This is important to study in order to

understand if the potential benefits of performing exercise in the overnight-fasted state apply to both sexes.

Exercise has been found to alter energy balance by increasing energy expenditure as well as by modifying energy intake (King et al., 1994, Imbeault et al., 1997). Evidence has shown that, following a single bout of exercise, individuals will typically either reduce energy intake acutely or wait an extended time before initiating food consumption, leading to a short-term energy deficit (Martins et al., 2007, Li et al., 2014). Whilst there does not appear to be evidence of energy compensation when breakfast is omitted in the context of exercise in lean individuals, this has not been examined in people with obesity (Clayton et al., 2016). If performing exercise in the overnight-fasted state in obese women promoted greater fat oxidation than fed-state exercise, it would be important in terms of long-term body weight management to ensure there was no subsequent increase in food intake (i.e., compensation).

The first purpose of the present study was to determine the influence of overnight-fasted versus fed state exercise on substrate metabolism during exercise in obese or overweight/centrally obese women. A secondary purpose was to examine how subjective appetite responses and subsequent energy intake at an *ad libitum* style lunch are modulated by exercise performed in the overnight-fasted or fed state in the same study population. It was hypothesized that a single exercise bout performed in the fasted state will promote greater fat oxidation than fed-state exercise and energy intake across the study period will be similar regardless of whether exercise is performed in the overnight-fasted or fed state. Finally, in

Chapter 2, it was observed that overall glucose and insulin responses (i.e., AUC across the trial) were higher when exercise was performed in the overnight-fasted versus fed state despite matched exercise energy expenditures and identical prescribed food intake. Thus, the final aim of the present study was to explore if differential metabolite and hormone responses across the entire study period are also observed when obese women undertake exercise in the overnight fasted or fed state.

3.3 Methods

3.3.1 Participants

This present study followed a randomized; cross-over design and was carried out in Birmingham, UK. Participants were recruited through the university portal (my.bham), poster and newspaper advertisement. Participants were selected on the basis that they were apparently healthy, not physically active, having a regular menses (non-oral contraceptive users) and they were obese (white European, BMI 30-34.9 kg/m^2 or Asian BMI 27.5-35 kg/m^2) or overweight/centrally obese (BMI, white European 25-29.9 kg/m^2 or Asian 23-27.4 kg/m^2) with a high waist circumference (>80 cm [31.5 inches]) (NICE 2014).This population was selected as they there were identified as at risk of cardiovascular disease and type II diabetes based on the anthropometry (NICE, 2014). A total of 12 individuals completed the study protocol. The present study was approved by the University of Birmingham's Ethical Review Committee (ERN_16-0421). Written informed consent was also obtained prior to the study from participants after detailed information of study procedures, risks and benefits of the research were verbally explained to them. The participant characteristics are presented in **Table 3.1**.

Table 3.1 General characteristics of participants

	n = 12
Age (years)	26.3 ± 7.0
Weight (kg)	79.6 ± 12.8
Height (m)	1.62 ± 0.07
BMI (kg/m^2)	30.1 ± 3.8
Waist circumference (cm)	94.4 ± 8.7
Hip circumference (cm)	111.4 ± 10.0
Systolic blood pressure (mmHg)	120 ± 8
Diastolic blood pressure (mmHg)	71 ± 6
$\dot{V}O_{2peak}$ (ml/kg/min)	24.0 ± 5.1
Physical activity MET (min/week)	383 ± 193
Estimated energy expenditure (kcal/d)	2327 ± 167
Fasting blood glucose (mmol/L)	5.3± 0.4
2-h blood glucose (OGTT) (mmol/L)	5.4± 0.9
Ethnicity, *n*	
White	3
Indian	2
Bangladeshi	1
Other Asian	6

Mean ± SD

3.3.2 Study design

This study involved of four visits to the laboratory. The first visit was a screening visit, during which participants completed a GHQ and their BMI, waist circumference, blood pressure and ECG were assessed before undertaking OGTT. The second visit involved preliminary testing to measure $\dot{V}O_{2peak}$ as well as familiarisation to the exercise testing procedures to be used in the final two visits.

The third and fourth visits were the main experimental visits. These two visits were separated by one month. Two days before these visits, participants were provided with a standardized food package based on individual energy requirements. These visits lasted for ~8 hours, and included exercise performed either in overnight fasted or fed state (randomized order), with indirect calorimetry undertaken during exercise and venous blood samples obtained before, during and in recovery from exercise, followed by provision of an *ad libitum* lunch. Participant's appetite was obtained through appetite questionnaire across the study period and subsequent energy intake during lunch was recorded.

3.3.3 Experimental procedures

Visit 1 (Screening): This visit was used for consenting and screening based on inclusion criteria which included initially being: healthy as assessed by successful completion of a GHQ; aged between 18 and 49 years; Class I obese BMI (white European, BMI 30-34.9 kg/m^2 or Asian BMI 27.5-35 kg/m^2) or overweight (white

European, BMI 25-29.9 kg/m^2 or Asian, BMI 23-27.4 kg/m^2) with a high waist circumference (≥ 80 cm [31.5 inches]); not physically active as assessed by International Physical Activity Questionnaire short form [IPAQ-SF]; stable weight (± 2 kg) for more than 3 months before enrolment; have regular menstrual cycle.

On the screening day, participants came to the laboratory in the morning in an overnight fasted state. Upon arrival, a verbal explanation about the purpose of the study and procedures involved was given by an investigator to the participants before they signed informed consent. They then completed the GHQ (**Appendix 3**) to determine their health history and IPAQ-SF (**Appendix 6**) to determine their physical activity level [PAL] (Craig et al., 2003). Body mass was measured using an electronic weighing scale (OHaus Champ II Scales, USA) and height was measured using a stadiometer (Stadiometer, SECA, UK) to the nearest 0.1 kg and 0.1 cm respectively. BMI was calculated as weight (kg) divided by height squared (m^2), while waist and hip circumferences were measured to the nearest 0.1 cm using a measuring tape. Resting blood pressure was determined using an automatic blood pressure monitor (Omron M6, Netherlands). Participants who successfully completed the Screening Form and met the eligibility requirements with respect to BMI, waist circumference and blood pressure (<140/90 mmHg), they were asked to undertake a 12-lead ECG examination (Oxycon Pro, Jaeger, Wurzburg, Germany) to check for potential cardiac abnormalities. Following this, an OGTT was performed to ensure none of the participants were undiagnosed diabetic. It involved comparing the levels of glucose in the blood before and after drinking a glucose drink (containing 75 g glucose). For this, 5 ml blood was drawn via venepuncture at pre

OGTT (fasting) and 2 hour after ingestion of glucose. The diagnostic criteria for diabetes is fasting plasma glucose ≥7 mmol/L or 2 hours plasma glucose ≥11.1 mmol/L and for impaired glucose tolerance are fasting plasma glucose <7.0 mmol/L and 2-hour plasma glucose ≥7.8 and <11.1mmol/L (WHO and IDF, 2006).

Visit 2 (Baseline Assessments): The second visit was conducted at least one week before the main experiment trials (Visits 3 and 4). Upon arrival to the laboratory, they were asked to perform an incremental exercise test on a stationary bicycle (Lode Sport Excalibur, Groningen, Netherlands) to determine their level of aerobic fitness ($\dot{V}O_{2peak}$) and also the workload required to elicit 65% $\dot{V}O_{2peak}$ for use in later experimental trials. Participants started cycling at 35 W and were asked maintaining a pedal rate of between 60-70 rev/min with the power increased by 35 W every 3 minutes until they reached voluntary exhaustion (i.e. they stop on their own accord or the pedal rate dropped below 50 rev/min despite verbal encouragement from the investigator). Their heart rate was monitored continuously throughout the testing using a heart rate monitor (Polar A300, Finland) and was recorded at the final 10 seconds of each exercise stage, as well as their self-rated perceived exertion using the Borg scale (Borg, 1982). Breath-by-breath measurements of $\dot{V}O_2$ and $\dot{V}CO_2$ during the exercise were collected using a computerised gas analysis system (Oxycon Pro, Jeager, Wurzburg, Germany). The data obtained was used to determine $\dot{V}O_{2peak}$ which was taken as the highest value of $\dot{V}O_2$ attained during a 10 second period on this test. After an approximately 30 minute rest period, participants were asked to perform a 30-minute moderate intensity (65% $\dot{V}O_{2peak}$) exercise bout

to familiarise themselves with the exercise bout to be employed during subsequent experimental trials.

Visits 3 and 4 (Experimental trial; ***Figure 3.1****):* The third and fourth visits were separated by a month during consecutive months to ensure testing was undertaken during the same phase of the menstrual cycle, which is mid follicular phase (6 or 7 days after initiation of menses). **Table 3.2** shows the serum estrogen and progesterone concentrations determined from blood samples collected at baseline during Fasted and Fed trials, which confirmed that women participants were in the correct phase of the cycle.

Table 3.2 Baseline value of estrogen and progesterone in women participants

	Fasted	Fed
Estrogen	204 ± 152	203 ± 130
Progesterone	0.89 ± 0.38	1.06 ± 0.62

Mean ± SD. Typical ranges for follicular phase; estrogen: 87.60 – 224.68 pmol•L^{-1}, progesterone: 0.32 – 1.29 nmol•L^{-1} (Stricker et al., 2006).

These visits were preceded for two days by a standardised weight-maintaining diet consisting of 50% carbohydrate, 30% fat and 20% protein based on their estimated energy expenditure that was calculated using the Institute of Medicine equations assuming a low physical activity coefficient (see Section 3.3.6 for calculation). In addition, they were asked to maintain their normal habitual diet and refrain from vigorous exercise and caffeine and alcohol consumption during this period. On the

day of the experimental trials, participants were asked to drink a glass of water upon waking up to ensure there were adequately hydrated and reported to the laboratory in the morning (approximately 7.30am) after an overnight fast (~12 hours).

Upon arrival, an indwelling cannula (to allow for repeated blood collections) was inserted into a forearm vein and a blood sample was collected (~12 ml). On one occasion (Fed), a standardised breakfast was consumed (25% daily energy intake; 65%, 20% and 15% energy from carbohydrate, fat and protein, respectively) within 15 minutes, while on the other visit (Fasted) participants received breakfast immediately after cessation of the exercise bout. A second blood sample (~10ml) was collected at 45 minutes after breakfast. Approximately 90 minutes post-breakfast, participants then performed a 60-minute steady state at moderate intensity (65% $\dot{V}O_{2peak}$) cycling exercise bout. During the exercise tests, water was provided *ad libitum*. Heart rate was monitored continuously during exercise and recorded every 10 minutes (Polar A300, Finland), as well as self-rated perceived exertion using the standard Borg scale (Borg, 1982). Breath-by-breath measurements were collected using indirect calorimetry at 30- and 60-minute of exercise bouts. Blood samples were collected before exercise, at 30- and 60- minute of exercise bout and every 1 hour during 3-h post-exercise (~10 ml each time). The environmental condition of the laboratory during the exercise bout was recorded and there was no significant difference between the two visits with humidity at 34 ± 2 % and 34 ± 3 %; temperature at 24.0 ± 1.2 °C and 24.1 ± 0.7 °C for fasted and fed conditions, respectively.

After the last blood sampling, participants were provided with lunch (in excess) and instructed to eat until they were comfortably full. The lunch meal was adapted from previous study (Gonzalez et al., 2015) and consisted of a homogenous mixed meal consisting of pasta (Sainsbury), tomato pasta sauce (Prego), cheddar cheese (Sainsbury) and virgin olive oil (Sainsbury) and provided 205 kcal of energy per 100g of food (52 % carbohydrate, 34 % fat, 14 % protein). The subjects were asked to consume until comfortably full. The food intake was measured using a kitchen scale. An appetite questionnaire (**Appendix 5**) was administered at baseline immediately before and after exercise and immediately before lunch.

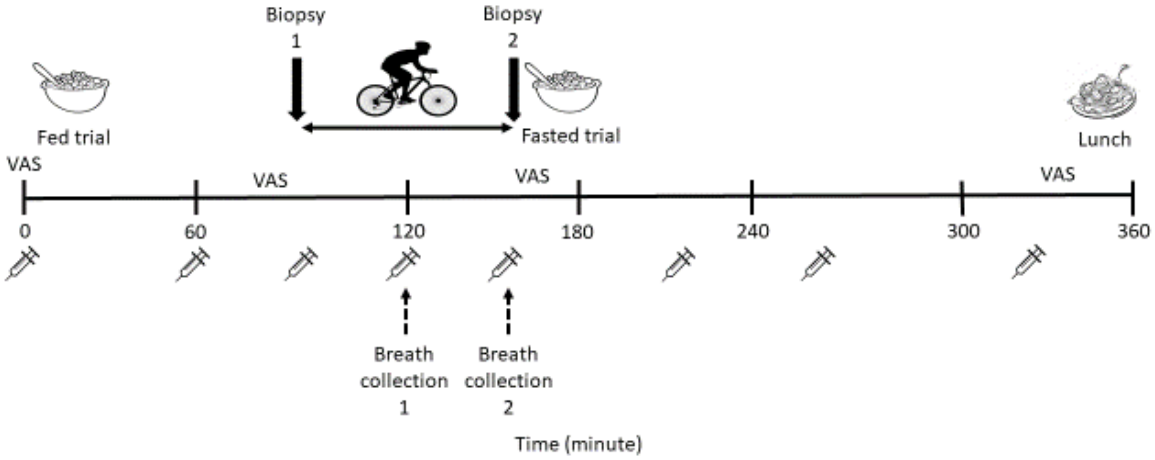

Figure 3.1 A schematic representation of the Experimental Trials.

3.3.4 Appetite Questionnaire

Self-rated VAS were used to assess hunger, satiety, fullness, prospective food consumption as previously described (Flint et al., 2000). The VAS was 100 mm in length with the words anchored at each end, expressing the most positive and the most negative rating. Participants were asked to rate their appetite sensations at baseline, before and after exercise and before the *ad libitum* lunch for both trials.

3.3.5 Blood sampling and analysis

Venous blood samples were obtained from an antecubital forearm vein at various time points as explained above. Approximately 6 ml blood samples were transferred into vacutainer containing EDTA and immediately the tubes were placed in the ice, while the remaining sample was allowed to clot in a plain vacutainer. At the end of trial, samples were centrifuged at 1361 g for 15 minutes at 4 ºC. Aliquots of plasma or serum were flash frozen in the liquid nitrogen then stored at -80ºC until analysed.

Plasma samples were analysed for glucose (Glucose Oxidase, Instrumentation Laboratories, Cheshire, UK), glycerol (GLY, Randox, London, UK), NEFA (NEFA, Randox, London, UK) and TG (TG kit, Instrumentation Laboratory, Warrington, UK) using an ILAB 650 Clinical Chemistry Analyser (Instrumentation Laboratory, Warrington, UK). The average intra-assay coefficient of variation for glucose was 1.5 %, glycerol was 2.8 %, NEFA was 3.3 % and TG was 1.3 %, while the inter-assay coefficient of variation for glucose and glycerol were 5.9 %, NEFA was 3.1 % and TG was 3.5 % based on analysis of 20 duplicate plasma samples ran across two assays.

Serum insulin concentrations were measured by using a human insulin ELISA kit (Invitrogen, Paisley, UK) and a Biotek ELx800 analyser (Biotek Instruments, Vermont, USA). The average intra-assay coefficient of variation was 3.6 % based on 96 duplicate samples with the inter-assay coefficient of variation 7.3 % based on 10 samples ran across two assays. In addition, fasting serum estrogen and progesterone concentrations were measured (**Table 3.2**) in separate analyses quantified by immunoassays using a Roche e602 unit on a Cobas 8000 modular analyser (Roche Diagnostics Ltd, Rotkreuz, Switzerland).

3.3.6 Calculations

The weight maintenance diet that was given to the participants two days before their experimental visit was based on their estimated total energy expenditure (TEE), which was calculated using the Institute of Medicine equations by assuming a low physical activity coefficient (Brooks et al., 2004).

For women aged ≥19 y:

TEE = 354 – [6.91*age (y)] + 1.12 x [9.36*Weight (kg) + 726*Height (m)]

For the experimental trials (Visit 3 and 4), the $\dot{V}O_2$ and $\dot{V}CO_2$ were used to quantify RER by diving $\dot{V}CO_2$ over $\dot{V}O_2$, while the whole body carbohydrate and fat oxidation were calculated by using equations from Frayn (1983), with the assumption that the urinary nitrogen excretion rate was negligible.

$$\text{Carbohydrate oxidation (g/min)} = 4.55*\dot{V}CO_2 - 3.21*\dot{V}O_2$$

$$\text{Fat oxidation (g/min)} = 1.67*\dot{V}O_2 - 1.67*\dot{V}CO_2$$

For plasma and serum metabolite and hormone concentrations, an AUC was calculated using the trapezoid method:

$$AUC = AUC_{0\text{-}1} + AUC_{1\text{-}2} + \ldots + AUC_{x\text{-}last}$$

$$= ((C_0 + C_1)/2)*(T_1 - T_0) + ((C_1 + C_2)/2)*(T_2 - T_1) + \ldots + ((C_x + C_{last})/2)*(T_{last} - T_x)$$

Where;

C = plasma concentration

T = time

3.3.7 Statistical analysis

Data were analysed using Statistical Package for the Social Sciences [SPSS] software package (version 22, SPSS Inc. Chicago, IL) and the post-hoc test was performed manually in excel. Normality of the data was checked by Shapiro Wilk tests. Homogeneity of variances was confirmed by Mauchley's test of sphericity and a Greenhouse-Geisser correction was applied to the degrees of freedom if the sphericity assumption was violated. A paired-sample t-test was used to compare differences between trials in heart rate, rating of perceived exertion [RPE], $\dot{V}O_2$,

$\dot{V}CO_2$, RER, fat oxidation (g/min and % energy from fat oxidation), carbohydrate oxidation (g/min and % energy from carbohydrate oxidation), AUC for blood metabolites and insulin and energy intake during lunch time. A two-way ANOVA with repeated measures (trial x time) was used to compare differences in blood metabolites and insulin and appetite variables between trials and over time. Further, a paired-sample t-test was used to test the difference of each time point from baseline during each trial separately and the difference between trials for each time point. Where a significant effect (i.e., $P<0.05$) was indicated for these variables, the Holm-Bonferroni stepwise correction was made for location of variance post-hoc (Holm, 1979). Normally distributed data are presented as means ± standard deviation unless otherwise stated. The 95% confidence intervals (CI) for mean absolute pairwise differences between trials were calculated using the t-distribution and degrees of freedom (n – 1). Absolute standardised effect sizes (ES) were calculated for within-measures comparisons to supplement important findings as follows:

$$\text{ES} = \frac{\text{Mean v2} - \text{Mean v1}}{CON\ SD} \text{ (Cumming and Finch, 2001)}$$

In the absence of a clinical anchor, an ES of 0.2 was considered the minimum important difference for all outcome measures, 0.5 moderate and 0.8 large (Cohen, 1988). Statistical significance was accepted as $P<0.05$.

3.4 Results

3.4.1 Physiological and perceptual response to exercise

The exercise intensity attained was similar between experimental trials (67 ± 2 vs. 67 ± 2 % $\dot{V}O_{2peak}$ for Fasted and Fed, respectively). In addition, the RPE was also similar in both experimental trials (14 ± 2 vs. 14 ± 2 for Fasted and Fed, respectively) which qualitatively corresponded to a perception of somewhat hard to hard. Furthermore, the mean heart rate was similar in both conditions (136 ± 11 vs. 139 ± 13 beat/min for Fasted and Fed, respectively). There was no significant difference in $\dot{V}O_2$ during exercise between Fasted and Fed conditions (1252 ± 203 and 1261 ± 204 ml/min, respectively; P=0.084). In contrast, $\dot{V}CO_2$ was significantly higher during exercise in Fed state as compared to Fasted (1157 ± 191 vs. 1078 ± 170 ml/min for Fed and Fasted, respectively; P<0.001).

3.4.2 Substrate utilisation during exercise

There were significant differences observed for RER and fat and carbohydrate oxidation (**Table 3.3**). RER was significantly lower during the Fasted than the Fed conditions (95% CI -0.07 to -0.03, ES = 1.96, P<0.001). Accordingly, the calculated fat oxidation rate, expressed as gram per minute, was significantly higher during Fasted exercise (95% CI 0.08 to 0.15, ES = 1.71, P<0.001). In contrast, the carbohydrate oxidation rate was significantly lower in Fasted exercise when compared with Fed exercise (95% CI -0.43 to -0.22, ES = 1.55, P<0.001).

Consistently, as presented in **Figure 3.2**, the percentage of energy from fat was higher during Fasted exercise as compared to Fed exercise (42.2 ± 5.2% vs. 24.6 ± 8.3%, respectively, 95% CI -22.2 to -12.9, ES = 2.54, *P*<0.001). In contrast the percentage of energy from carbohydrate was higher during exercise in Fed than Fasted trials (57.8 ± 5.2% vs. 75.4 ± 8.3%, respectively, 95% CI 12.9 to 22.2, ES = 2.54, *P*<0.001). Additionally, the results showed that the carbohydrate was the main source used as a fuel during exercise in both trials with mean percentage of carbohydrate contribution to energy expenditure were 58 ± 5 % during Fasted and 75 ± 8 % during Fed exercise.

Table 3.3 Gas exchange and substrate oxidation during exercise

	Fasted state	Fed state
RER	0.87 ± 0.02	0.92 ± 0.03*
Fat oxidation (g/min)	0.29 ± 0.07	0.17± 0.07*
Carbohydrate oxidation (g/min)	0.89 ± 0.15	1.22 ± 0.26*

Data are means ± SD. Significant difference from fasted state at *P<0.001

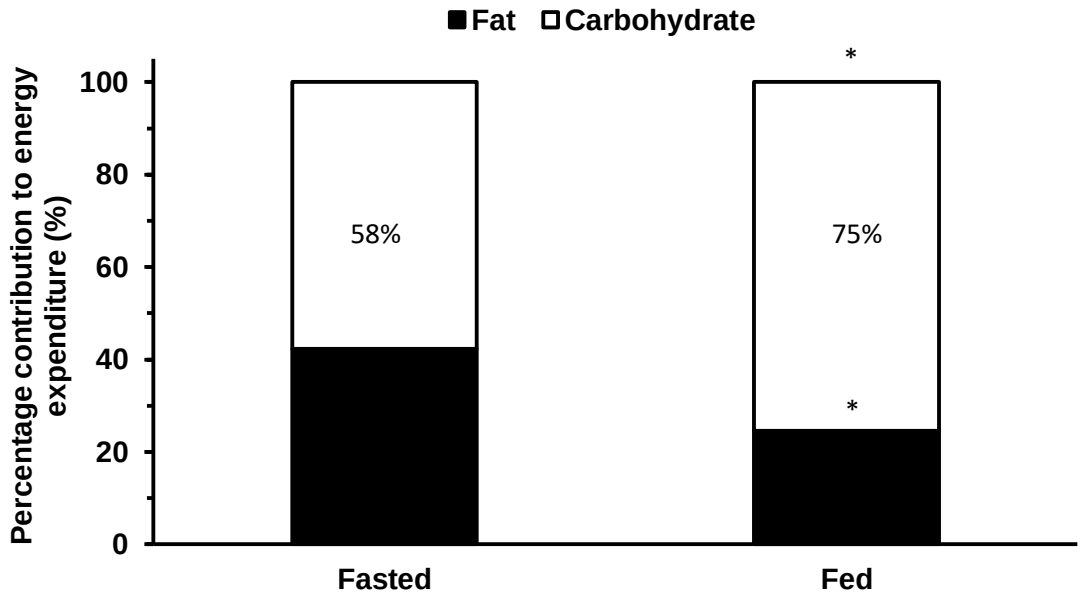

Figure 3.2 Percentage contributions to energy expenditure during exercise. Data are means ± SEM. *Significant difference from fasted state at $P<0.001$.

3.4.3 Blood metabolites and hormone responses

At baseline, plasma glucose, glycerol, NEFA, triglyceride and serum insulin concentrations were not statistically different from one trial to another. There was however significant trial x time interactions for plasma glucose, glycerol, NEFA, triglyceride and serum insulin concentrations ($P<0.001$).

During the Fasted trial, plasma glucose concentrations were constant during pre-exercise and exercise period, with no significant difference from baseline. However, the plasma glucose concentration increased significantly after post-exercise meal ingestion ($P<0.001$), and had returned to almost baseline value 3-hour of after exercise had finished (**Figure 3.3**). In contrast, during the Fed trial, glucose

concentration was significantly increased after the pre-exercise meal ingestion (*P*<0.001), returned to baseline values after 30 minutes of exercise and remained stable until the end of the trial. Plasma glucose concentration was significantly higher in the Fed trial at 45-minute post-meal before exercise than the Fasted trial (*P*=0.008), while the glucose concentration was significantly higher in the Fasted trial as compared to the Fed trial during 3-hour post exercise (1-hour, *P*<0.001; 2-hour, *P*<0.001; 3-hour, *P*=0.016). AUC for glucose was 14.4% higher in Fasted as compared to Fed (5.98 ± 0.22 mmol•L^{-1} vs. 5.23 ± 0.39 mmol•L^{-1}, respectively, 95% CI 0.51 to 0.99, ES = 2.37 *P*<0.001).

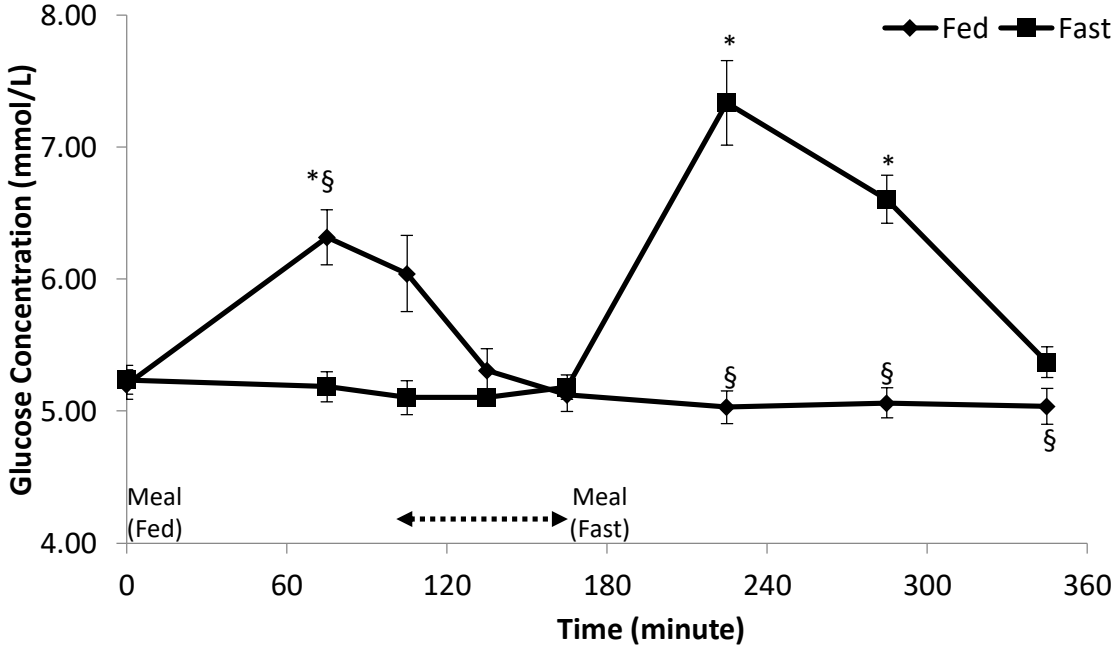

Figure 3.3 Changes in glucose concentrations in response to feeding and exercise (*n*=12). Data are means ± SEM. Arrow represents the exercise period. *Significant difference from baseline. §Significant difference from Fasted.

A similar pattern with glucose concentration was shown in serum insulin concentration (**Figure 3.4**), with the concentration stable before and during exercise but markedly increased following the meal ingestion after exercise (P<0.001) during the Fasted trial and remaining significantly elevated above baseline for the duration of the trial (2-hour, P<0.001 and 3-hour, P=0.007 post-exercise). On the other hand, during the Fed trial, serum insulin concentration increased significantly from baseline following ingestion of food before exercise (P<0.001) and remained elevated at the onset of exercise (P<0.001) and after 30 minutes of exercise (P<0.001). However, it returned to baseline values after the exercise bout was completed and then remained stable until the end of the trial. Insulin concentration was significantly higher in the Fed than Fasted trial in the period before and during exercise (P<0.001), in contrast insulin concentration was higher in the Fasted vs. Fed trial at post exercise (P<0.001) until the end of trial (P=0.016). AUC for insulin was 30.6% higher in the Fasted than Fed trial (318.6 ± 95.54 pmol•L^{-1} vs. 243.95 ± 57.27 pmol•L^{-1}, respectively, 95% CI 28.43 to 120.87, ES = 0.95, P=0.005).

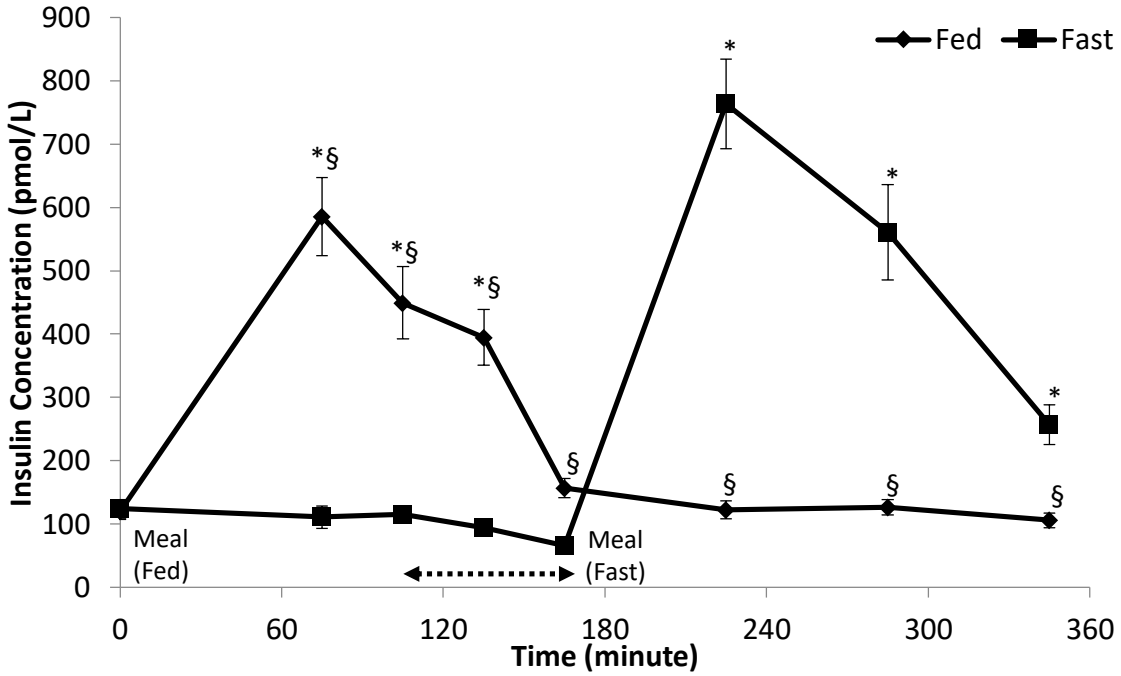

Figure 3.4 Changes in insulin concentrations in response to feeding and exercise (*n*=12). Data are means ± SEM. Arrow represents the exercise period. *Significant difference from baseline. §Significant difference from Fasted.

During the Fasted trial, plasma glycerol concentration, as shown in **Figure 3.5**, was significantly increased during exercise (*P*<0.001) and at the end of exercise bout (*P*<0.001) as compared to baseline. The concentration was then markedly decreased after the post-exercise meal and became significantly lower than baseline at 2-hour post exercise until the end of the trial (*P*=0.028). In contrast, in the Fed trial, glycerol concentration was significantly decreased from baseline at 45-minute and 90-minute after the pre-exercise meal (*P*<0.001) but slightly increased during exercise and the concentration was higher from baseline by the end of the trial. Plasma glycerol concentration was significantly higher in the Fasted than Fed trial at 45-minute pre-exercise (*P*=0.021), during exercise (*P*<0.001) and at end of exercise (*P*<0.001), but significantly lower in the Fasted than Fed trial at post-

exercise (2-hour, *P*=0.024; 3-hour, *P*=0.008). AUC for glycerol was 30% higher in the Fasted than Fed trial (84.87 ± 13.78 μmol•L^{-1} vs. 65.31 ± 22.23 μmol•L^{-1}, respectively, 95% CI 3.26 to 22.81, ES = 1.06, *P*=0.014)

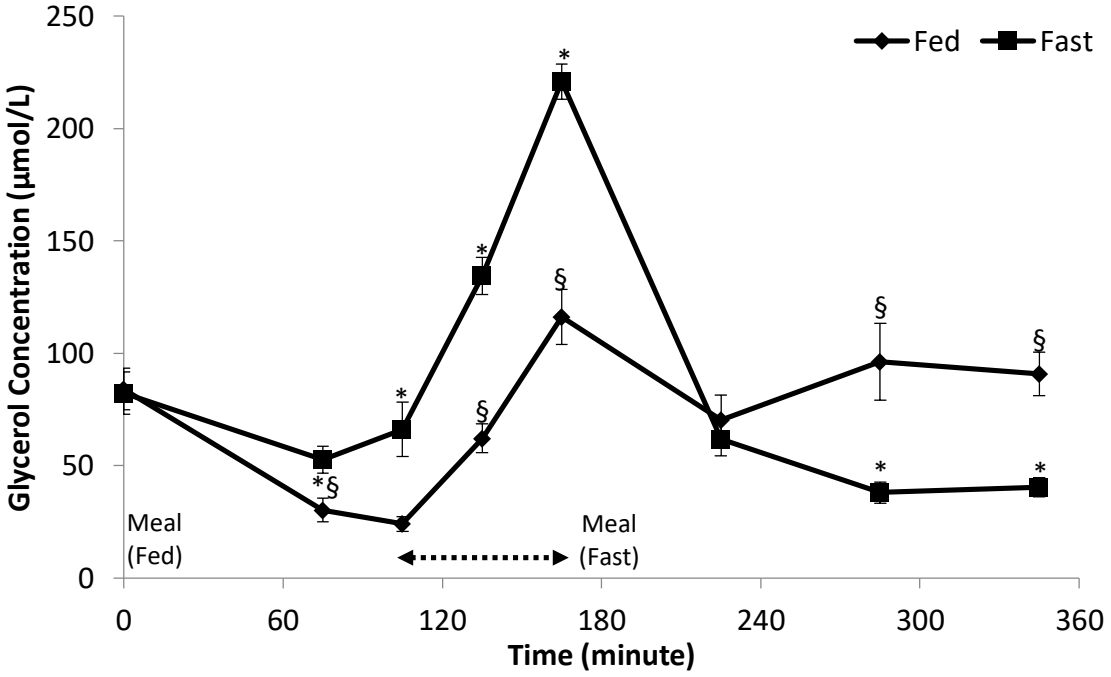

Figure 3.5 Changes in glycerol concentrations in response to feeding and exercise (*n*=12). Data are means ± SEM. Arrow represents the exercise period. *Significant difference from baseline. §Significant difference from Fasted.

In the Fasted trial, plasma NEFA concentration was stable before exercise, but significantly increased from baseline at the end of exercise (*P*=0.007) as shown in **Figure 3.6**. The post-exercise meal induced a significant decline in NEFA concentration (*P*=0.021) and remained lower than baseline until the end of the trial (*P*<0.001). In contrast, in the Fed trial, the pre-exercise meal significantly reduced plasma NEFA concentration below baseline value before exercise (45-minute post-meal, *P*=0.004; 90-minute post-meal, *P*<0.001), and it stayed low during exercise

(30-minute, *P*<0.001; 60-minute, *P*<0.05). However, it then started increasing above baseline at 2-hour post-exercise and remained elevated until the end of the trial but was not significantly different from baseline. NEFA concentration was higher in the Fasted trial during pre-exercise (*P*<0.001) and exercise (*P*<0.001) but lower during post-exercise (*P*<0.001) as compared to the Fed trial. AUC for NEFA was 20.5% higher in Fasted than Fed trial (0.53 ± 0.11 mmol•L^{-1} vs. 0.44 ± 0.14 mmol•L^{-1}, respectively, 95% CI 0.02 to 0.16, ES = 0.72, *P*=0.016).

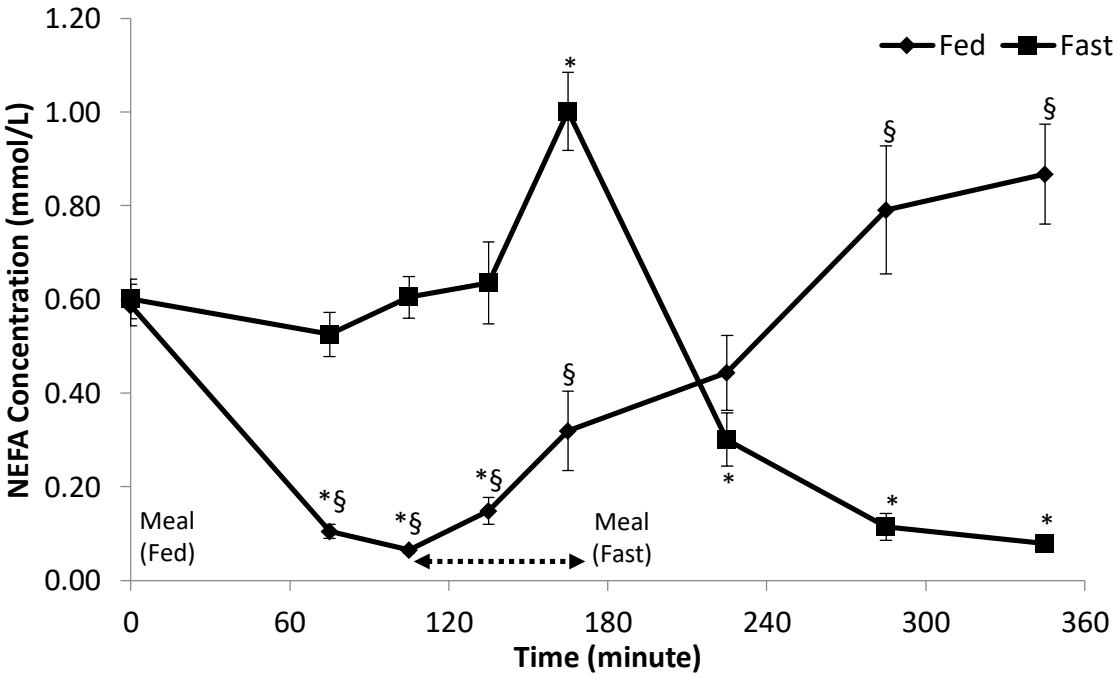

Figure 3.6 Changes in NEFA concentrations in response to feeding and exercise (*n*=12). Data are means ± SEM. Arrow represents the exercise period. *Significant difference from baseline. §Significant difference from Fasted.

In the Fasted trial, plasma TG concentration was significantly decreased from baseline before the onset of exercise (*P*<0.001), but after that not significantly different from baseline for the whole trial (**Figure 3.7**). Conversely, in the Fed trial,

TG concentration was significantly higher than baseline at the end of the exercise (*P*=0.042). TG concentration was generally higher, albeit not statistically different, in the Fed trial as compared to the Fasted trial at most of the time points except at baseline and 3-h of recovery. Accordingly, AUC for TG was 18.3% higher in Fed than Fasted trial (1.03 ± 0.35 mmol•L^{-1} vs. 1.26 ± 0.45 mmol•L^{-1}, respectively, 95% CI -0.29 to -0.01, ES = 0.57, *P*=0.037).

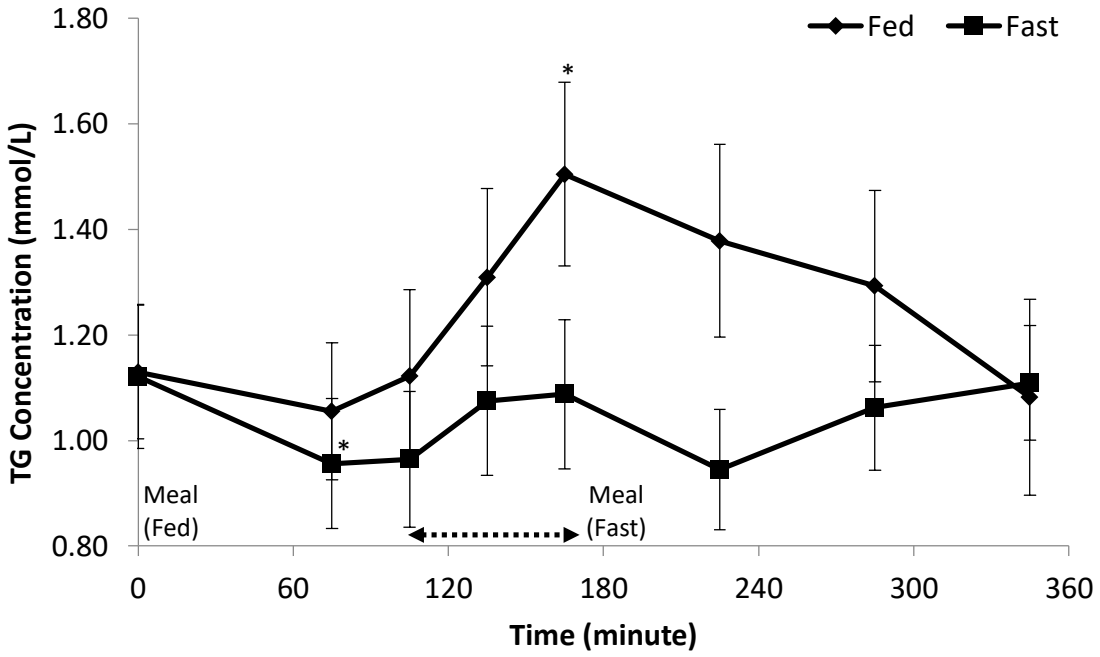

Figure 3.7 Changes in TG concentrations in response to feeding and exercise (*n*=12). Data are means ± SEM. Arrow represents the exercise period. *Significant difference from baseline. §Significant difference from Fasted.

3.4.4 Appetite rating and energy intake during lunch

At baseline, the response for hunger, satiety, fullness and prospective food consumption was not statistically different between trials. There was however

significant trial x time interactions for hunger, satiety, fullness and prospective food consumption (*P*<0.001).

During the Fasted trial, feelings of hunger, albeit not statistically significant, were slightly increased from baseline when assessed before and after exercise, while the rating was lower before lunchtime, as presented in **Figure 3.8**. In contrast, feelings of hunger before exercise (*P*<0.001) was suppressed with pre-exercise feeding but was significantly increased compared with baseline right before lunchtime (*P*<0.001) in the Fed trial. The feeling of hunger was suppressed in the Fed versus Fasted immediately before exercise (*P*<0.001), while the feeling was significantly suppressed in the Fasted vs. Fed trial before the lunchtime (*P*<0.001). There was no significant difference of hunger rating immediately post-exercise period between trials.

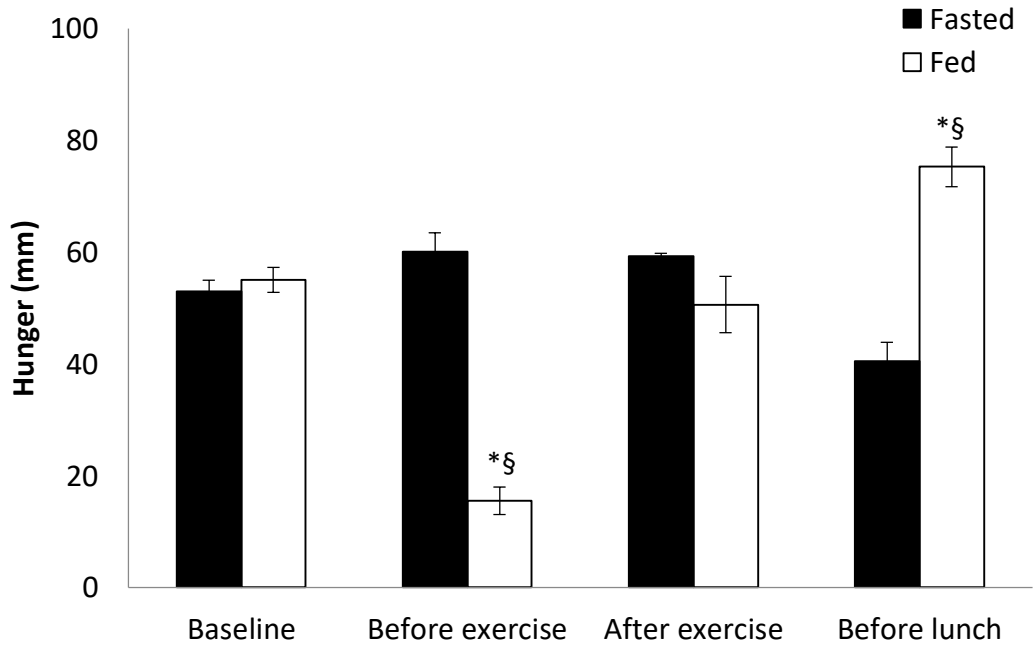

Figure 3.8 Hunger scores (*n*=12) in response to exercise. Data are means ± SEM. *Significant difference from baseline. §Significant difference from Fasted.

For prospective consumption (**Figure 3.9**), in the Fasted trial, the score at pre- (*P*=0.003) and post-exercise (*P*=0.006) period was significantly higher than baseline value and then returned to baseline value before lunch time. In the Fed trial the rating was significantly decreased from baseline before exercise (*P*<0.001) but significantly higher than baseline before lunch time (*P*=0.003). The score of prospective food consumption was significantly higher in the Fasted vs. Fed trial before exercise (*P*<0.001), but significantly lower before lunch time (*P*=0.024). Similarly, there was no significant difference of prospective food consumption rating immediately post-exercise period between trials.

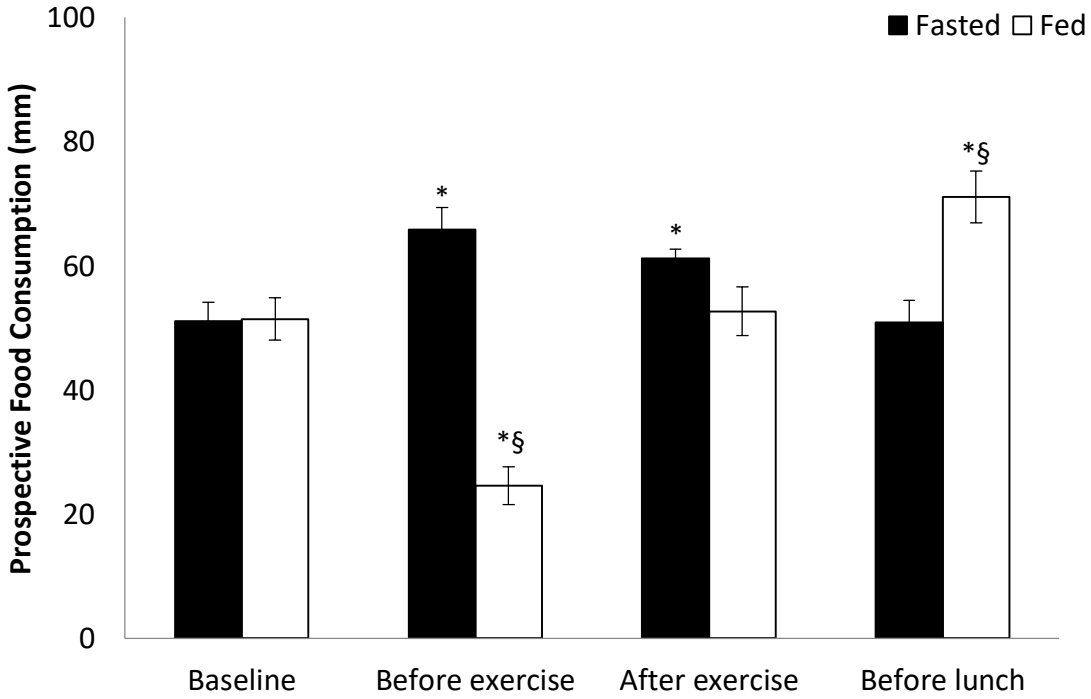

Figure 3.9 Prospective food consumption scores (*n*=12) in response to exercise. Data are means ± SEM. *Significant difference from baseline. §Significant difference from Fasted.

During the Fasted trial, the score for satiety was significantly lower than baseline at post-exercise (*P*=0.003), while significantly higher than baseline before lunch time (*P*=0.003) (**Figure 3.10**). Contrary, in the Fed trial satiety score was significantly higher than baseline before (*P*<0.001) and after exercise (*P*=0.018), but the score was non-significantly lower than baseline before lunchtime. The satiety score was significantly higher in the Fed than Fasted trial before and after exercise (*P*<0.001), while significantly higher in the Fasted vs. Fed trial before the lunch time (*P*<0.001).

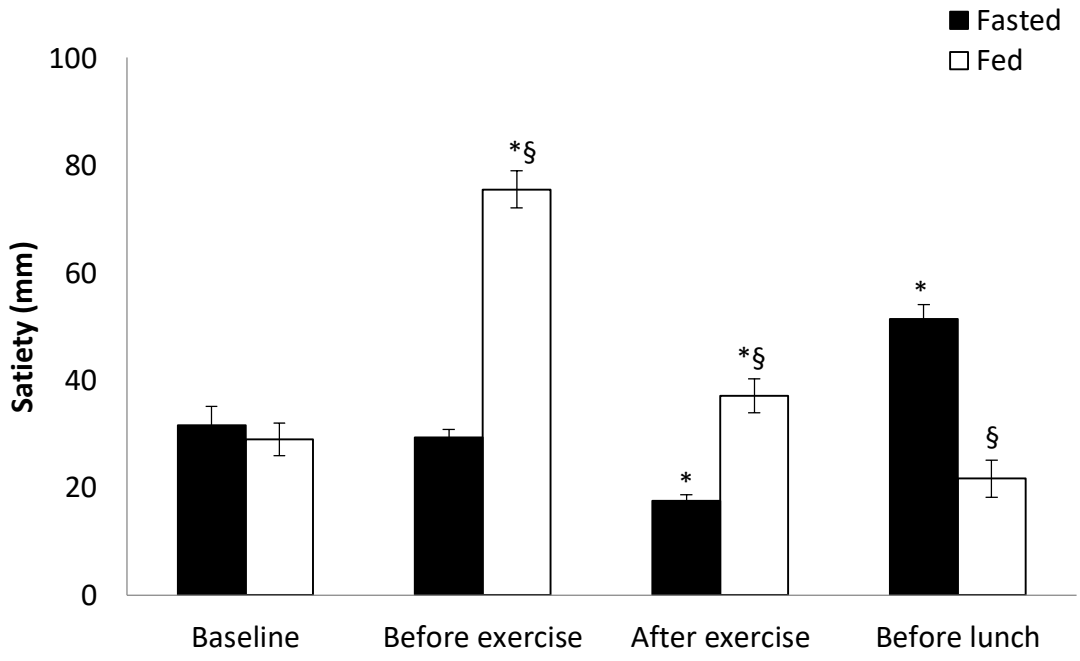

Figure 3.10 Satiety scores (*n*=12) in response to exercise. Data are means ± SEM. *Significant difference from baseline. §Significant difference from Fasted.

The score for fullness showed a similar pattern with satiety (**Figure 3.11**), where the score was non-significantly lower than baseline at post-exercise, but significantly higher than baseline before lunch time in the Fasted trial (P<0.001). In contrast, in the Fed trial, the fullness score was significantly higher before exercise (P<0.001) than baseline score, but non-significantly lower right before lunch time. Fullness score was significantly higher in the Fed than Fasted trial before exercise (P<0.001), whereas the score was significantly higher in the Fasted vs. Fed trial before the lunch time (P=0.004). There was no significant difference of fullness rating at post-exercise period between trials.

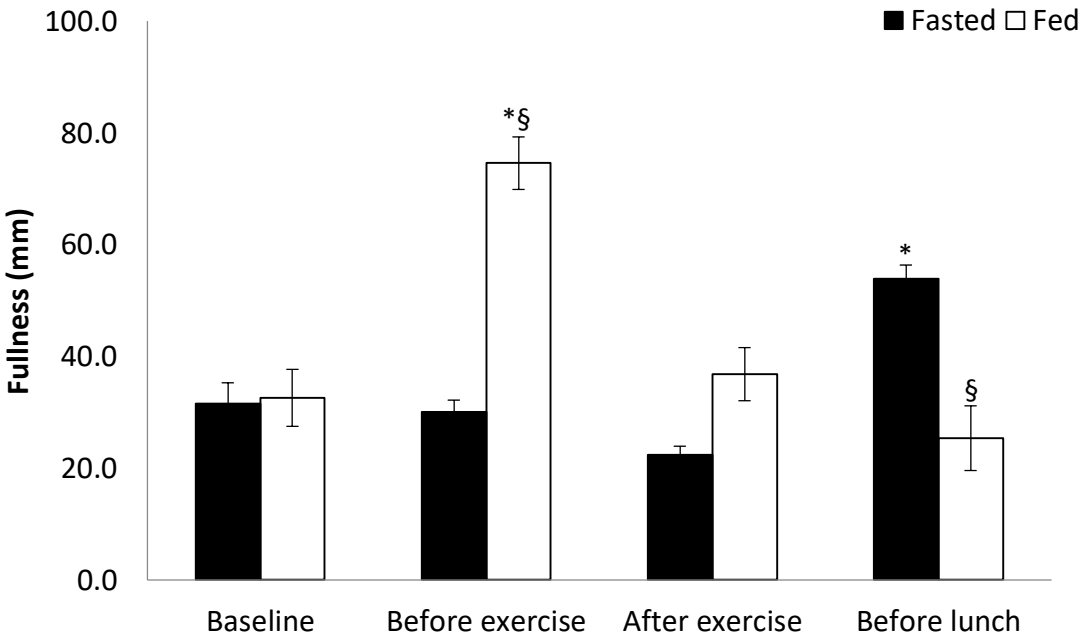

Figure 3. 11 Fullness scores (n=12) in response to exercise. Data are means ± SEM. *Significant difference from baseline. §Significant difference from Fasted.

During lunch time, the total energy intake was significantly different between trials (**Figure 3.12**), with a 17% higher calorie intake observed in the Fed trial as

compared to the Fasted trial (897 ± 241 vs. 767 ± 211 kcal, respectively, 95% CI -181.17 to -78.83, ES = 0.57, *P*<0.001).

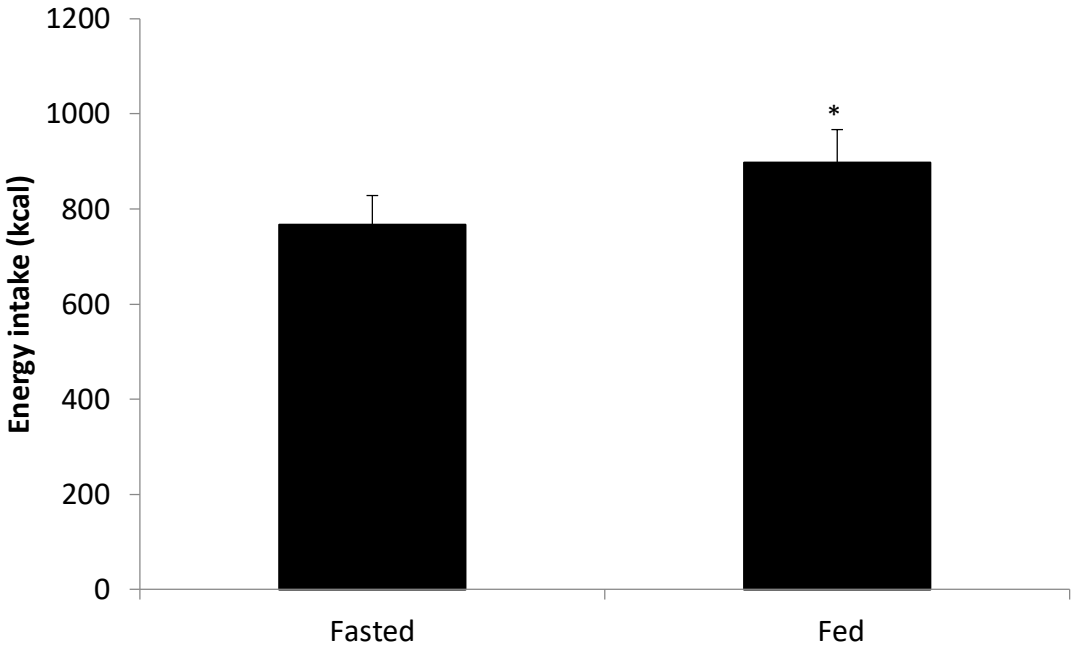

Figure 3.12 Energy intake during lunch (*n*=12). Data are means ± SEM. *Significant difference from Fasted.

3.5 Discussion

The main purpose of the present study was to investigate the effect of performing exercise in the fed vs. overnight-fasted state on substrate utilisation during exercise in obese or overweight/centrally obese women. The results showed that exercise performed in the overnight-fasted state resulted in higher fat oxidation and lower carbohydrate oxidation during exercise as compared to when exercise was commenced 90 minutes after meal consumption. The study also aimed to investigate the effects of feeding before or after exercise on overall metabolic and hormonal responses, appetite sensations and subsequent food intake. Exercise performed in the overnight-fasted state with food provided immediately after exercise, as compared to exercise performed after food intake, resulted in higher overall glucose, insulin, glycerol and NEFA responses and lower plasma TG responses across the measurement period. As well, exercise in the overnight fasted-state resulted in lower energy intake during an *ad libitum* lunch as compared to fed-state exercise.

A key finding from this present study was that fasted exercise induced a significantly greater reliance on fat and lesser reliance on carbohydrate towards exercise energy expenditure as compared with fed exercise. Such responses are consistent with an overall higher potential for lipid oxidation and lesser carbohydrate oxidation in the fasted exercise condition as observed in lean men and women and overweight and obese men (Vieira et al., 2016). The greater contribution of fat as fuel during exercise in the fasted state was paralleled with higher concentrations of plasma

glycerol and NEFA during exercise in fasted state, with insulin being higher during exercise in the fed condition. The data further demonstrates the potency of fasted as compared with fed exercise in augmenting exercise fat oxidation in obese populations regardless of biological sex.

As **Chapter 2 and 3** were conducted in men and women, respectively, under similar experimental conditions it was of interest to explore whether the effect of pre-exercise feeding on fat oxidation during exercise was similar, given that sex-differences in substrate utilisation during exercise have been reported (Horton et al., 1998, Henderson et al., 2007, Henderson and Alderman, 2014). Indeed, this secondary analysis showed that the suppression in fat oxidation with fed vs. fasted state exercise was higher in the women than the men (**Table 3.4**). Specifically, a reduction of 17% in energy contributions from fat during exercise, from fasted to fed trial, was seen in women participants, whereas only 9% of reduction was seen in men participants, in which suggested that the change in fat oxidation (and carbohydrate oxidation) with fed vs. fasting is greater in women as compared to men. The present study also showed a greater difference of AUC plasma glycerol and NEFA at pre- and during exercise between fasted and fed state in women when comparing with men participants (**Table 3.5**). In line with current study, sex differences in the metabolic response to fasting have also been observed in humans, in which the increase in plasma fatty acids that normally occur during fasting are greater in women than in men who were matched on percent body fat in response to fasting (Mittendorfer et al., 2001). It is acknowledged that the secondary data analysis employed herein is based on groups with similar in recruitment criteria

and characteristics but the men and women were not matched accordingly based on recommended matching criteria for sex-difference studies (Tarnopolsky, 2000). Even so, the observations that the effects of fasted vs. fed exercise appears to show greater differences for substrate utilisation and some blood markers opens up the interesting possibility that there could be sex-differences in metabolic flexibility. This idea became the subject of further investigation in **Chapter 4**.

Table 3.4 Differences of energy contributions during exercise between sexes

	Fasted	Fed	Differences between trials
Carbohydrate			
Men (n=12)	69%	78%	9%
Women (n=12)	58%	75%	17%
P value	0.001	0.431	0.046
Fat			
Men (n=12)	31%	22%	9%
Women (n=12)	42%	25%	17%
P value	0.001	0.431	0.046

Table 3.5 Differences of AUC of plasma lipids at pre- and during exercise between sexes

	Fasted	Fed	Differences between trials
Glycerol			
Men (n=12)	12693 ± 4831	8402 ± 4588	34%
Women (n=12)	19706 ± 2430	7447± 2896	42%
P value	0.001	0.609	0.052
NEFA			
Men (n=12)	102.3 ± 36.4	44.3 ± 14.2	57%
Women (n=12)	135.3 ± 24.5	39.8 ± 7.0	71%
P value	0.016	0.339	0.003

Mean ± SD

In Chapter 2, a higher response of glucose and insulin was seen with fasted-state exercise than fed-state exercise was observed in obese men. Indeed, the same observation was seen in women obese in this present study. In addition, a higher NEFA and a lower triglyceride response in fasted state as compared to fed state was observed. This observation was similar in a study by Enevoldsen and colleagues (2004), who determined blood metabolite and hormone responses in young healthy men who undertook exercise either before or after mixed macronutrient meal ingestion. A lower plasma TAG and very low-density-lipoprotein-TAG [VLDL-TAG] concentrations was observed when exercise was performed before as compared after to meal ingestion in the period of 5.5 hours of

trial. A similar result was also observed in a study of overweight men, whereby plasma TAG was significantly lowered when exercise in exercise followed by food intake but not food intake followed by exercise (Farah and Gill, 2013). It is well established that high concentrations of triglycerides were regarded as a cardiovascular risk factor (Hokanson and Austin, 1996), thus exercise in fasted state might be a strategy to maximise the benefits of exercise on blood triglyceride reduction. Regardless, it is important to note that the triglyceride responses observe here and in the studies cited were measured over a transient period, thus the long-term significance remains unclear.

In addition to assessing metabolic responses, it was relevant to consider the potential for fasted vs. fed state exercise to affect food intake at a subsequent meal, as exercise tends to modify energy intake (King et al., 1994, Imbeault et al., 1997). A previous study in young, lean active men demonstrated that fasting before exercise decreased 24-hour energy intake (Bachman et al., 2016). In line with that, the present study indicated that energy intake at lunch time was lower in the fasted trial as compared to fed trial. However, it was contrary with previous study among lean populations (Gonzalez et al., 2013). Gonzalez and colleagues conducted a study in young physically active men who undertook one hour moderate intensity treadmill running exercise performed in the overnight-fasted state or two hours after breakfast consumption. After exercise, all participants consumed a standardised mixed-macronutrient drink, followed by provision of an *ad libitum* test lunch at 90 minutes postprandial. The authors reported that despite the absence of breakfast in the overnight-fasted exercise, energy intake during the test lunch was similar when

exercise was performed in the fed-state. This discrepancy might be explained by the different time used in the study protocol, as previous work provided lunch at 90 minutes after exercise, while present work provided lunch at 180 minutes after exercise. This might also be because they provided a post-exercise snack (test drink) in each trial condition, 90 minutes before the ad libitum lunch was provided. Nonetheless, they observed that, energy balance was least positive when exercise performed in breakfast omission state. Interestingly, they also found that a negative fat balance is achieved when exercise is performed without taking breakfast.

Nevertheless, the present energy intake data was supported by the VAS data suggesting there are clear effects on both subjective feelings and behaviour. To the best of our knowledge, this is the first study to demonstrate the effect of exercise in fasted and fed conditions on appetite sensations and subsequent energy intake among overweight and obese women. It is acknowledged that while a strength of the present study design is that overall energy balance up to the point of the lunch was designed to be similar, the duration since the last meal, which was shorter during the fasted trial, may have influenced the subsequent energy intake. Thus, how fasted vs. fed exercise affects energy intake and also subsequent expenditure when participants are allowed to self-select timing and quantity of all subsequent meals would be important to study in order to fully understand the potential benefit of exercising in the overnight-fasted state in the obese population. Further, previous research has shown that acute exercise alters appetite hormones (e.g., lower concentrations of ghrelin, higher PYY3-36) in a direction expected to suppress

energy intake (Broom et al., 2007, Hagobian et al., 2008, Broom et al., 2009, King et al., 2011), which is another area that should be explored in the future research.

In conclusion, the current study showed that an acute bout of aerobic exercise performed in the overnight-fasted state, as compared to fed-state, augmented whole-body exercise fat oxidation and appeared to decrease subsequent energy intake in obese or overweight/centrally obese women. This provides a metabolic and behavioural basis for further exploring the long-term consequences of performing exercise in the overnight fasted versus fed state in obese or overweight centrally obese women. In addition, the current study provided initial evidence that women may be more metabolically flexible than men in response to carbohydrate-containing food ingestion with respect to changes in substrate utilisation and the metabolite and hormonal response. This opens up the need to more carefully understand the potential for sex-differences in metabolic flexibility in response to nutrient ingestion.

3.6 References

BACHMAN, J. L., DEITRICK, R. W. & HILLMAN, A. R. 2016. Exercising in the fasted state reduced 24-hour energy intake in active male adults. *Journal of Nutrition and Metabolism***,** 1-7.

BORG, G. 1982. Ratings of perceived exertion and heart rates during short-term cycle exercise and their use in a new cycling strength test. *International Journal of Sports Medicine,* 3**,** 153-158.

BROOKS, G. A., BUTTE, N. F., RAND, W. M., FLATT, J.-P. & CABALLERO, B. 2004. Chronicle of the Institute of Medicine physical activity recommendation: how a physical activity recommendation came to be among dietary recommendations. *The American Journal of Clinical Nutrition,* 79**,** 921S-930S.

BROOM, D. R., BATTERHAM, R. L., KING, J. A. & STENSEL, D. J. 2009. Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology,* 296**,** R29-R35.

BROOM, D. R., STENSEL, D. J., BISHOP, N. C., BURNS, S. F. & MIYASHITA, M. 2007. Exercise-induced suppression of acylated ghrelin in humans. *Journal of Applied Physiology,* 102**,** 2165-2171.

CLAYTON, D. J., STENSEL, D. J. & JAMES, L. J. 2016. Effect of breakfast omission on subjective appetite, metabolism, acylated ghrelin and GLP-17-36 during rest and exercise. *Nutrition,* 32**,** 179-185.

COHEN, J. 1988. Statistical power analysis for the behavioural sciences. 2nd ed. Hillsdale (NJ): *Lawrence Erlbaum Associates*, 22–25.

COLBERG, S. R., HAGBERG, J. M., MCCOLE, S. D., ZMUDA, J. M., THOMPSON, P. D. & KELLEY, D. E. 1996. Utilization of glycogen but not plasma glucose is reduced in individuals with NIDDM during mild-intensity exercise. *Journal of Applied Physiology,* 81**,** 2027-2033.

CRAIG, C. L., MARSHALL, A. L., SJÖSTRÖM, M., BAUMAN, A. E., BOOTH, M. L., AINSWORTH, B. E., PRATT, M., EKELUND, U., YNGVE, A., SALLIS, J. F. & OJA, P. 2003. International physical activity questionnaire: 12-country reliability and validity. *Medicine and Science in Sports and Exercise,* 35**,** 1381-1395.

CUMMING, G. & Finch, S. 2001. A primer on the understanding, use, and calculation of confidence intervals that are based on central and noncentral distributions. *Educational and Psychological Measurement,* 61(4), 532–574.

DERAVE, W., MERTENS, A., MULS, E., PARDAENS, K. & HESPEL, P. 2007. Effects of post-absorptive and postprandial exercise on glucoregulation in metabolic syndrome. *Obesity,* 15**,** 704-711.

ENEVOLDSEN, L. H., SIMONSEN, L., MACDONALD, I. A. & BÜLOW, J. 2004. The combined effects of exercise and food intake on adipose tissue and splanchnic metabolism. *The Journal of Physiology,* 561**,** 871-882.

FARAH, N. & GILL, J. 2013. Effects of exercise before or after meal ingestion on fat balance and postprandial metabolism in overweight men. *British Journal of Nutrition,* 109**,** 2297-2307.

FLINT, A., RABEN, A., BLUNDELL, J. E. & ASTRUP, A. 2000. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity,* 24**,** 38-48.

GONZALEZ, J. T., FUCHS, C. J., SMITH, F. E., THELWALL, P. E., TAYLOR, R., STEVENSON, E. J., TRENELL, M. I., CERMAK, N. M. & VAN LOON, L. J. 2015. Ingestion of glucose or sucrose prevents liver but not muscle glycogen depletion during prolonged endurance-type exercise in trained cyclists. *American Journal of Physiology, Endocrinology and Metabolism,* 309**,** E1032-E1039.

GONZALEZ, J. T., VEASEY, R. C., RUMBOLD, P. L. S. & STEVENSON, E. J. 2013. Breakfast and exercise contingently affect postprandial metabolism and energy balance in physically active males. *The British Journal of Nutrition,* 110**,** 721-732.

HAGOBIAN, T. A., SHAROFF, C. G. & BRAUN, B. 2008. Effects of short-term exercise and energy surplus on hormones related to regulation of energy balance. *Metabolism: Clinical and Experimental,* 57**,** 393-398.

HAINER, V., KUNESOVÁ, M., PARÍZKOVÁ, J., STICH, V., MIKULOVÁ, R. & SLABÁ, S. 2000. Respiratory quotient in obesity: its association with an ability to retain weight loss and with parental obesity. *Sbornik Lekarsky,* 101**,** 99-104.

HENDERSON, G. C. & ALDERMAN, B. L. 2014. Determinants of resting lipid oxidation in response to a prior bout of endurance exercise. *Journal of Applied Physiology,* 116**,** 95-103.

HENDERSON, G. C., FATTOR, J. A., HORNING, M. A., FAGHIHNIA, N., JOHNSON, M. L., MAU, T. L., LUKE-ZEITOUN, M. & BROOKS, G. A. 2007. Lipolysis and fatty acid metabolism in men and women during the postexercise recovery period. *The Journal of Physiology,* 584**,** 963-981.

HOKANSON, J. E. & AUSTIN, M. A. 1996. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. *Journal of Cardiovascular Risk,* 3**,** 213-219.

HORTON, T. J., PAGLIASSOTTI, M. J., HOBBS, K. & HILL, J. O. 1998. Fuel metabolism in men and women during and after long-duration exercise. *Journal of Applied Physiology,* 85**,** 1823-1832.

IMBEAULT, P., SAINT-PIERRE, S., ALMÉRAS, N. & TREMBLAY, A. 1997. Acute effects of exercise on energy intake and feeding behaviour. *The British Journal of Nutrition,* 77**,** 511-521.

KELLEY, D. E., WILLIAMS, K. V., PRICE, J. C., MCKOLANIS, T. M., GOODPASTER, B. H. & THAETE, F. L. 2001. Plasma fatty acids, adiposity, and variance of skeletal muscle insulin resistance in type 2 diabetes mellitus. *The Journal of Clinical Endocrinology and Metabolism,* 86**,** 5412-5419.

KIM, J. Y., HICKNER, R. C., CORTRIGHT, R. L., DOHM, G. L. & HOUMARD, J. A. 2000. Lipid oxidation is reduced in obese human skeletal muscle. *American Journal of Physiology, Endocrinology and Metabolism,* 279**,** E1039-E1044.

KING, J. A., WASSE, L. K., EWENS, J., CRYSTALLIS, K., EMMANUEL, J., BATTERHAM, R. L. & STENSEL, D. J. 2011. Differential acylated ghrelin, peptide YY3-36, appetite, and food intake responses to equivalent energy deficits created by exercise and food restriction. *The Journal of Clinical Endocrinology andM,* 96**,** 1114-1121.

KING, N. A., BURLEY, V. J. & BLUNDELL, J. E. 1994. Exercise-induced suppression of appetite: effects on food intake and implications for energy balance. *European Journal of Clinical Nutrition,* 48**,** 715-724.

LI, J., O'CONNOR, L. E., ZHOU, J. & CAMPBELL, W. W. 2014. Exercise patterns, ingestive behaviors, and energy balance. *Physiology and Behavior,* 134**,** 70-75.

MARTINS, C., MORGAN, L. M., BLOOM, S. R. & ROBERTSON, M. D. 2007. Effects of exercise on gut peptides, energy intake and appetite. *The Journal of Endocrinology,* 193**,** 251-258.

MITTENDORFER, B., HOROWITZ, J. F. & KLEIN, S. 2001. Gender differences in lipid and glucose kinetics during short-term fasting. *American Journal of Physiology, Endocrinology and Metabolism,* 281**,** E1333-E1339.

NICE 2014. Obesity - identification, assessment and management. https://www.nice.org.uk/guidance/cg189/chapter/1ecommendations#identification-and-classification-of-overweight-and-obesity. *National Institute for Health and Care Excellence*.

SEIDELL, J. C., MULLER, D. C., SORKIN, J. D. & ANDRES, R. 1992. Fasting respiratory exchange ratio and resting metabolic rate as predictors of weight gain: the Baltimore Longitudinal Study on Aging. *International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity,* 16**,** 667-674.

STRICKER, R., EBERHART, R., CHEVAILLER, M.-C., QUINN, F. A., BISCHOF, P. & STRICKER, R. 2006. Establishment of detailed reference values for luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone during different phases of the menstrual cycle on the Abbott ARCHITECT analyzer. *Clinical Chemistry and Laboratory Medicine,* 44**,** 883-887.

TARNOPOLSKY, M. A. 2000. Gender differences in metabolism; nutrition and supplements. *Journal of Science and Medicine in Sport,* 3**,** 287-298.

VIEIRA, A. F., COSTA, R. R., MACEDO, R. C. O., COCONCELLI, L. & KRUEL, L. F. M. 2016. Effects of aerobic exercise performed in fasted v. fed state on fat and carbohydrate metabolism in adults: a systematic review and meta-analysis. *The British Journal of Nutrition,* 116**,** 1153-1164.

WHO & IDF 2006. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. *World Health Organization and International Diabetes Federation*.

ZURLO, F., LILLIOJA, S., ESPOSITO-DEL PUENTE, A., NYOMBA, B. L., RAZ, I., SAAD, M. F., SWINBURN, B. A., KNOWLER, W. C., BOGARDUS, C. & RAVUSSIN, E. 1990. Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ. *American Journal of Physiology, Endocrinology and Metabolism,* 259**,** E650-E657.

CHAPTER 4

Metabolic Flexibility: Comparison of Substrate Metabolism between Men and Women after Glucose Feeding

In this chapter, there were several individuals involved during the data collection and analysis other than author of this thesis:

1. *Nurul Fadhilah Abdullah (author) conducted all data collection, sample analysis for substrate utilisation, blood samples and performed all statistical analysis.*
2. *Tim Podlogar contributed in urine sample analysis for urea.*
3. *Dr Konstantinos Manolopoulos gave medical oversight including inspection of ECG traces.*
4. *For sex-hormone analysis (estrogen and progesterone), they were outsourced to Randox Clinical Laboratory Services, Co Antrim, UK.*

4.1 Summary

Metabolic flexibility has been defined as the capacity to switch between carbohydrate and fat as primary fuel sources in response to changing fuel availability and energy demand. Evidence has shown that poor metabolic flexibility is associated with obesity and type 2 diabetes. There is also emerging evidence showing that men and women respond differently to a range of metabolic stressors (e.g., exercise, starvation), and women might, therefore, be more metabolically flexible in response to dietary manipulation than men. Therefore, the aim of this study was to investigate the influence of biological sex on metabolic flexibility (substrate oxidation responses) in response to the provision of a carbohydrate drink (1 g glucose/kg lean body mass [LBM]) among 8 well-matched (based on age, BMI, ethnicity and $\dot{V}O_{2peak}$ relative to LBM) young men and women who were healthy and reported low habitual physical activity. Indirect calorimetry was used to measure whole body substrate utilisation, and venous blood was collected routinely over a 5 hour period. There were significant sex-time interactions for RQ (P<0.001) and carbohydrate (P<0.001) and fat oxidation rate (P<0.001). RQ and carbohydrate oxidation rate had increased significantly by 1 hour and remained elevated above baseline levels for at least 2 hours in women and 3 hours in men, and returned to baseline by 3 hours in women and 4 hours in men, with statistically significant sex-differences at 1 hour (P<0.001). Additionally, women also had significantly higher carbohydrate oxidation rate at baseline (2.61 ± 0.46 vs. 1.79 ± 0.34 mg/min/kg LBM, respectively, 95% CI -1.25 to -0.04, ES = 2.03, P=0.012) than men. Fat oxidation was reduced significantly at 1 hour (P<0.001) and remained so until 2 hours

(*P*=0.005) in women as compared to baseline, whereas significantly reduced at 1 hour until 3 hour (*P*=0.032) in men. Fat oxidation returned to baseline values by 3 hours in women and 4 hours in men, with statistically significant sex-differences (higher in women) at 3 and 4 hours (*P*=0.030). In women, the RQ and carbohydrate oxidation peaked earlier (at 1 hour; RQ: 0.93 ± 0.02 vs. 0.87 ± 0.04, respectively, 95% CI -0.09 to -0.02, ES = 1.90, *P*=0.012; CHO oxidation: 4.67 ± 0.58 vs. 2.21 ± 0.38 mg/min/kg LBM, respectively, 95% CI -3.07 to -3.07 to -2.02, ES = 5.01, *P*<0.001) as compared to men (at 2 hours). Similarly, in women, glucose and insulin concentration peaked (at 1 hour) and returned to baseline (at 3 hours) earlier in women as compared to men (at 2 and 4 hours, respectively). There was no significant sex difference found in NEFA concentration responses (AUC women: 0.44 ± 0.10 mmol•L^{-1} *vs*. AUC men: 0.39 ± 0.10 mmol•L^{-1}, *P*=0.405). The present study has revealed substantial differences in the response of postprandial substrate metabolism to glucose feeding in men and women, whereby women appear to exhibit higher levels of metabolic control than men. This suggests that women cannot be assumed to respond in the same manner as men to nutritional interventions, which has implications for research and potentially practice in nutrition and exercise.

4.2 Introduction

Metabolic flexibility has been defined as the capacity to switch between carbohydrate and fat as the primary fuel sources for energy production in response to changing fuel availability and energy demand (Storlien et al., 2004). For example, someone who is metabolically flexible would easily be able to switch from using fat as the primary fuel source in the overnight-fasted state to carbohydrate as the primary fuel source following ingestion of a carbohydrate containing drink or meal. However, when the body is metabolically inflexible, the capacity to switch between fat and carbohydrate as the primary source of fuels based on the availability of nutrients and energy demand is impaired (Blaak et al., 2000, Corpeleijn et al., 2009). This metabolic inflexibility is often seen in patients with insulin resistance (Galgani et al., 2008a), and has been considered to play a crucial role in the development of type 2 diabetes (Corpeleijn et al., 2009). Previous studies in obese and type 2 diabetes populations have shown that the RQ is not responsive or blunted to hyperinsulinemic euglycaemic clamps or after a meal, indicating an impairment in the capacity to increase muscle and whole body glucose oxidation and storage during insulin stimulation (Kelley et al., 1999, Kelley and Mandarino, 2000, Wohl et al., 2004, Galgani et al., 2008b). Therefore, it is essential to understand factors that may influence metabolic flexibility and thereby susceptibility to metabolic disease.

Interestingly, there appears to be a distinct sex difference in the prevalence and prognoses of type 2 diabetes (The DECOTE Study Group, 2003), where the global prevalence is reported to be higher in men than women (Wild et al., 2004). Whether

sex-differences in metabolic flexibility are related to this is unknown. Indeed, in **Chapter 3** of this thesis, data were presented to suggest that the magnitude of change in exercise fuel utilisation (e.g., greater suppression of fat oxidation, greater stimulation of carbohydrate oxidation) in response to fed versus fasted exercise was greater in women than men. The possibility that sex-differences in metabolic flexibility was suggested.

Several lines of evidence indicate that men and women may respond differently to a variety of metabolic stressors such as fasting, exercise or lipid infusion (Mittendorfer et al., 2001, Devries et al., 2006, Høeg et al., 2011, Henderson, 2014). There is substantial evidence that suggests, for a given submaximal exercise intensity performed in the overnight-fasted state, women as compared to men rely more heavily on lipid and less on carbohydrate oxidation (Horton et al., 1998, Carter et al., 2001, Tarnopolsky, 2008, Lundsgaard and Kiens, 2014), which is consistent with the comparisons made in **Chapter 3**. In addition, lipolytic rate (assessed by glycerol rate of appearance [Ra]) was found to be greater in women as compared to men during overnight-fasting (14 hours), however when the period of fasting is longer (22 hours), the relative increase in glycerol Ra was higher in men than in women (Mittendorfer et al., 2001). As well, intra-lipid infusion has been shown to cause less insulin resistance of muscle glucose uptake in women than in men (Høeg et al., 2011). However, the effect of sex on metabolic flexibility in response to carbohydrate nutrition as a more ecologically valid intervention has to the authors' knowledge not been directly investigated.

While the results of **Chapter 3** implied a sex-difference in metabolic response, it was acknowledged that comparisons were made between studies that were not designed to investigate sex-differences *per se*. Investigating metabolic differences as a function of biological sex is difficult, as confounding variables such as adiposity, fat distribution, hormonal status, and aerobic fitness level might complicate interpretations of the data. Thus, matching of men and women in regard to variables such as age, BMI, $\dot{V}O_{2peak}$ relative to LBM, physical activity status, and with consideration of menstrual cycle status has been suggested to be crucial in metabolic studies of sex differences (Tarnopolsky et al., 1990, Tarnopolsky, 2000, Tarnopolsky, 2008). As well, ethnicity can influence metabolic responses (Hall et al., 2010) and thus matching for race is an important consideration. Finally, the provision of nutrients in doses expressed relative to lean body mass as a surrogate for the most relevant site of glucose disposal (i.e., skeletal muscle) is likely to represent a fairer approach to making sex-based comparisons, as previously highlighted (Rattarasarn et al., 2010).

The purpose of the present study was to investigate sex-differences in metabolic responses (fuel selection, plasma glucose/NEFA and insulin responses) to acute oral glucose feeding in men and women pair-matched for relevant variables known to affect metabolic responses, with glucose provided in doses relative to LBM. In order to investigate metabolic flexibility *per se*, responses in this study were focused on effects of resting postprandial metabolism. Based on the comparisons made in **Chapter 3** and through pilot work (**Appendix 1**) it was hypothesized that women

would display an improved homeostatic response of fuel selection (i.e., improved metabolic flexibility) following glucose feeding than men.

4.3 Methodology

4.3.1 Participants

Participants were recruited through the university portal (my.bham), poster and flyers advertisement. This study was conducted among apparently healthy adults aged 18 to 45 years. The other basic selection criteria for participants were BMI ranged between 18.5 – 29.99 kg/m^2, sedentary or low habitual physical activity level and no food intolerances or allergies that restricted participants from consuming the foods to be provided in the study. Exclusion criteria were weight change >2 kg within 3 months before the study start, any metabolic diseases, any known family history of diabetes mellitus and smoking. Women participants had to have a regular menstrual cycle or be on a monophasic contraceptive pill, and not be pregnant or currently breast-feeding.

A total of 29 participants were initially screened, from which 10 men and 14 women were eligible. From those eligible participants, eight men and eight women were pair matched based on the age, ethnicity, BMI and $\dot{V}O_{2peak}$ expressed as relative to LBM and thus were included in the experimental visit. The present study was approved by the University of Birmingham's Ethical Review Committee (ERN_16-1272). Written informed consent was obtained prior to the study from participants after detailed information of study procedures, risks and benefits of the research were verbally explained to them. The characteristics of the 16 participants that completed the study are shown in **Table 4.1**.

Table 4.1 Baseline characteristics of participants based on sexes

	Men (*n* = 8)	Women (*n* = 8)
Age (years)	22.4 ± 1.7	23.4 ± 2.2
BMI (kg/m^2)	23.6 ± 2.2	22.6 ± 2.3
Lean body mass (LBM) (kg)	62.3 ± 3.7	41.7 ± 3.3***
Body fat (%)	17.0 ± 3. 3	28.6 ± 3.9***
Waist circumference (cm)	87.3 ± 5.8	75.2 ± 3.5***
Systolic blood pressure (mmHg)	114 ± 3	110 ± 8
Diastolic blood pressure (mmHg)	68 ± 7	68 ± 7
$\dot{V}O_{2peak}$ (ml/min)	2628 ± 545	1755 ± 367**
$\dot{V}O_{2peak}$/body weight (ml/kg/min)	35.3 ± 8.6	29.9 ± 5.5
$\dot{V}O_{2peak}$/LBM (ml/kg/min)	42.4 ± 9.5	41.8 ± 6.3
Total physical activity, MET (min/week)[§]	546 ± 88	513 ± 89
Ethnicity (*n)*		
White	4	4
Chinese	1	1
Bangladeshi	1	1
Indian	1	1
Other Asian	1	1

Mean ± SD. Significant from men at $^{**}P<0.01$ and $^{***}P<0.001$

[§]Number of hours dedicated to each activity class multiply by the specific MET score for that activity (MET = Metabolic Equivalent Task)

4.3.2 Study design

The study followed a between subjects design. It involved three visits to the School of Sport, Exercise and Rehabilitation Sciences. Visit 1 included informed consent, eligibility screening and familiarisation with measurement of resting substrate oxidation. Visit 2 involved a fitness assessment. Visit 3 was preceded by provision of a standardised diet for two days and involved determination of substrate oxidation and blood metabolite/hormone levels in the overnight-fasted state and throughout the 5-hour period following consumption of a glucose drink.

4.3.3 Experimental procedures

Visit 1 (Consenting and Screening): This visit was used for consenting and screening based on inclusion criteria which included initially being: healthy as assessed by successful completion of a GHQ; aged between 18 and 45 years; BMI ranged from 18.5 – 29.99 kg/m^2; not physically active (i.e., sedentary or low physical activity level) based on IPAQ-SF, have a stable weight (± 2 kg) for more than 3 months before enrolment, have resting blood pressure <140/90 mmHg and have regular menses cycle or on contraceptive pill for women participants.

On the screening day, participants came to the laboratory at any time of the day as convenient for them. On arrival, an investigator gave a verbal explanation about the purpose of the study procedures involved and risks and benefits of the study to the participants before they signed informed consent. They then completed GHQ

(**Appendix 4**) to determine their health history and also completed IPAQ-SF (**Appendix 6**) (Craig et al., 2003) to determine their PAL. The short form questionnaire records the activity of four intensity levels, which consist of vigorous-intensity activity such as aerobics, moderate-intensity activity such as leisure cycling, low-intensity which is light walking, and sedentary which is sitting. The participants were asked to recall and report their activity for the last seven (7) days.

Participant's weight and height then was measured in light clothes to calculate their BMI. Body weight was measured using an electronic weighing scale (OHaus Champ II Scales, USA) and height was measured using a stadiometer (Stadiometer, SECA, UK) to the nearest 0.1 kg and 0.1 cm respectively. BMI was calculated as weight (kg) divided by height squared (m^2). Waist circumferences was measured to the nearest 0.1 cm using a measuring tape. Thereafter, their body composition (percentage body fat [%BF], fat mass [FM] and LBM) was determined by 4-site (bicep, tricep, subscapular and suprailliac) skinfold thickness measurements using Harpenden skinfold calliper and calculated based on the updated sex and race/ethnicity specific equations (Davidson et al., 2011). The skinfold thickness measurement were done by a kinanthropometist accredited by International Society for the Advancement of Kinanthropometry [ISAK].

Next, participants were familiarized with the process to be used in Visit 3 for measuring fuel utilisation. For this, the participant lay in a supine position for 10 minutes with a ventilated hood placed over their head and upper torso, which was

then connected to an indirect calorimeter (described below). During this period, they were asked to undertake a 12-lead ECG examination (Oxycon Pro, Jaeger, Wurzburg, Germany) to check for potential cardiac abnormalities that would contraindicate maximal exercise testing. While participants are rested, their resting blood pressure was also determined using an automatic blood pressure monitor (Omron M6, Netherlands). If participants successfully completed the Screening Form and met the eligibility requirements with respect to BMI, PAL, blood pressure and normal ECG outcome, they were asked to return for Visit 2.

Visit 2 (Fitness Assessment): This visit was used to assess their fitness level ($\dot{V}O_{2peak}$), which was one of the matching criteria used for pair-matching men and women participants. During this visit, they were asked to perform an incremental exercise test on a stationary bicycle (Lode Sport Excalibur, Groningen, Netherlands) to determine their $\dot{V}O_{2peak}$. The exercise test was commenced with the power set at low intensity (35 W) and this was be maintained for 5 minutes in order to allow participants to adequately warm-up. Additionally, they were asked to maintain a constant pedal rate of between 60-70 rev/min during the test. After a short break, participants recommenced cycling with the power set at 60 W and cycled continuously for 3 minutes maintaining a pedal rate of between 60-70 rev/min with the power increased by 35 W every 3 minutes until they reached voluntary exhaustion (i.e. they stop on their own accord or the pedal rate drops below 50 rev/min despite verbal encouragement from the investigator).

Their heart rate was monitored continuously throughout the testing using a heart rate monitor (Polar A300, Finland) and was recorded at the final 10 seconds of each exercise stage, as well as their self-rated perceived exertion using the Borg's RPE scale (Borg, 1982). Breath-by-breath measurements of $\dot{V}O_2$ and $\dot{V}CO_2$ during the exercise were collected using a computerised gas analysis system (Oxycon Pro, Jeager, Wurzburg, Germany). The data obtained was used to determine $\dot{V}O_{2peak}$ that was taken as the highest value of $\dot{V}O_2$ attained during a 10-second period on this test.

Upon completion of Visit 2, eight men and eight women were pair matched from the group screened based on the following matching criteria:

a. Age (≤5 years difference)
b. Ethnicity
c. BMI (≤10 % difference)
d. $\dot{V}O_{2peak}$ relative to LBM (≤10 % difference)

Those individuals who were pair-matched were called to attend the third visit to the research site for further assessments – this was within one (1) month of their screening / baseline assessment visit. Participants who were not pair-matched were informed that they were not required for the remained of the study.

The pair-matched were asked to undertake a 3-day weighed food intake survey to estimate habitual dietary intake before returning for Visit 3. Participants were

provided with weighing scales and a food diary (**Appendix 7**) to complete their 3-day weighed food intake. An explanation on how to record the food diary was given verbally as well as example of food diary as a guideline was provided.

*Visit 3 (Experimental Trial, **Figure 4.1**)*: Women participants who were not undergoing hormonal contraception had experimental visits scheduled within the mid follicular phase of the menstrual cycle, on day 6 or 7 after initiation of menses. Meanwhile those taking monophasic hormonal contraception were tested between day 1 and 21 of the active pill consumption period. Overall, only one women participant was on monophasic hormonal contraception, while the other women participants were on regular menses cycle. **Table 4.2** shows the serum estrogen and progesterone concentrations determined from blood samples collected at Visit 3 at baseline, which confirmed that women participants were in the correct phase of the cycle. On the other hand, men participants were scheduled for testing based at their convenience.

Table 4.2 Baseline value of estrogen and progesterone in women participants

	Mean ± SD
Estrogen (pmol/L)	191 ± 63
Progesterone (nmol/L)	1.10 ± 0.48

(n=7). Typical ranges for follicular phase; estrogen: 87.60 – 224.68 pmol/L, progesterone: 0.32 – 1.29 nmol/L (Stricker et al., 2006).

For the 48 hours prior to visit, participants were provided with a standardized weight maintaining diet (50% carbohydrate, 35% fat, 15% protein) based on estimated individual energy requirements (see Section 4.3.8 for calculation). Participants were required to not exercise for those 48 hours, with drinking being limited to plain water (*ad libitum*) to keep them hydrated.

On the trial day, participants arrived at the laboratory between 7.00 to 8.00 a.m. following a 12 hour overnight fast. Upon arrival, before the baseline measurement started, participants were asked to empty their bladder. After 10 minutes of rest in a supine position, resting substrate oxidation was measured over 20-30 minutes using indirect calorimetry to measure the $\dot{V}O_2$ and $\dot{V}CO_2$ (see Section 4.3.4 for detailed explanation).

An indwelling cannula (to allow for repeated blood collections) was then inserted into an arm vein and a blood sample was collected (~10ml). After that, participants consumed a glucose drink and they were asked to finish it within 10 minutes. The glucose drink contained of 1g glucose (Dextrose, MyProtein) per kg lean body mass diluted in a 4:1 solution of water to glucose. Post feeding substrate oxidation was determined 5 times for a period of 20-30 minutes at 1-hour intervals. Further blood samples (~5 ml) was collected at 15 and 30 minutes after drink consumption and then at 30 min intervals for the duration of the 5 hour protocol. During the trial period, participants remained semi-supine when not undergoing indirect calorimetry with activities restricted to reading or mobile phone use and they were wheeled to the

toilet when needed. Additionally, drinking was allowed but being limited to plain water (*ad libitum*) to keep them hydrated.

The data from the indirect calorimeter was used to quantify the RQ and also compute substrate oxidation in response to the test drink using stoichiometric equations as previously described (Frayn, 1983). Stable measurements of 15 minutes for both resting and postprandial substrate oxidation were used for data analysis to ensure measurements were valid and a true representative of the oxidative responses. Collected blood was centrifuged and the plasma or serum was stored for analysis.

The environmental condition of the laboratory during the trial period was recorded for each individual and there was no significant difference between time points throughout the trial and also between participants with the average of humidity at 44 ± 5 % and 44 ± 5 % and temperature at 19.0 ± 2.0 °C and 18.9 ± 1.5 °C for men and women, respectively during the trial.

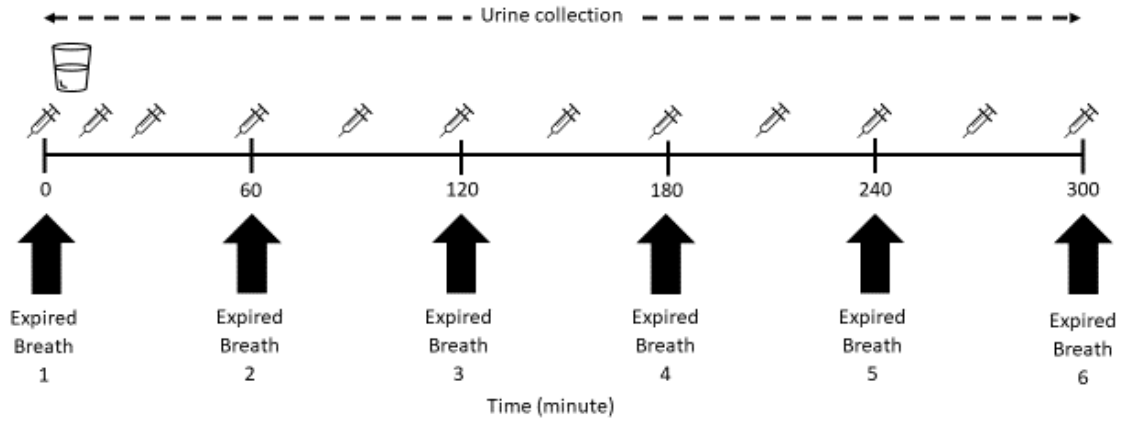

Figure 4.1 A schematic representation of the Experimental Trials.

4.3.4 Breath sample and substrate utilisation analysis

Breath was measured by using an open circuit GEM Indirect Calorimeter fitted with a ventilated hood (GEM Nutrition Ltd. Cheshire, UK). The measurement took place in a dimly light room where subjects lay awake, undisturbed and motionless in a supine position, for about 20-30 minutes whilst exhaled gases were collected over a constant airflow of 40L/min. Next, $\dot{V}O_2$ and $\dot{V}CO_2$ measurements were averaged over 30 second periods, with values discarded from the first 10 and last 2 minutes, with the average of the remaining 15-18 minutes used to determine the substrate oxidation provided the CV for $\dot{V}O_2$ and $\dot{V}CO_2$ was <10%. The gas analysers were automatically calibrated immediately before each test by using certified reference gases (1.00% CO_2, 21.00% O_2, balance Nitrogen) (BOC Gases, Surrey, UK).

4.3.5 Blood sampling and analysis

Venous blood samples were obtained from an antecubital forearm vein at various time points as explained above. Part of the blood samples were transferred into vacutainer containing EDTA and the remaining samples were transferred into tube with no additive to allow clotting. EDTA tubes were immediately stored on ice, with serum tubes left to clot at room temperature, after which all the tubes were centrifuged at 1361 g for 15mins at 4 °C. Aliquots containing plasma or serum were then flash frozen in liquid nitrogen and immediately stored at -80°C until analysed.

Plasma samples were analysed for glucose (Glucose Oxidase, Instrumentation Laboratories, Cheshire, UK) and non-esterified fatty acids (NEFA, Randox, London, UK) using an ILAB 650 Clinical Chemistry Analyser (Instrumentation Laboratory, Warrington, UK). The average intra-assay coefficient of variation for glucose was 1.5 % and NEFA was 3.3%, while the inter-assay coefficient of variation for glucose was 5.9 % and NEFA was 3.1% based on analysis of 20 duplicate plasma samples ran across 2 assays.

Serum insulin concentrations were measured by using a human insulin ELISA kit (Invitrogen, Paisley, UK) and a Biotek ELx800 analyser (Biotek Instruments, Vermont, USA). The average intra-assay coefficient of variation for insulin was 3.6 % based on 10 duplicate samples with the inter-assay coefficient of variation 7.3 % based on 10 samples ran across two assays. In addition, in women participants, fasting serum estrogen and progesterone concentrations were measured (**Table**

4.2) in separate analyses by an external laboratory (Randox Clinical Laboratory Services, Co. Antrim, UK) quantified by immunoassays using an Immulite 1000 Immunoassay System (Siemens Healthcare Ltd, Surrey, UK).

4.3.6 Urine sampling and analysis

When they arrived to the laboratory, they were asked to empty their bladder and urine was collected throughout the trial period in a disposable urine container. The collected urine volume was measured using digital weighing scales (Model 323, Salter, Kent, UK) and was analysed for urea concentrations (Urea kit, Randox, Co. Antrim, UK) using a semi-automated clinical chemistry analyser (Randox Daytona+, Randox, Co. Antrim, UK).

4.3.7 3-day weighed food diary

Participants were provided with comprehensive written and verbal instructions explaining how to complete a three-day weighed food diary. They were asked to keep this diary of everything they eat and drink over two weekdays and one weekend days and these need not be consecutive. Digital weighing scales, one portable pocket sized (Swees, Digital Pocket Mini Gold, Kent, UK) and one standard sized (Macallen TM Digital LCD Electronic Kitchen Weighing Scales 10kg, UK), were provided to allow all food and drink consumed to be weighed to the nearest gram. The researcher checked each food diary, with any uncertainties clarified by the participant. The diaries were analysed using Dietplan 6.70.67 (Forestfield

Software Ltd. Horsham, West Sussex, UK) to produce a comprehensive report of energy and macronutrient intake. When a consumed food item was missing from the database, the nutritional data were located from the manufacturer and entered manually.

4.3.8 Calculations

The weight maintenance diet that was given to the participants two days before their experimental visit was based on their estimated total energy expenditure (TEE), which was calculated using the Institute of Medicine equations by assuming a low physical activity coefficient (Brooks et al., 2004).

For men aged ≥19 y:

TEE = 662 – [9.53*age (y)] + 1.11*[15.9*Weight (kg) + 540*Height (m)]

For women aged ≥19 y:

TEE = 354 – [6.91*age (y)] + 1.12*[9.36*Weight (kg) + 726*Height (m)]

Urinary nitrogen production was calculated using the urinary urea based formula below:

$$N\ (g/min) = [((((CH_4NO_2/16.6) * 0.466) * Volume) / Time) * 1.2]$$

Where,

CH_4NO_2 = urinary urea (mmol/L)

Volume = urine volume (L)

As sex might have influences on protein metabolism, hence it is important to use non-protein RQ ($RQ_{non\text{-}protein}$). The non-protein RQ were quantified using the $\dot{V}O_2$ consumption and $\dot{V}CO_2$ production based on step below:

Step 1

First, the contribution of protein oxidation to $\dot{V}O_2$ and $\dot{V}CO_2$ was estimated with knowledge that 1 g nitrogen (N) corresponds to 6.25 g protein:

$$\dot{V}O_{2(protein)} = N \times 6.25 \times 0.97$$

$$\dot{V}CO_{2(protein)} = N \times 6.25 \times 0.77$$

Where $\dot{V}O_{2(protein)}$ and $\dot{V}CO_{2(protein)}$ refer to the rates of O2 consumption and CO2 production, respectively, from protein oxidation; 0.97 and 0.77 are the respective volumes (L) of O_2 consume and CO_2 produced by the biological oxidation of 1 g of protein. N is urinary nitrogen production in grams.

Step 2

Next, the contribution of protein oxidation was subtracted from the measured $\dot{V}O_2$ and $\dot{V}CO_2$ to obtain non-protein $\dot{V}O_2$ ($\dot{V}O_{2(non\text{-}protein)}$) and non-protein $\dot{V}CO_2$ ($\dot{V}CO_{2(non\text{-}protein)}$) as follow:

$$\dot{V}O_{2(non\text{-}protein)} = \dot{V}O_2 - \dot{V}O_{2(protein)}$$

$$\dot{V}CO_{2(non\text{-}protein)} = \dot{V}CO_2 - \dot{V}CO_{2(protein)}$$

Step 3

The straightforward output from indirect calorimetry allows the quantitation of RQ ($\dot{V}CO_2$ / $\dot{V}O_2$), but it is the non-protein RQ ($RQ_{(non\text{-}protein)}$) that is required:

$$RQ_{(non\text{-}protein)} = \dot{V}CO2_{(non\text{-}protein)} / \dot{V}O2_{(non\text{-}protein)}$$

The whole body carbohydrate, fat and protein oxidation were calculated by using equations from Frayn (1983) with urinary nitrogen correction.

$$\text{Carbohydrate oxidation (g/min)} = 4.55 * \dot{V}CO_2 - 3.21 * \dot{V}O_2 - 2.87 * N$$

$$\text{Fat oxidation (g/min)} = 1.67 * \dot{V}O_2 - 1.67 * \dot{V}CO_2 - 1.92 * N$$

$$\text{Protein oxidation (g/min)} = N \text{ (g/min)} * 6.25$$

For blood metabolite/hormone concentrations, an AUC was calculated using the trapezoid method as shown below:

$$AUC = AUC_{0\text{-}1} + AUC_{1\text{-}2} + \ldots + AUC_{x\text{-}last}$$

$$= ((C_0 + C_1)/2)*(T_1 - T_0) + ((C_1 + C_2)/2)*(T_2 - T_1) + \ldots + ((C_x + C_{last})/2)*(T_{last} - T_x)$$

Where,

C = plasma concentration

T = time

4.3.9 Statistical analysis

Data were analysed using Statistical Package for the Social Sciences [SPSS] software package (version 22, SPSS Inc. Chicago, IL) and the post-hoc test was performed manually in excel. Normality of the data was checked by Shapiro Wilk tests. Homogeneity of variances was confirmed by Mauchley's test of sphericity and a Greenhouse-Geisser correction was applied to the degrees of freedom if the sphericity assumption was violated. An independent t-test was used to compare differences between sexes in baseline characteristics, energy and macronutrient intake and AUC for RQ and blood metabolites and insulin. A two-way ANOVA with repeated measures (sex x time) was used to compare differences in RQ, substrates oxidation, blood metabolites and insulin variables between gender and over time. Further, a paired-sample t-test was used to test the difference of each time point from baseline during each trial separately and the difference between trials for each time point. Where a significant effect (i.e., $P<0.05$) was indicated for these variables, the Holm-Bonferroni stepwise correction was made for location of variance post-hoc (Holm, 1979). Normally distributed data are presented as means ± standard deviation unless otherwise stated. The 95% confidence intervals (CI) for mean absolute pairwise differences between trials were calculated using the t-distribution and degrees of freedom (n – 1). Absolute standardised effect sizes (ES) were calculated for within-measures comparisons to supplement important findings as follows:

$$ES = \frac{\text{Mean v2} - \text{Mean v1}}{CON\ SD}$$ (Cumming and Finch, 2001)

In the absence of a clinical anchor, an ES of 0.2 was considered the minimum important difference for all outcome measures, 0.5 moderate and 0.8 large (Cohen, 1988). Statistical significance was accepted as $P<0.05$.

4.4 Result

4.4.1 Participant characteristics and nutritional intake

Participant characteristics at baseline and their nutritional status were reported in **Table 4.1** and **Table 4.3** respectively. As expected, percent body fat was greater (P<0.001) in women than men, whereas lean body mass (P<0.001) and waist circumference (P<0.001) was lower in women than men. Meanwhile, maximal oxygen consumption ($\dot{V}O_{2peak}$) were higher (P=0.001) in men than in women, however when $\dot{V}O_{2peak}$ was expressed relative to body mass and lean body mass, no significant difference was found between sexes. In addition, there was no significant difference between sexes for other baseline characteristics, which suggests that the participants were well matched as per study design.

For the nutritional status, significant sex-differences were found in all measured parameters. Men's total absolute energy intake (P<0.001) and estimated energy expenditure (P<0.001) were higher than the women's, with men reporting a greater absolute intake of carbohydrate (P<0.001), fat (P<0.001) and protein (P<0.001). Further, when expressing as percentage of daily energy intake, men had a greater percentage contribution to total energy intake from protein (P<0.001), with no significant difference in carbohydrate and fat contribution to daily energy intake from their habitual intake based on 3-day diet record.

Table 4.3 Energy and macronutrient intake of participants based on sexes

	Men (*n* = 8)	Women (*n* = 8)
Estimated energy expenditure (kcal/d)	2844 ± 135	2119 ± 108***
Energy intake (kcal/day)	2989 ± 179	2227 ± 152***
% Carbohydrate to Energy intake	45.1 ± 7.9	47.5 ± 6.8
% Fat to Energy intake	33.1 ± 6.9	33.3 ± 6.8
% Protein to Energy intake	19.2 ± 5.1	15.6 ± 3.5***
Carbohydrate intake (g/day)	348 ± 35	293 ± 31***
Fat intake (g/day)	113 ± 33	86 ± 29***
Protein intake (g/day)	141 ± 21	86 ± 23***

Mean ± SD. Significant from men at ***P<0.001

4.4.2 Substrate utilisation in response to feeding

At baseline, RQ and fat oxidation rate were not statistically different between sexes. On the contrary, there was a significant difference at baseline for carbohydrate oxidation rate, where women had higher rate than men (P=0.012). Further, there were significant sex x time interactions for RQ (P<0.001), carbohydrate oxidation rate (P<0.001) and fat oxidation rate (P<0.001). For protein oxidation, estimated for the entire 5-hour study period, there was no significant difference between men and women with mean 1.11 ± 0.52 vs. 0.95 ± 0.37 mg/min/kg LBM, respectively.

The RQ (**Figure 4.2**) had increased significantly by 1 hour (P<0.001) and remained elevated above baseline levels for at least 2 hours (P<0.001) then returned to

baseline level by 3 hours after ingestion of the carbohydrate drink and remained stable until end of trial in women. However in men, the RQ was significantly increased from baseline at 1 hours (*P*=0.015), 2 hours (*P*=0.005) and 3 hours (*P*=0.006), returned to baseline at 4 hours after drink ingestion then fell below baseline level (*P*=0.029) by the end of trial. RQ was significantly higher in women at 1 hour even after post hoc correction (RQ: 0.93 ± 0.02 vs. 0.87 ± 0.04, respectively, 95% CI -0.09 to -0.02, ES = 1.90, *P*=0.012) as compared to men.

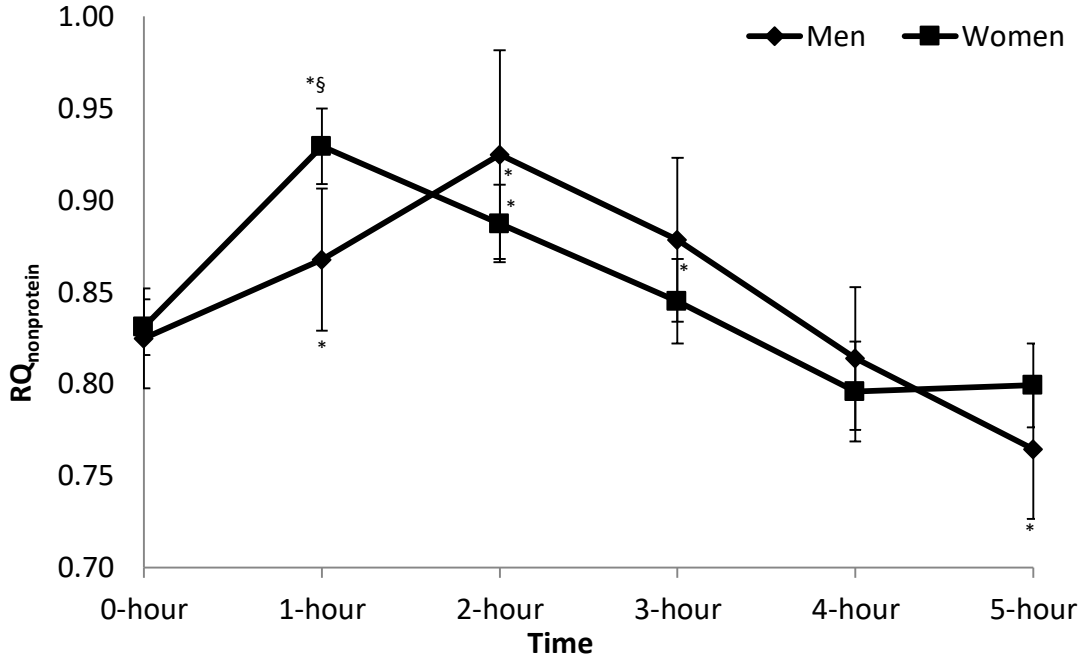

Figure 4.2 Changes in RQ in response to feeding based on sexes. Data are means ± SEM. *Significant difference from baseline. §Significant difference from men. The significance indicators above and below line reflect women and men, respectively.

A similar pattern as RQ was seen in carbohydrate oxidation rates in response to glucose feeding except that the sex-difference at baseline as shown in **Figure 4.3**. Carbohydrate oxidation rates were significantly increased by 1 hour (*P*<0.001) and

remained higher at 2 hour (*P*<0.001) from baseline and then returned to baseline value by 3 hour and then remained below baseline value at 4 hour (*P*=0.005) until at the end of trial (*P*=0.005) in women. In contrast, carbohydrate oxidation rates had increased by 2 hours after glucose ingestion, but this increase was not significantly different from baseline after post-hoc correction (*P*=0.080) and then significantly decreased below baseline by 4 hours (*P*=0.014) until the end of the trial (*P*=0.004) in the men. Women had significantly higher carbohydrate oxidation rate at baseline (2.61 ± 0.46 vs. 1.79 ± 0.34 mg/min/kg LBM, respectively, 95% CI -1.25 to -0.04, ES = 2.03, *P*=0.012) and after 1 hour (CHO oxidation: 4.67 ± 0.58 vs. 2.21 ± 0.38 mg/min/kg LBM, respectively, 95% CI -3.07 to -3.07 to -2.02, ES = 5.01, *P*<0.001) of carbohydrate ingestion as compared to men, whereas men had significantly higher carbohydrate oxidation rate at the end of trials (*P*=0.018)

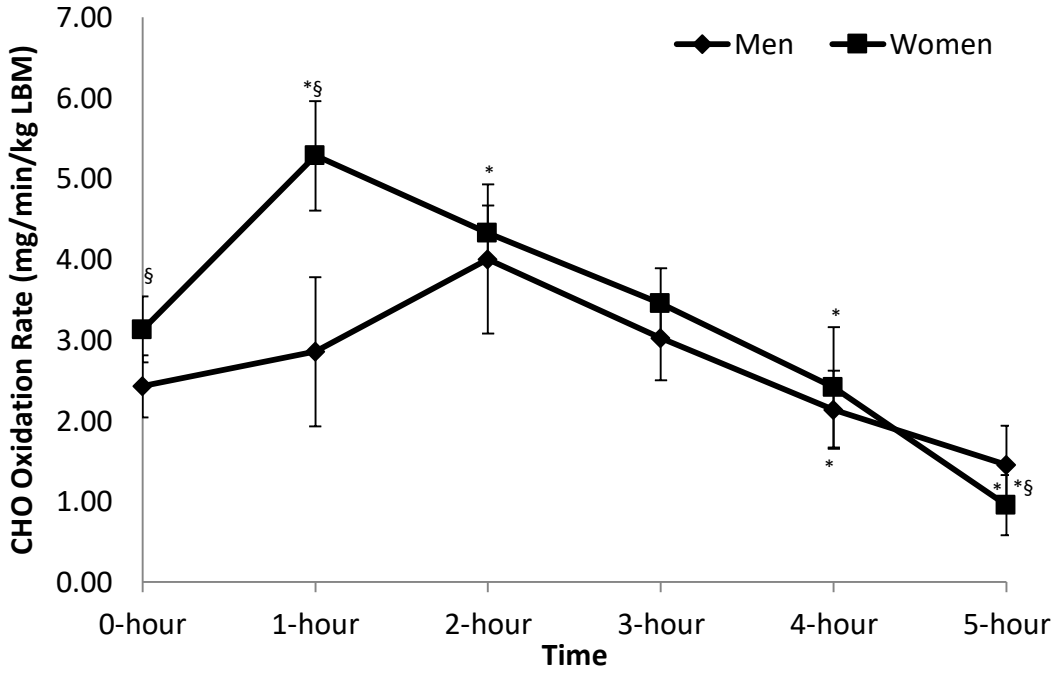

Figure 4.3 Changes in carbohydrate oxidation in response to feeding based on sexes. Data are means ± SEM. *Significant difference from baseline. §Significant

difference from men. The significance indicators above and below line reflect women and men, respectively.

Fat oxidation rate in women was significantly decreased from baseline by 1 hour (P<0.001) and remained so until 2 hour (P=0.005) and then gradually increased above baseline until the end of trial, but was not significantly different after post-hoc adjustment during this period (**Figure 4.4**). Contrary, fat oxidation rates were significantly decreased below baseline at 1 (P=0.032), 2 (P=0.006) and 3 (P=0.012) hours post feeding, and returned to baseline by 4 hours in men. A sex-difference was found at 3 and 4 hours after carbohydrate ingestion. A higher rate of fat oxidation was seen in women than men (3-hour: 2.61 ± 0.46 vs. 1.79 ± 0.34 mg/min/kg LBM, respectively, 95% CI -1.25 to -0.04, ES = 2.03, P=0.030; 4-hour: 2.61 ± 0.46 vs. 1.79 ± 0.34 mg/min/kg LBM, respectively, 95% CI -1.25 to -0.04, ES = 2.03, P=0.030).

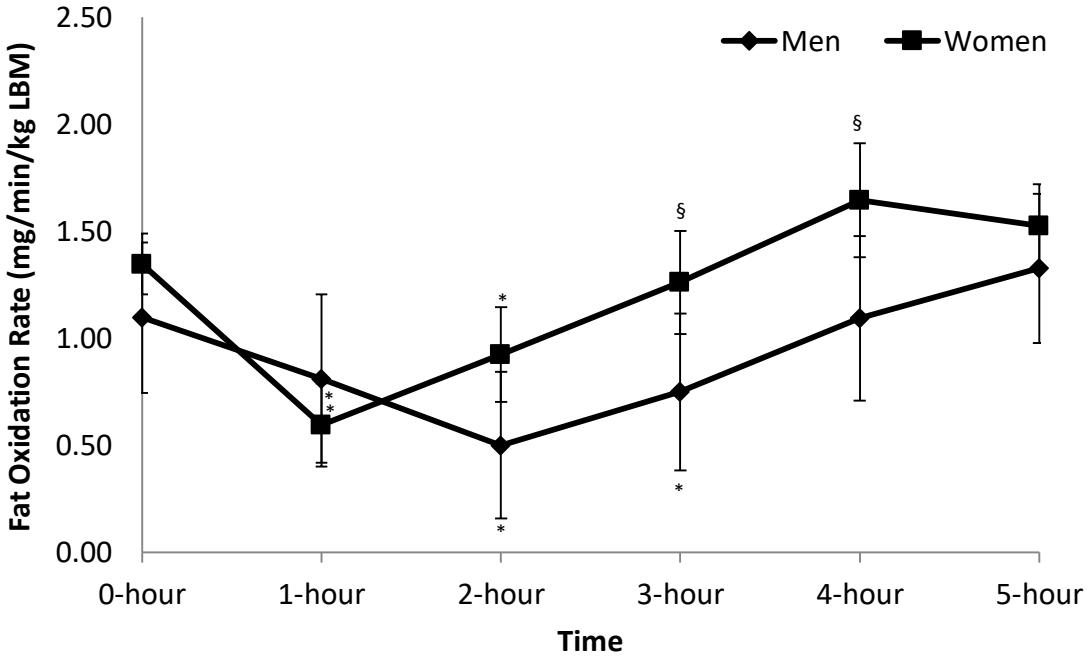

Figure 4.4 Changes in fat oxidation in response to feeding based on sexes. Data are means ± SE. *Significant difference from baseline. §Significant difference from men. The significance indicators above and below line reflect women and men, respectively.

4.4.3 Blood metabolites and hormone response

At baseline, plasma glucose, NEFA and serum insulin concentrations were not statistically different between men and women. There were however significant sex x time interactions for plasma glucose (P<0.001) and serum insulin concentrations (P<0.001), but not for NEFA.

In women, the plasma glucose concentration was significantly elevated from baseline value at 15, 30 and 60 minutes after glucose ingestion (P<0.001) before declining and completely returning to baseline values by 150 minutes (2 hours 30 minutes) and remaining stable until end of trial (**Figure 4.5**). On the other hand, in men, plasma glucose concentration was elevated from baseline value only after 30 minutes of feeding (not significant after post-hoc correction, P=0.198) and became significantly higher than baseline from 60 (P=0.007) to 150 minutes (P=0.005), and had completely returned to baseline values by 240 minutes. Plasma glucose concentration was higher in women at 15 minutes but not significant after post-hoc correction (P=0.060) as compared to men, while men had significantly higher glucose concentration at 120 and 150 minutes (P<0.001) than women. AUC for

glucose was 14.0% higher in men as compared to women (6.29 ± 0.44 mmol•L^{-1} and 5.52 ± 0.35 mmol•L^{-1}, respectively, 95% CI 0.35 to 1.20, ES = 1.94, *P*=0.002).

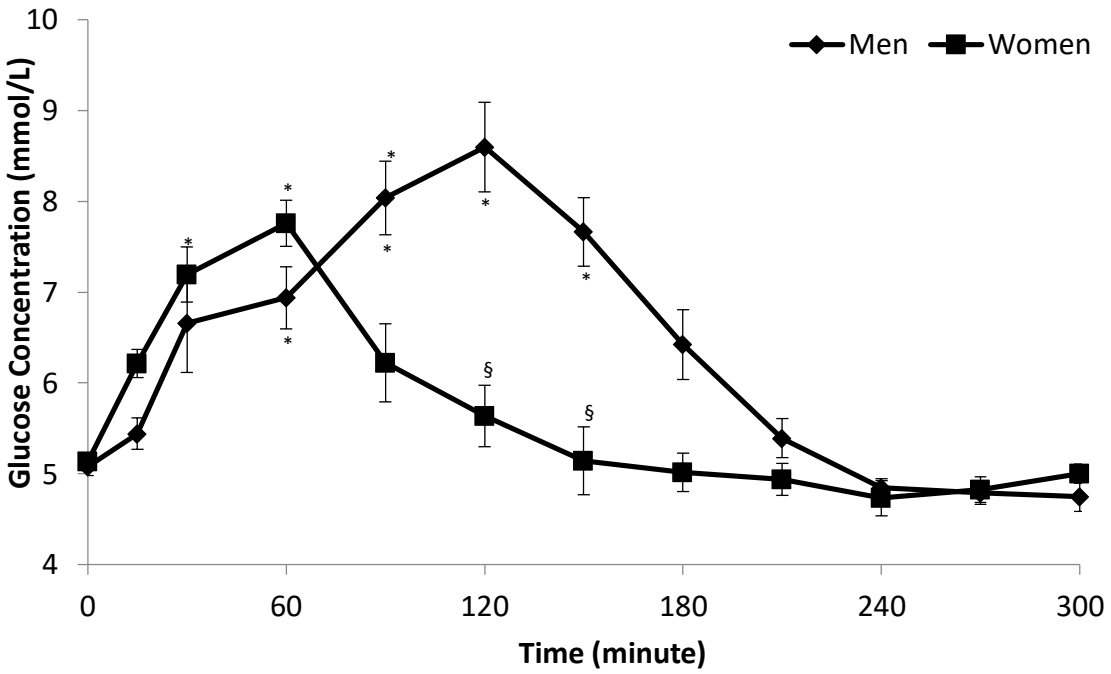

Figure 4.5 Changes in glucose concentrations in response to feeding based on sexes. Data are means ± SE. *Significant difference from baseline. §Significant difference from men. The significance indicators above and below line reflect women and men, respectively.

A similar pattern was seen in the insulin response to glucose feeding, in which the insulin concentration was significantly elevated from baseline value at 15 (P<0.032), 30 (P<0.022) and 60 minutes (P<0.042) after ingestion before declining to be not different from baseline by 120 min and for the remainder of the trial in women (**Figure 4.6**). On the contrary, in men, insulin concentration was elevated from baseline value only after 30 minutes of feeding (P<0.001) remaining so until 120 minutes (P=0.001) before declining and remaining no different from baseline by 180. The insulin concentration was higher in women at 15 minutes but not significant after post-hoc correction (P=0.154) as compared to men, while men had significantly

higher insulin concentration at 120 minutes (P<0.001) than women. AUC for insulin concentration was 37.4% higher in men than women with average AUC were 257.49 ± 97.01 pmol•L^{-1} and 187.44 ± 90.82 pmol•L^{-1}, respectively (95% CI -30.72 to 170.83). However it is not statistically significant (P=0.158) with moderate effect size (0.75).

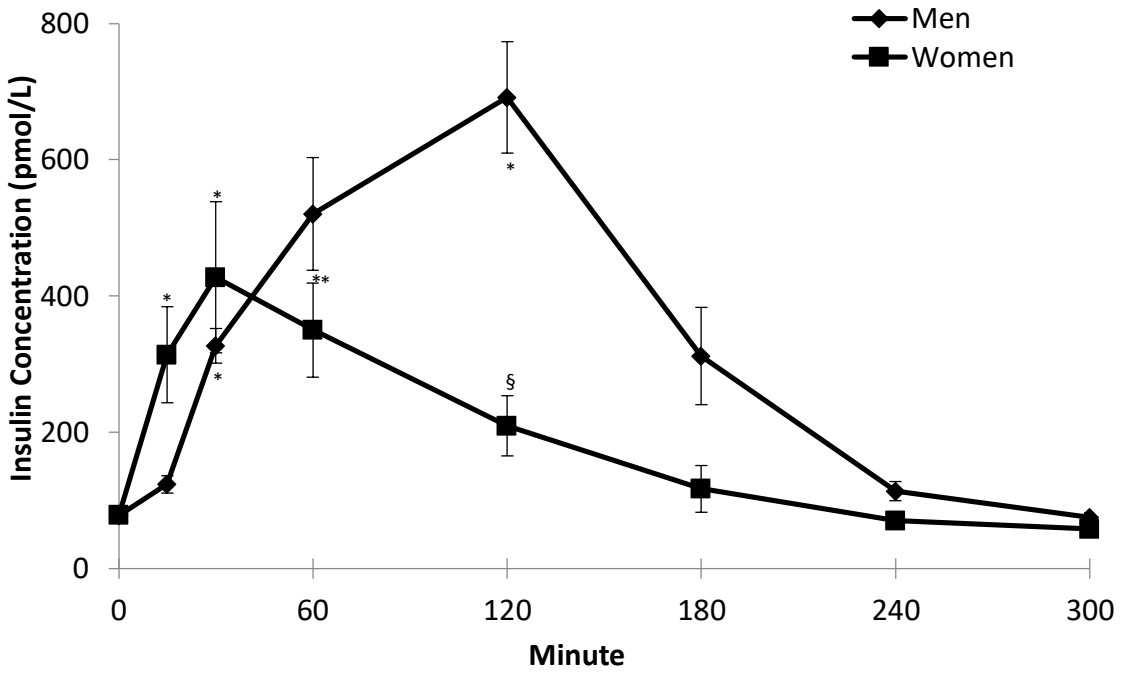

Figure 4.6 Changes in insulin concentrations in response to feeding based on sexes. Data are means ± SE. *Significant difference from baseline. §Significant difference from men. The significance indicators above and below line reflect women and men, respectively.

NEFA concentration response to glucose feeding was not significantly different between men and women as shown in **Figure 4.7**. In women, the concentration significantly reduced below baseline values at 60 until 150 minutes (P<0.001 to P=0.011). After that, the concentration increased and become significantly higher

from baseline by end of trial (*P*=0.033). Similar response was seen in men, in which the concentration went below baseline value right after the feeding and became significant by 60 minutes (*P*<0.001) until 150 minutes (*P*=0.008) before it increase above the baseline value and become significant at 270 minutes (*P*=0.049) until end of trial (*P*=0.002). The NEFA concentration was significantly higher in women at 120 minutes (*P*=0.048) as compared to men. Even though it is not statistically significant (P=0.405), AUC for NEFA concentration was 10.8% higher in women than men with average AUC were 0.44 ± 0.10 mmol•L^{-1} and 0.39 ± 0.10 mmol•L^{-1}, respectively (-0.15 to 0.07), with moderate effect size (0.50).

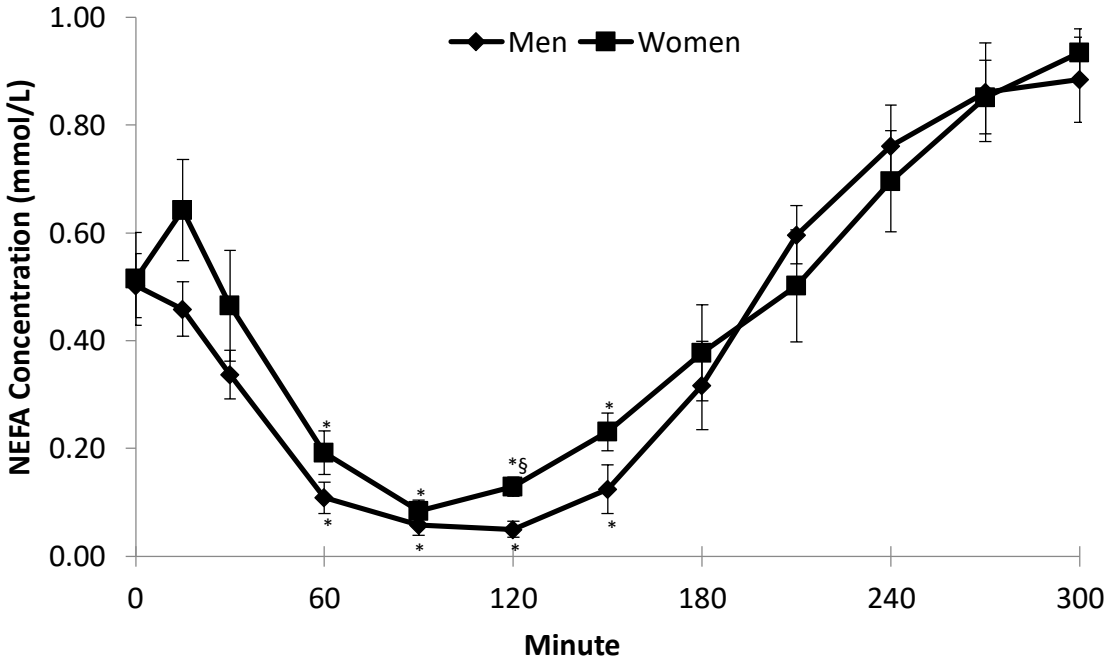

Figure 4.7 Changes in NEFA concentrations in response to feeding based on sexes. Data are means ± SE. *Significant difference from baseline. §Significant difference from men. The significance indicators above and below line reflect women and men, respectively.

4.5 Discussion

The main purpose of the present study was to investigate sex-differences in fuel selection, plasma glucose/NEFA and insulin responses to acute oral glucose feeding in men and women carefully matched with respect to BMI, age, ethnicity and fitness level/LBM and investigated on a controlled diet in the mid follicular phase of the menstrual cycle, with glucose provided in doses relative to LBM. The result revealed that the pattern of changes in substrate use and glucose and insulin responses appear to be different between sexes, with changes occurring earlier and returning to baseline more quickly in women than men. This confirms the hypothesis that women appear to have better homeostatic regulation than men.

This observation of improved capacity to handle an oral glucose load is consistent with other studies suggesting that women have greater metabolic control. As reviewed by Henderson (2014) and others (Clausen et al., 1996, Kuhl et al., 2005, Høeg et al., 2009, Høeg et al., 2011), women appear to show better management of metabolic perturbations as compared to men. In the present study, changes in glucose and insulin but not NEFA appear to underpin and match the substrate oxidation changes, which may provide some insight into the metabolic mechanisms of the observed sex-difference in RQ response. It is possible that glucose delivery and/or insulin-stimulated glucose uptake was activated faster in the women than men, and thus changes in RQ were facilitated more rapidly. Some prior evidence for faster absorption of ingested carbohydrate in women has been shown in a previous study using a dual-isotope approach and kinetic modelling (Robertson et

al., 2002)**.** They observed a higher fractional rate of glucose appearance in plasma from a high carbohydrate meal immediately after ingestion in women as compared to men. In contrast, Anderwald and colleagues (Anderwald et al., 2011) reported the opposite, that women had a more prolonged absorption of glucose following an oral glucose tolerance test as compared to men, and a worsening of postprandial glycaemic regulation. However, both of these studies did not apply adequate matching of men and women nor did they consider the carbohydrate dose relative to body or lean body mass. Regardless of the exact mechanism, in the present study it appears the response to glucose feeding occurred sooner and was restored to baseline sooner, suggesting enhanced responsiveness or flexibility to glucose feeding in women.

The significance of enhanced metabolic flexibility to glucose feeding in general in women vs. men might be relevant for type 2 diabetes risk. In line with the current study, a previous study showed that women had higher insulin-stimulated leg glucose uptake as compared to well-matched men (Høeg et al., 2009). In addition, improved metabolic flexibility was observed during intralipid infusion whereby whole-body insulin sensitivity decreased by 38% in men but only 26% in women (Høeg et al., 2011). As mentioned previously, the global prevalence of diabetes type 2 has been reported to be higher in men than women (Wild et al., 2004), and the evidence from the present study and others suggests some of this difference may be underpinned by sex differences in metabolic flexibility.

It is acknowledged that the sex differences in metabolic response shown in the present study were observed at rest, and the concept was developed from an exercise observation (**Chapter 3**). However, this finding could be used to inform further study on the potential for sex-differences in nutrient-exercise interactions, particularly in respect of nutrient timing. For example, if there are benefits to optimising fat oxidation during exercise, and fasted state exercise is not possible or practical, it could mean that women may have returned to basal metabolic status and thus could optimise fuel utilisation during exercise by 3 hours after nutrient provision, but men may need to wait longer (i.e., 4 hours). Montain and colleagues showed that substrate use and lipolysis during exercise can be altered for at least 6 h after a carbohydrate containing meal, but the meal was almost double the carbohydrate dose used in present study, and no sex differences were investigated (Montain et al., 1991). Hence, future work may investigate if substrate use in exercise after feeding differs as a function of time from meal between the sexes (or even between individuals within the same sex), and whether the dose of carbohydrate and/or presence of other macronutrients is influential.

This study has several limitations that should be acknowledged. Firstly, the sample size of the study was relatively small. However, despite the small sample size, the pattern of metabolic response to feeding was clearly seen and the participants were well matched. Nonetheless, the results cannot be generalised to other situations like differing menstrual status (e.g. luteal phase and post-menopause). Secondly, due to limited time, the secretion of incretins (gastric inhibitory polypeptide [GIP], glucagon-like peptide-1 [GIP-1]) that are responsible for enhancing glucose

stimulated insulin secretion from the pancreatic beta cells (Vilsbøll and Holst, 2004, Sanlioglu et al., 2013) were not analysed. This may have added further explanatory data to the present study (i.e., perhaps informing on the differential insulin response between men and women).

Nevertheless, there were several strengths in this present study that need to be highlighted. The participants were carefully matched on possible factors that might influence metabolic response, which has been suggested to be important in order to determine the effect of biological sex *per se* (Tarnopolsky et al., 1990, Tarnopolsky, 2000, Tarnopolsky, 2008). Participants were given a dose of glucose relative to their lean body mass, which means they received an equal dose relatively to the major site of postprandial glucose disposal (i.e., skeletal muscle) and hence the design was adequate to accurately compare postprandial glucose homeostasis between men and women (Rattarasarn et al., 2010). In addition, established scientific evidence shows that men typically oxidise more protein than women (Tarnopolsky, 2004). Therefore, it was important to quantify the protein oxidation rate and use non-protein RQ as presented in the present study. Finally, there is growing evidence suggesting that the sex hormones (estrogen and progesterone) play a role in substrate metabolism regulation (Campbell and Febbraio, 2001). Hence, women participants were selected in a specific phase, where they were tested only during their mid follicular phase of menstrual cycle (day 6 or 7 from day of initial menses). Indeed, this was confirmed by the low level of basal sex hormones as presented in **Table 4.2**, which minimises the influence of menstrual cycle variation on the outcomes of the study.

In conclusion, the metabolic response following ingestion of carbohydrate resulted in significant sex differences in substrate utilisation and glucose and insulin responses, with women exhibiting an earlier response to feeding and return to baseline quicker than men. Therefore, these findings suggest that women cannot be assumed to respond in the same manner as men to feeding/nutrition, which is relevant in implications for research practice (i.e., study design) and for guidance on nutrient timing around physical activity, which warrant further investigation. For example, it may be that men and women need to exercise at different times post-feeding to accommodate differences in time to return to basal metabolism. Furthermore, nutritional recommendations and exercise training prescriptions perhaps can be planned by considering the potential for sex-specific responses. Further studies are needed to determine the physiological and clinical importance of the sex differences observed in the present study.

4.6 References

ANDERWALD, C., GASTALDELLI, A., TURA, A., KREBS, M., PROMINTZER-SCHIFFERL, M., KAUTZKY-WILLER, A., STADLER, M., DEFRONZO, R., PACINI, G. & BISCHOF, M. 2011. Mechanism and effects of glucose absorption during an oral glucose tolerance test among females and males. *The Journal of Clinical Endocrinology and Metabolism,* 96(2)**,** 515-524.

BLAAK, E. E., VAN AGGEL-LEIJSSEN, D. P., WAGENMAKERS, A. J., SARIS, W. H. & VAN BAAK, M. A. 2000. Impaired oxidation of plasma-derived fatty acids in type 2 diabetic subjects during moderate-intensity exercise. *Diabetes,* 49**,** 2102.

BORG, G. 1982. Ratings of perceived exertion and heart rates during short-term cycle exercise and their use in a new cycling strength test. *International Journal of Sports Medicine,* 3**,** 153-158.

BROOKS, G. A., BUTTE, N. F., RAND, W. M., FLATT, J.-P. & CABALLERO, B. 2004. Chronicle of the Institute of Medicine physical activity recommendation: how a physical activity recommendation came to be among dietary recommendations. *The American Journal of Clinical Nutrition,* 79**,** 921S-930S.

CAMPBELL, S. E. & FEBBRAIO, M. A. 2001. Effects of ovarian hormones on exercise metabolism. *Current Opinion in Clinical Nutrition and Metabolic Care,* 4**,** 515-520.

CARTER, S., MCKENZIE, S., MOURTZAKIS, M., MAHONEY, D. J. & TARNOPOLSKY, M. A. 2001. Short-term 17beta-estradiol decreases glucose R(a) but not whole body metabolism during endurance exercise. *Journal of Applied Physiology,* 90**,** 139-146.

CLAUSEN, J. O., BORCH-JOHNSEN, K., IBSEN, H., BERGMAN, R. N., HOUGAARD, P., WINTHER, K. & PEDERSEN, O. 1996. Insulin sensitivity index, acute insulin response, and glucose effectiveness in a population-based sample of 380 young healthy Caucasians. Analysis of the impact of gender, body fat, physical fitness, and life-style factors. *The Journal of Clinical Investigation,* 98**,** 1195-1209.

COHEN, J. 1988. Statistical power analysis for the behavioural sciences. 2nd ed. Hillsdale (NJ): *Lawrence Erlbaum Associates*, 22–25.

CORPELEIJN, E., SARIS, W. & BLAAK, E. 2009. Metabolic flexibility in the development of insulin resistance and type 2 diabetes; effects of lifestyle. *Obesity Reviews,* 10**,** 16.

CRAIG, C. L., MARSHALL, A. L., SJÖSTRÖM, M., BAUMAN, A. E., BOOTH, M. L., AINSWORTH, B. E., PRATT, M., EKELUND, U., YNGVE, A., SALLIS, J. F.

& OJA, P. 2003. International physical activity questionnaire: 12-country reliability and validity. *Medicine and Science in Sports and Exercise,* 35**,** 1381-1395.

CUMMING, G. & Finch, S. 2001. A primer on the understanding, use, and calculation of confidence intervals that are based on central and noncentral distributions. *Educational and Psychological Measurement,* 61(4), 532–574.

DAVIDSON, L. E., WANG, J., THORNTON, J. C., KALEEM, Z., SILVA-PALACIOS, F., PIERSON, R. N., HEYMSFIELD, S. B. & GALLAGHER, D. 2011. Predicting fat percent by skinfolds in racial groups: Durnin and Womersley revisited. *Medicine and Science in Sports and Exercise,* 43**,** 542-549.

DEVRIES, M. C., HAMADEH, M. J., PHILLIPS, S. M. & TARNOPOLSKY, M. A. 2006. Menstrual cycle phase and sex influence muscle glycogen utilization and glucose turnover during moderate-intensity endurance exercise. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology,* 291**,** R1120-8.

FRAYN, K. N. 1983. Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology,* 55**,** 628-634.

GALGANI, J. E., MORO, C. & RAVUSSIN, E. 2008a. Metabolic flexibility and insulin resistance. *American Journal of Physiology, Endocrinology and Metabolism,* 295**,** E1009-E1017.

GALGANI, J. E., PI-SUNYER, X., HEILBRONN, L. K., SMITH, S. R., AZUMA, K. & RAVUSSIN, E. 2008b. Metabolic flexibility in response to glucose is not impaired in people with type 2 diabetes after controlling for glucose disposal rate. *Diabetes,* 57**,** 5.

HALL, L. M. L., MORAN, C. N., MILNE, G. R., WILSON, J., MACFARLANE, N. G., FOROUHI, N. G., HARIHARAN, N., SALT, I. P., SATTAR, N. & GILL, J. M. R. 2010. Fat oxidation, fitness and skeletal muscle expression of oxidative/lipid metabolism genes in South Asians: implications for insulin resistance? *PloS One,* 5**,** e14197.

HENDERSON, G. C. 2014. Sexual dimorphism in the effects of exercise on metabolism of lipids to support resting metabolism. *Frontiers in Endocrinology,* 5**,** 1-8.

HØEG, L., ROEPSTORFF, C., THIELE, M., RICHTER, E. A., WOJTASZEWSKI, J. F. P. & KIENS, B. 2009. Higher intramuscular triacylglycerol in women does not impair insulin sensitivity and proximal insulin signaling. *Journal of Applied Physiology,* 107**,** 824-831.

HØEG, L. D., SJØBERG, K. A., JEPPESEN, J., JENSEN, T. E., FRØSIG, C., BIRK, J. B., BISIANI, B., HISCOCK, N., PILEGAARD, H., WOJTASZEWSKI, J. F. P., RICHTER, E. A. & KIENS, B. 2011. Lipid-induced insulin resistance

affects women less than men and is not accompanied by inflammation or impaired proximal insulin signaling. *Diabetes,* 60**,** 64-73.

HOLM, S. 1979. A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics,* 6**,** 65-70.

HORTON, T. J., PAGLIASSOTTI, M. J., HOBBS, K. & HILL, J. O. 1998. Fuel metabolism in men and women during and after long-duration exercise. *Journal of Applied Physiology,* 85**,** 1823-1832.

KELLEY, D., GOODPASTER, B., WING, R. & SIMONEAU, J. 1999. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *American Journal of Physiology, Endocrinology and Metabolism,* 277(96)**,** E1130-E1141.

KELLEY, D. E. & MANDARINO, L. J. 2000. Fuel selection in human skeletal muscle in insulin resistance. *Diabetes,* 49(5)**,** 677-683.

KUHL, J., HILDING, A., OSTENSON, C. G., GRILL, V., EFENDIC, S. & BÅVENHOLM, P. 2005. Characterisation of subjects with early abnormalities of glucose tolerance in the Stockholm Diabetes Prevention Programme: the impact of sex and type 2 diabetes heredity. *Diabetologia,* 48**,** 35-40.

LUNDSGAARD, A.-M. & KIENS, B. 2014. Gender differences in skeletal muscle substrate metabolism - molecular mechanisms and insulin sensitivity. *Frontiers in endocrinology,* 5**,** 1-6.

MITTENDORFER, B., HOROWITZ, J. F. & KLEIN, S. 2001. Gender differences in lipid and glucose kinetics during short-term fasting. *American Journal of Physiology, Endocrinology and Metabolism,* 281**,** E1333-E1339.

MONTAIN, S. J., HOPPER, M. K., COGGAN, A. R. & COYLE, E. F. 1991. Exercise metabolism at different time intervals after a meal. *Journal of Applied Physiology,* 70**,** 882-888.

RATTARASARN, C., LEELAWATTANA, R. & SOONTHORNPUN, S. 2010. Contribution of skeletal muscle mass on sex differences in 2-hour plasma glucose levels after oral glucose load in Thai subjects with normal glucose tolerance. *Metabolism: Clinical and Experimental,* 59**,** 172-176.

ROBERTSON, M. D., LIVESEY, G. & MATHERS, J. C. 2002. Quantitative kinetics of glucose appearance and disposal following a 13C-labelled starch-rich meal: comparison of male and female subjects. *The British Journal of Nutrition,* 87**,** 569-577.

SANLIOGLU, A. D., ALTUNBAS, H. A., BALCI, M. K., GRIFFITH, T. S. & SANLIOGLU, S. 2013. Clinical utility of insulin and insulin analogs. *Islets,* 5**,** 67-78.

STORLIEN, L., OAKES, N. & KELLEY, D. 2004. Metabolic flexibility. *Proceeding of the Nutrition Society,* 63**,** 363-368.

STRICKER, R., EBERHART, R., CHEVAILLER, M.-C., QUINN, F. A., BISCHOF, P. & STRICKER, R. 2006. Establishment of detailed reference values for luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone during different phases of the menstrual cycle on the Abbott ARCHITECT analyzer. *Clinical Chemistry and Laboratory Medicine,* 44**,** 883-887.

TARNOPOLSKY, L. J., MACDOUGALL, J. D., ATKINSON, S. A., TARNOPOLSKY, M. A. & SUTTON, J. R. 1990. Gender differences in substrate for endurance exercise. *Journal of Applied Physiology,* 68**,** 302-308.

TARNOPOLSKY, M. 2004. Protein requirements for endurance athletes. *Nutrition,* 20**,** 662-668.

TARNOPOLSKY, M. A. 2000. Gender differences in metabolism; nutrition and supplements. *Journal of Science and Medicine in Sport,* 3**,** 287-298.

TARNOPOLSKY, M. A. 2008. Sex differences in exercise metabolism and the role of 17-beta estradiol. *Medicine and Science in Sports and Exercise,* 40**,** 648-654.

The DECOTE Study Group. 2003. Age- and Sex-specific prevalence of diabetes and impaired glucose regulation in 13 European Cohorts. *Diabetes Care,* 26, 61-69.

VILSBØLL, T. & HOLST, J. J. 2004. Incretins, insulin secretion and Type 2 diabetes mellitus. *Diabetologia,* 47**,** 357-366.

WILD, S., ROGLIC, G., GREEN, A., SICREE, R. & KING, H. 2004. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care,* 27**,** 1047-1053.

WOHL, P., GIRMAN, P. & PELIKANOVA, T. 2004. Inflexibility of energy substrate oxidation in type 1 diabetic patients. *Metabolism - Clinical and Experimental,* 53(5), 655-659.

CHAPTER 5

General Discussion

5.1 Summary of key findings

Nutrition and physical activity are the main strategies to improve metabolic health among individuals who are obese or overweight/centrally obese. The predominant theme of this thesis was to investigate the metabolic responses to feeding and exercise in men and women. Specifically, **Chapter 2** intended to understand how performing aerobic exercise in the fed or overnight-fasted state affected substrate metabolism responses in obese or overweight/centrally obese men. The main result revealed that, in obese or overweight/centrally obese men, an acute bout of aerobic exercise performed in the overnight-fasted state augmented whole-body exercise fat oxidation and showed for the first time in this population that Type I fibre IMTG utilisation was promoted as compared to exercise performed in the fed state.

Most scientific evidence available largely focuses on men and rarely investigates women. It was therefore relevant to explore whether women responded to manipulation of nutrient-exercise timing in a similar manner to men. Therefore, **Chapter 3** explored the response of substrate oxidation and blood metabolites to exercise and recovery when food was provided before or after exercise in obese or overweight/centrally obese women. Additionally, **Chapter 3** examined the subsequent appetite responses and subsequent energy intake in response to eating

before or immediately after exercise. Whilst it had not been shown previously in obese women, the result revealed a directionally similar response as men in which the acute bout of aerobic exercise performed in the overnight-fasted state, as compared to fed-state, augmented whole-body exercise fat oxidation. Additionally, consuming breakfast after exercise as compared to before exercise appeared to suppress appetite sensations and decrease subsequent energy intake at a later *ad libitum* style lunch.

Interestingly, while the metabolic responses were directionally similar between men and women, the study provided initial evidence that the magnitude of change in substrate utilisation with pre-exercise feeding differed between men and women (i.e., greater suppression of lipid utilisation in women). This suggested the need to explore in a more controlled manner the potential influence of biological sex in the response to metabolic challenges, such a nutrient ingestion. Hence, **Chapter 4** was designed to characterize potential sex-differences in metabolism after glucose feeding in men and women pair-matched for relevant variables known to affect the metabolic responses. The result revealed, for the first time using gold-standard approaches for between-sex metabolic comparisons, that women exhibit an earlier metabolic response to feeding and return to baseline quicker than men, providing initial evidence to suggest higher levels of metabolic control in response to carbohydrate feeding in women than in men.

The remainder of this Chapter will expand upon these key new contributions to the knowledge base by critical discussion and consideration of their implications for future research and practice.

5.2 Fed vs. overnight-fasted exercise and whole-body fat utilisation

Established scientific evidence proposes that a low capacity to oxidise fat is an important determinant in the aetiology of obesity and consequently development of chronic metabolic disease. For instance, a low relative fat oxidation, indicated by a high RQ, is predictive of future weight gain (Zurlo et al., 1990, Marra et al., 2004, Ellis et al., 2010, Shook et al., 2015), and development of type 2 diabetes, hypertension and atherosclerosis (Ferro et al., 2013, Montalcini et al., 2013, Pujia et al., 2016). In fact, Kelley and colleagues found that in the fasted state fat oxidation was lower and fatty acid storage was higher in the obese as compared with lean subjects (Kelley et al., 1999). They also observed differences between lean and obese subjects in insulin-stimulated substrate utilization. In lean subjects, fat oxidation and storage decreased in response to insulin; however, in obese subjects, those same responses to insulin were blunted, demonstrating a reduced capacity for fat oxidation and inflexibility in regulating fat oxidation. Thus, increasing utilisation of fat as fuel might be beneficial for long-term metabolic health especially in obese population.

Exercise is a good way to acutely increase energy expenditure but its use as a means to increase fat oxidation in obesity requires understanding of the interaction

between nutrient intake and metabolic responses in this population. A key finding from **Chapter 2** and **Chapter 3** showed that when exercise is performed in the overnight-fasted state, it induced significantly greater reliance on fat and lesser reliance on carbohydrate as main fuel source as compared when exercising in fed state. This is consistent with previous works in lean or overweight men (De Bock et al., 2005, Derave et al., 2007, Derave et al., 2008, Farah and Gill, 2013) but the present thesis extends this to obese or centrally obese men *and* women. In parallel, a higher circulating concentration of lipolytic markers including glycerol and NEFA and lower insulin was observed during exercise undertaken in the overnight-fasted state, confirming this as a successful strategy to increase fat mobilisation and utilisation during exercise in obese populations.

Despite the result of this thesis showing differences in fat utilisation during exercise commenced under different nutritional states, a limitation is that substrate use in the periods before or after exercise was not determined; hence, it is not possible to make inferences about longer-term potential of overnight fasted exercise to influence fat balance. A recent review has suggested that exercise performed in the overnight-fasted state could altered 24 h fat oxidation in obesity (Wallis and Gonzalez, 2019) as shown in previous works among lean and active young adults men and women (Iwayama et al., 2015, Iwayama et al., 2017). Another important element in obesity is reduction of body fat, in which the same review suggested that acute exercise or short-term (i.e., 4-6 weeks) overnight-fasted versus fed-state exercise training was unable to show any reduction of body fat. Therefore, a relevant next step is might be to perform 24 hour calorimetry studies in obese population to

see if findings in lean translate, and this might underpin a longer term exercise training study (i.e., >12 weeks) of body composition changes with regular overnight-fasted versus fed state exercise.

5.3 Fed vs. overnight-fasted exercise and IMTG utilisation

Additionally, in **Chapter 2**, the use of IMTG during exercise was determined, as elevations in IMTG content have also been linked to the development of metabolic diseases associated with obesity, such as insulin resistance and type 2 diabetes (Phillips et al., 1996a, Pan et al., 1997). The balance between synthesis and breakdown, also called IMTG turnover, determines the IMTG content. Thus, IMTG concentrations can only change when there is an imbalance between lipolysis (during exercise) and synthesis (after exercise and/or after feeding). The interaction of fat and carbohydrate oxidation during exercise depends on the feeding status. In lean men, it has been shown that provision of carbohydrate before and during exercise negates the IMTG use (Coyle et al., 1997, De Bock et al., 2005). The result from **Chapter 2** demonstrates that Type I IMTG is only used when exercise performed in overnight-fasted state in obese men. Interestingly, as far as authors' knowledge, this current work is the first one that established directly the use of IMTG during exercise in fasted state in obesity, with some study have shown using indirect methods like stable isotopes (Goodpaster et al., 2000). Indeed, other works has fail to show exercise-induced degradation of IMTG in obese (Nellemann et al., 2014) and overweight populations (Larson-Meyer et al., 2006). Differences in study methodology, including the technique used to measure IMTG content from muscle

samples might explain the discrepancies. For example, no fibre specific use was measured and this is often cited as the reason why even in lean people some studies do not see evidence of net IMTG use during exercise (van Loon et al., 2003).

Nonetheless, despite evidence showing a reduction in Type I fibre IMTG in overnight-fasted state exercise, the IMTG contents did not reduce to lower levels than those observed at the end of the fed-state exercise. Hence, the significance of this finding is unclear; whether the potential benefits of IMTG utilisation requires reduction to a certain absolute level or whether stimulation of net utilisation *per se* is sufficient. Furthermore, the significant difference in pre-exercise IMTG between fasted and fed trials might have contributed to the changes observed in IMTG use over the exercise period between trials. Such an assumption is consistent with earlier studies showing that resting IMTG content was correlated with subsequent utilisation during exercise (Standl et al., 1980, Essen-Gustavsson & Tesch, 1990, Steffensen et al., 2002, Van Loon et al., 2003). Another limitation that needs to be acknowledged is that this was an acute study, so the consistent effects of training in these divergent nutritional states is not known. Previous work in lean men shows overnight fasted training (that stimulates IMTG turnover) prevents high fat diet induced glucose intolerance (Van Proeyen et al., 2010). This suggests fasted-exercise might be protective in conditions of excess nutrient provision, i.e., in the obese state; therefore a training study in more at-risk populations with health outcome measures would be an important follow-up.

5.4 Food intake before or after exercise and subsequent energy intake

Most studies of weight control and exercise training would not typically consider feeding status around the exercise sessions, particularly in obese populations. To authors' knowledge, this is the first cross over study to demonstrate the effect of exercise in fasted and fed conditions on appetite sensations and subsequent energy intake among overweight and obese women. The result from **Chapter 3** of this thesis showed that a single exercise session performed in the overnight-fasted state decreased the subsequent energy intake as compared when performing exercise in fed state, with the difference being approximately 15%. This was supported by the VAS data that suggested there are clear effects on subjective appetite ratings. These findings indicate that overnight-fasted exercise did not result in a significant compensatory response in energy intake.

One strength of the study was that that food intake and exercise energy expenditure over study period up to the consumption of the *ad libitum* lunch was balanced. However, the simple duration since the last meal was shorter in the overnight-fasted exercise condition, which may have influenced the subsequent energy intake response at lunch. Thus, how fasted vs. fed exercise affects energy intake and also expenditure when participants are allowed to self-select timing and quantity of all subsequent meals would be a good follow up. Meanwhile, a limitation that also needs to be acknowledged is that no appetite hormones were investigated in this thesis and also, the experiment was of relatively short-term and appetite responses only investigated in women, not also in obese men. Nonetheless, a study recently

published in physically active young men concluded that, pre-exercise breakfast omission is not fully compensated post-exercise (Edinburgh et al., 2019). In fact, pre-exercise breakfast omission created a more negative daily energy balance compared with when breakfast is consumed before exercise, at least over a 24-h time period. The authors suggest that pre-exercise breakfast is a useful strategy to induce short-term energy deficit in healthy young men in order to increase weight loss. Therefore, this should be done in obese to see if it is the same, as well as investigating the long-term consequences of fasted vs. fed under free-living conditions. The potential for improved energy deficit plus the possibility for high IMTG turnover raises the possibility that fasted vs. fed exercise could be strikingly beneficial for body weight and metabolic health in obese populations.

5.5 Sex-difference in substrate metabolism in response to feeding

The results from **Chapter 2 and Chapter 3** revealed an apparent sex-difference in terms of metabolic response, in which the magnitude of reduction in fat oxidation was greater in women as compared to men upon feeding before exercise in overnight-fasted state. This was followed up by investigating among well-controlled pair-matched participants during rest and indeed, it was then found that there was a significant sex-difference in response to acute glucose feeding as presented in **Chapter 4**. The findings from this chapter show that metabolic control was improved in women vs. men after glucose feeding. Collectively, the thesis provides evidence for different metabolic responses to feeding and feeding plus exercise in men and women. The finding from this aspect of the thesis convey a notion that in

metabolism, biological sex plays an important role in the response towards any metabolic stressors such as exercise and nutrition timing. However, a more comprehensive manner of investigation that determines mechanisms responsible for sex-differences in the metabolic response to feeding or feeding and exercise is now warranted.

5.6 Practical implication

The studies in this thesis have advanced the evidence base in men and women, especially overweight and obese population, regarding the potential efficacy of performing exercise in the overnight-fasted vs. fed state. The research findings presented may have practical implications in term of guiding future experimental research, promoting healthy lifestyle behaviours and informing public health policy. The suggested recommendations discussed in this section are based on the research findings in specific populations (**Chapter 2 and 3** – overweight and obese adults; **Chapter 4** – healthy adults with low physical activity level); therefore, further work is required to determine the applicability of the recommendations to more diverse populations.

Promoting a physically active lifestyle may stimulate transient benefit to overweight and obese population in lipid metabolism and have long-term implications by improving their metabolic health. Thus, manipulation of nutrition and increased physical activity seems great strategy to use in the treatment of obesity in managing their weight and body composition. Additionally, higher accumulation of fat in the

body is known as aetiology of obesity and higher fat accumulation, particularly in the skeletal muscle, is known to decrease insulin sensitivity; hence, the manipulations are also needed to maximize the use of fat, specifically the use of IMTG, so the benefits could be on body composition and insulin sensitivity. From a translational perspective, the result of this thesis would suggest that to increase fat oxidation / utilisation, particularly IMTG, exercising in the overnight-fasted could be more beneficial as compared to exercising in the fed-state.

Furthermore, performing exercise in the overnight-fasted state was beneficial in controlling the amount of calorie intake during a subsequent meal, which could be an advantage for obese populations in order to lose weight. Restricting calorie intake has been used as a strategy to reduce body fat. Surprisingly, it is generally unsuccessful as more than 90% of obese individuals regain lost body fat within 2 years (Vogels et al., 2005). Perhaps, sustaining the restriction diet might be quite challenging for obese individuals. Hence, exercising in fasted state seems a great alternative way in helping this population to manage their weight loss. Nevertheless, since this part of study was only done in women with obesity, further study in men is warranted in future work as a sex difference in the way exercise alters hormones and appetite related to energy intake has been suggested in previous work (Hagobian et al., 2009).

The identification of sex differences in metabolic responses to feeding and/or feeding plus exercise suggest the fundamental role of biological sex that may have a variety of implications. For instance, it might explain sex-differences in disease

risk and understanding underlying mechanisms may help with development of relevant sex-based therapeutic approaches obesity, diabetes and metabolic syndrome. There also may be significant implications for nutritional advice in terms of exercise, specifically on timing of nutrition around exercise. If overnight-fasted exercise is preferable, but not possible, it is important to know how long after feeding to wait in order to optimise metabolic responses, and this could well necessitate waiting longer for men than women. Collectively, the data from this thesis suggest that both sexes should be studied to elucidate the determinants of these fundamental biological sex differences in future research.

5.8 Conclusion

In conclusion, the results from this thesis have developed new insights in the understanding of the impact of feeding timing on substrate utilisation during exercise and the influences of biological sexes in metabolic responses to feeding in high risk populations, which is important in order to improve their metabolic health. The first aim of this thesis was to investigate the effect of a single exercise bout performed either before or after feeding on whole-body lipid and IMTG utilisation during exercise in obese or overweight/centrally obese men. The finding shows that fat oxidation and IMTG use was higher in overnight-fasted exercise as compared to fed exercise. The second aim was to determine the influence of overnight-fasted versus fed state exercise on substrate metabolism during exercise and appetite responses after exercise, in obese or overweight centrally obese women. The findings show a similar directional response to men for substrate utilisation during exercise but also

revealed a potential benefit for overnight-fasted exercise in reducing subsequent energy intake later in the day. The third aim was to investigate potential sex-differences in metabolic responses to feeding and exercise. The findings show there was a significant sex-difference in metabolic response to feeding both at rest and during exercise, whereby women appear to exhibit higher metabolic flexibility than men. Nevertheless, future work is warranted to focus on further understanding the mechanisms underlying the observations made in this thesis. Additionally, through longer-term training studies, their practical implications for the optimisation of health via nutrition and exercise in men and women should be further investigated.

In term of self-reflection, the researcher, who is from an epidemiology nutrition background, has developed numerous research skills and laboratory techniques throughout the PhD work. For example, as the primary methods of this work, the researcher mastered the techniques of indirect calorimetry, phlebotomy exercise testing, blood analysis including enzyme assays and ELISA, and nutritional intervention. The researcher also managed to run IMTG analysis by herself after extensive practical training from an expert, with the analysis involving sectioning muscle samples, staining, image acquisition and quantification the IMTG content. Nevertheless, it acknowledged that there are few skills or techniques that contributed in this thesis but was completed by other staff due to some reasons in which in researcher view is reasonable. For example, other skilled staff did the muscle biopsy. As acknowledged in each experimental chapter, some biological sample analysis was performed by other staff due to the end of scholarship whereby

researcher required go back to her hometown (Malaysia), but the techniques used during the analysis had already been already mastered by the researcher.

5.8 References

COYLE, E. F., JEUKENDRUP, A. E., WAGENMAKERS, A. & SARIS, W. 1997. Fatty acid oxidation is directly regulated by carbohydrate metabolism during exercise. *American Journal of Physiology, Endocrinology and Metabolism - Clinical and Experimental,* 273**,** E268-E275.

DE BOCK, K., RICHTER, E. A., RUSSELL, A. P., EIJNDE, B. O., DERAVE, W., RAMAEKERS, M., KONINCKX, E., LÉGER, B., VERHAEGHE, J. & HESPEL, P. 2005. Exercise in the fasted state facilitates fibre type-specific intramyocellular lipid breakdown and stimulates glycogen resynthesis in humans. *The Journal of Physiology,* 564**,** 649-660.

DERAVE, K. D. B., EIJNDE, B., HESSELINK, M., KONINCKX, E., ROSE, A., SCHRAUWEN, P., BONEN, A., RICHTER, E. & HESPE, P. 2008. Effect of training in the fasted state on metabolic responses during exercise with carbohydrate intake. *Journal of Applied Physiology,* 10(4)**,** 1045-1055.

DERAVE, W., MERTENS, A., MULS, E., PARDAENS, K. & HESPEL, P. 2007. Effects of post-absorptive and postprandial exercise on glucoregulation in metabolic syndrome. *Obesity,* 15**,** 704-711.

EDINBURGH, R. M., HENGIST, A., SMITH, H. A., TRAVERS, R. L., BETTS, J. A., THOMPSON, D., WALHIN, J.-P., WALLIS, G. A., HAMILTON, D. L., STEVENSON, E. J., TIPTON, K. D. & GONZALEZ, J. T. 2019. Skipping breakfast before exercise creates a more negative 24-hour energy balance: A randomized controlled trial in healthy physically active young men. *The Journal of Nutrition,* 149**,** 1-9.

ELLIS, A. C., HYATT, T. C., HUNTER, G. R. & GOWER, B. A. 2010. Respiratory Quotient Predicts Fat Mass Gain in Premenopausal Women. *Obesity,* 18**,** 2255-2259.

ESSEN-GUSTAVSSON, B. & TESCH, P. A. 1990. Glycogen and triglyceride utilization in relation to muscle metabolic characteristics in men performing heavy-resistance exercise. *European Journal of Applied Physiology,* 61, 5–10.

FARAH, N. & GILL, J. 2013. Effects of exercise before or after meal ingestion on fat balance and postprandial metabolism in overweight men. *British Journal of Nutrition,* 109**,** 2297-2307.

FERRO, Y., GAZZARUSO, C., COPPOLA, A., ROMEO, S., MIGLIACCIO, V., GIUSTINA, A., PUJIA, A. & MONTALCINI, T. 2013. Fat utilization and arterial hypertension in overweight/obese subjects. *Journal of Translational Medicine,* 11(159), 1-6.

GOODPASTER, B. H., THERIAULT, R., WATKINS, S. C. & KELLEY, D. E. 2000. Intramuscular lipid content is increased in obesity and decreased by weight loss. *Metabolism - Clinical and Experimental,* 49**,** 467-472.

HAGOBIAN, T., SHAROFF, C., STEPHENS, B., WADE, G., SILVA, J., CHIPKIN, S. & BRAU, N. B. 2009. Effects of exercise on energy regulating hormones and appetite in men and women. *The American Journal of Physiology - Regulatory, Integrative and Comparative Physiology,* .296**,** R233–R242.

IWAYAMA, K., KAWABUCHI, R., NABEKURA, Y., KURIHARA, R., PARK, I., KOBAYASHI, M., OGATA, H., KAYABA, M., OMI, N., SATOH, M. & TOKUYAMA, K. 2017. Exercise before breakfast increases 24-h fat oxidation in female subjects. *PloS One,* 12**,** e0180472.

IWAYAMA, K., KURIHARA, R., NABEKURA, Y., KAWABUCHI, R., PARK, I., KOBAYASHI, M., OGATA, H., KAYABA, M., SATOH, M. & TOKUYAMA, K. 2015. Exercise Increases 24-h Fat Oxidation Only When It Is Performed Before Breakfast. *EBioMedicine,* 2**,** 2003-2009.

KELLEY, D., GOODPASTER, B., WING, R. & SIMONEAU, J. 1999. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *American Journal of Physiology, Endocrinology and Metabolism,* 277(6), E1130-E1141.

LARSON-MEYER, D. E., SMITH, S. R., HEILBRONN, L. K., KELLEY, D. E., RAVUSSIN, E., NEWCOMER, B. R. & LOOK, A. A. R. G. 2006. Muscle-associated triglyceride measured by computed tomography and magnetic resonance spectroscopy. *Obesity,* 14**,** 73-87.

MARRA, M., SCALFI, L., CONTALDO, F. & PASANISI, F. 2004. Fasting respiratory quotient as a predictor of long-term weight changes in non-obese women. *Annals of Nutrition and Metabolism,* 48**,** 189-192.

MONTALCINI, T., GAZZARUSO, C., FERRO, Y., MIGLIACCIO, V., ROTUNDO, S., CASTAGNA, A. & PUJIA, A. 2013. Metabolic fuel utilization and subclinical atherosclerosis in overweight/obese subjects. *Endocrine,* 44**,** 380-385.

NELLEMANN, B., SONDERGAARD, E., JENSEN, J., PEDERSEN, S. B., JESSEN, N., JORGENSEN, J. O. & NIELSEN, S. 2014. Kinetics and utilization of lipid sources during acute exercise and acipimox. *American Journal of Physiology, Endocrinology and Metabolism,* 307**,** E199-E208.

PAN, D., LILLIOJA, S., KRIKETOS, A., MILNER, M., BAUR, L., BOGARDUS, C., JENKINS, A. & STORLIEN, L. 1997. Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes,* 46**,** 983-988.

PHILLIPS, D. I., CADDY, S., ILIC, V., FIELDING, B. A., FRAYN, K. N., BORTHWICK, A. C. & TAYLOR, R. 1996. Intramuscular triglyceride and

muscle insulin sensitivity: evidence for a relationship in nondiabetic subjects. *Metabolism: Clinical and Experimental,* 45**,** 947-950.

PUJIA, A., GAZZARUSO, C., FERRO, Y., MAZZA, E., MAUROTTI, S., RUSSO, C., LAZZARO, V., ROMEO, S. & MONTALCINI, T. 2016. Individuals with Metabolically Healthy Overweight/Obesity Have Higher Fat Utilization than Metabolically Unhealthy Individuals. *Nutrients,* 8(2), 1-11.

SHOOK, R., HAND, G., PALUCH, A., WANG, X., MORAN, R., HEBERT, J., JAKICIC, J. & BLAIR, S. 2015. High respiratory quotient is associated with increases in body weight and fat mass in young adults. *European Journal of Clinical Nutrition,* 70**,** 1197-1202.

STANDL, E., LOTZ, N., DEXEL, T., JANKA, H. U. & KOLB, H. J. 1980. Muscle triglycerides in diabetic subjects. Effect of insulin deficiency and exercise. *Diabetologia,* 18, 463–469.

STEFFENSEN, C. H., ROEPSTORFF, C., MADSEN, M. & KIENS, B. 2002. Myocellular triacylglycerol breakdown in females but not in males during exercise. *American Journal of Physiology Endocrinology and Metabolism,* 282, E634–E642.

VAN LOON, L. J. C., KOOPMAN, R., STEGEN, J. H. C. H., WAGENMAKERS, A. J. M., KEIZER, H. A. & SARIS, W. H. M. 2003. Intramyocellular lipids form an important substrate source during moderate intensity exercise in endurance-trained males in a fasted state. *The Journal of Physiology,* 553**,** 611-625.

VAN PROEYEN, K., SZLUFCIK, K., NIELENS, H., PELGRIM, K., DELDICQUE, L., HESSELINK, M., VAN VELDHOVEN, P. P. & HESPEL, P. 2010. Training in the fasted state improves glucose tolerance during fat-rich diet. *The Journal of Physiology,* 588**,** 4289-4302.

VOGELS, N., DIEPVENS, K. & WESTERTERP-PLANTENGA, M. 2005. Predictors of long-term weight maintenance. *Obesity Research,* 13**,** 2162-2168.

WALLIS, G. A. & GONZALEZ, J. T. 2019. Is exercise best served on an empty stomach? *The Proceedings of the Nutrition Society,* 78**,** 110-117.

ZURLO, F., LILLIOJA, S., ESPOSITO-DEL PUENTE, A., NYOMBA, B. L., RAZ, I., SAAD, M. F., SWINBURN, B. A., KNOWLER, W. C., BOGARDUS, C. & RAVUSSIN, E. 1990. Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ. *American Journal of Physiology, Endocrinology and Metabolism,* 259**,** E650-E657.

APPENDIX

APPENDIX 1: PILOT STUDY

Sex-differences in Metabolic Flexibility

Outline

Metabolic flexibility has been defined as the capacity to switch between carbohydrate and fat as main fuel sources in response to changing fuel availability and energy demand. Evidence has shown that poor metabolic flexibility is associated with obesity and Type 2 Diabetes. There is also emerging evidence showing that men and women respond differently to a range of metabolic stressors (e.g., exercise, starvation), and women might therefore be more metabolically flexible in response to dietary manipulation than men. Therefore, two acute studies were carried out to investigate the influence of biological sex on metabolic flexibility. The first study was carried out to investigate the sex-difference of substrate oxidation in response to acute provision of carbohydrate or fat over a 3-hour postprandial period. Meanwhile, the second study investigated the sex-difference of substrate oxidation to acute provision of carbohydrate given in doses relative to lean body mass over a 5-hour postprandial period. The understanding of the sex-difference in metabolic flexibility is important to determine specific exercise and dietary intervention strategies for women and men in improving their health and performance.

Pilot Study 1: Comparison of Substrate Oxidation between Men and Women after High-Fat and High-CHO Feeding

Summary

Differences in the metabolic response to nutritional intake may confer susceptibility or resistance to obesity and diabetes mellitus type 2 in humans. Evidence has shown that men and women respond differently to a range of metabolic stressors (e.g., exercise, starvation), and women might therefore be more metabolically flexible in response to dietary manipulation than men. Thus, the purpose of this preliminary study was to investigate the influence of biological sex on metabolic flexibility (substrate oxidation responses) in response to acute provision of carbohydrate or fat. We measured the changes in RQ and substrate oxidation rate over a 3 hour period in response to high carbohydrate ([HCM] 1 g/kg body mass) or high fat meals [HFM] in 4 well-matched young men and women who reported low habitual physical activity. In response to HCM, RQ and carbohydrate oxidation had increased significantly by 1 hour and remained elevated above baseline levels for at least 3 hours in men and women, with no statistically significant sex-differences. Fat oxidation was reduced significantly at 1 and 2 hours but not after 3 hours as compared to baseline, with no sex differences observed. Nonetheless, substrate oxidation changes, particularly reflected in carbohydrate oxidation, appeared to be of greater magnitude and peak earlier in women versus men in response to HCM. In contrast, no substantial metabolic changes were observed in response to HFM in either sex. In conclusion, this preliminary study has revealed the potential for sex-

difference in the size and pattern of the carbohydrate oxidation response to carbohydrate feeding. However, women received higher dietary carbohydrate relative to lean body mass and neither sex showed a return to baseline substrate oxidation levels. Therefore, in order to fully characterize potential sex-differences further pilot work will explore longer term (5 hours) responses to carbohydrate feeding using doses relative to lean body mass.

Introduction

Obesity and type 2 diabetes have become major public health problems in both developed and developing countries (Gersh et al., 2010, Popkin, 2010). The prevalence of these diseases are increasing and it has been projected that 65 million more obese adults in the USA and 11 million more obese adults in the UK by 2030 (Wang et al., 2011), consequently it will increase the health and economic burden arising from obesity and type 2 diabetes.

Evidence has shown that these chronic diseases are associated with poor metabolic flexibility (Galgani et al., 2008, Corpeleijn et al., 2009). Metabolic flexibility has been defined as the capacity to switch between carbohydrate and fat as main fuel sources in response to changing fuel availability and energy demand (Storlien et al., 2004). However, when the system is metabolically inflexible, the capacity to switch between fat and glucose as the primary source of fuels based on the availability of nutrients and energy demand is impaired (Blaak et al., 2000, Corpeleijn et al., 2009). For example, a system that is metabolically inflexible to the provision of a high fat load

may lead to greater storage of fat, increasing the likelihood of developing obesity. If the excess fat is stored in tissues like the liver and skeletal muscle this could also result in insulin resistance (Krssak et al., 1999). Obesity and insulin resistance plays a crucial role in the pathogenesis of Type 2 Diabetes (Khan and Flier, 2000).

It has become increasingly apparent that biological sex has a profound influence on metabolism. Scientific evidences showing that men and women respond differently on to a range of metabolic stressors such as exercise (Henderson, 2014), lipid-infusion (Høeg et al., 2011) and insulin stimulation (Kuhl et al., 2005, Clausen et al., 1996). It has been also suggested that women might be more metabolically flexible in response to dietary manipulation than men (Lundsgaard and Kiens, 2014). Indeed, cross-sectional evidence from our group indicates dietary carbohydrate and fat independently exert a greater influence on substrate oxidation during exercise in women than in men. However, whether metabolic flexibility differs between well-matched men and women has not been investigated systematically.

Therefore, the purpose of the study is to investigate the influence of sex on metabolic flexibility (substrate oxidation responses) in response to acute provision of fat or carbohydrate in well-matched men and women.

Methodology

Study participant

This study was conducted among apparently healthy adults aged 18 to 45 years in Birmingham, United Kingdom. A total of 23 adults (9 men and 14 women) were recruited in this study. Recruitment was based on university portal announcement, poster advertisement, word of mouth and peer-to-peer referral in university areas. Eligibility participants were selected if they were healthy with normal body mass index (BMI, 19 – 24.9 kg/m_2), no family history of diabetes, sedentary of low habitual physical activity and no food intolerances or allergies that restrict them from consuming the test meals. Ten participants were excluded after the screening process due to various reasons that either they have family history of diabetes, their BMI higher than normal range or they were too active. From 13 eligible participants (6 men and 7 women), 4 men and 4 women were matched based on age, ethnicity, BMI and $\dot{V}O_{2peak}$ expressed as relative to lean body mass and thus were included in the experimental visits. General characteristics was presented in the **Table 1**. This study was approved by the Safety and Ethics Subcommittee of School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham. Written informed consent was obtained from participants prior to the study.

Table 1 General characteristics of participants

	Men (N=4)	Women (N=4)
	Mean ± SD	
Age (years)	23.8 ± 2.3	23.5 ± 1.7
BMI (kg/m^2)	21.5 ± 1.9	20.4 ± 1.4
Lean body mass, LBM (kg)	58.6 ± 8.3	43.1 ± 2.3*
Body fat (%)	11.4 ± 4.4	23.6 ± 3.1**
$\dot{V}O_{2peak}$ /body weight (ml/kg/min)	33.3 ± 4.5	29.0 ± 3.9
$\dot{V}O_{2peak}$ /LBM (ml/kg/min)	37.3 ± 3.7	38.1 ± 4.4

Significant difference from men at $^{*}p<0.05$, $^{**}p<0.01$

Study design

This preliminary study was a randomized and crossover design. It involved of 3 visits to the Human Performance Laboratory in the School of Sport, Exercise and Rehabilitation Sciences. Visit 1 included informed consent, health screening, familiarisation with resting measurement of substrate oxidation and estimation of $\dot{V}O_{2peak}$ through a sub maximal predictive test. Visits 2 and 3 involved of determination of resting substrate oxidation in the fasted state and in the period following consumption of HCM or a HFM for over 3 hours.

Visit 1: Screening and fitness test

During first visit, participants were completed the GHQ and IPAQ-SF (Craig et al. 2003). Participant's weight and height then was measured in light clothes to

calculate their BMI. Body weight was measured using an electronic scale (OHaus, Champ II scales, USA) to the nearest 0.1 kg and height was measured using a stadiometer (Seca, UK) to the nearest 0.1 cm, while waist circumferences were measured to the nearest 0.1 cm with a flexible but elastic measuring tape. BMI was calculated as weight (kg) divided by height squared (m^2). Thereafter, their body composition (%BF, FM and LBM was determined by 4-site (bicep, tricep, subscapular and suprailliac) skinfold thickness measurements using Harpenden skinfold caliper and calculated based on the updated sex and race/ethnicity specific equations (Davidson et al., 2011). The skinfold thickness measurement were done by an ISAK accredited kinanthropometist.

Next, participants were familiarized with the process to be used in Visit 2 and 3 for measuring fuel utilisation (described below). For this, the participant lay in a supine position for 10 minutes with ventilated hood placed over their head and upper torso, which was then connected to an indirect calorimeter (GEM Nutrition Ltd. Cheshire, UK). While participants are rested, their resting blood pressure was also determined using an automatic blood pressure monitor (Omron M6, Netherlands). If participants successfully completed the Screening Form and met the eligibility requirements with respect to BMI, PAL and blood pressure, they were asked to undergo the fitness test.

An exercise test on a stationary bicycle was used to determine fitness level of participants. The exercise test was commenced with the power set at low intensity (35 W) and this was be maintained for 5 minutes in order to allow participants to

adequately warm-up. They were asked to maintain a constant pedal rate of between 60-70 rev/min during the test. Then, participants were recommenced cycling with the power set at 60 W and cycled continuously for 3 minutes maintaining a pedal rate of between 60-70 rev/min with the power increased by 35 W every 3 minutes until they reached voluntary exhaustion (i.e. they stop on their own accord or the pedal rate drops below 50rpm despite verbal encouragement from the investigator). Depending on the fitness level of the individual, the duration of the test took approximately between 5 and 15 minutes. During the exercise test, participants wore a facemask connected to a computerized gas analysis system that enables the measurement of rates of $\dot{V}O_2$ and $\dot{V}CO_2$ during the exercise. The data obtained from the graded exercise test was used to determine $\dot{V}O_{2peak}$. Heart rate was monitored continuously during exercise and participants were self-rate their perceived exertion during exercise bout using the Borg's RPE scale (Borg, 1982).

Upon completion of visit 1, 4 men and 4 women were well-matched based on age, ethnicity, BMI and $\dot{V}O_{2peak}$ expressed as relative to LBM.

Visit 2 and 3: Experimental visit

Female volunteer's not undergoing hormonal contraception had experimental visits scheduled within the mid follicular phase of the menstrual cycle, day 6 or 7 after initiation of menses. Female volunteers taking monophasic hormonal contraception were tested between day 1 and 21 of the active pill consumption period. Experimental visits for male volunteers were scheduled 7 days apart. Twenty hours before each test visit, participants were provided with a one day standardised food

package consisting of 50% carbohydrate, 30% fat, 20% protein based on their estimated energy expenditure (estimated using the Institute of Medicine equations assuming a low physical activity coefficient (Brooks et al. 2009). Participants were required to not exercise on that day and drinking being limited to plain water (*ad libitum*) to keep them hydrated. Participants arrived at the laboratory at 8.00am following a 12 hour overnight fast. After 10 minutes of rest in supine position, resting substrate oxidation was measured over 20-30 minutes using indirect calorimetry. After that, participants consumed either HCM (glucose drink) or HFM (double cream in liquid form) and postprandial substrate oxidation was determined 3 times for a period of 20-30 minutes at 1-hour intervals.

Resting substrate oxidation measurement

During the measurement, subjects will lie in a supine position for 25-30 minutes with ventilated hood connected to an indirect calorimeter (GEM Nutrition Ltd. Cheshire, UK) to measure the consumption of oxygen and production of carbon dioxide. Stable measurements of 20 minutes for both resting and postprandial substrate oxidation were used for data analysis to ensure measurements were valid and a true representative of the oxidative responses. The data from the tests was used to quantify carbohydrate and fat oxidation in response to the differing meals using stoichiometric equations as previously described (Frayn 1983) assuming that the urinary nitrogen excretion rate is constant.

Test meal

For HFM, double cream with 21% fat/100grams was used. The macronutrient content of the HFM consisted of 97% fat, 1.5% CHO, and 1.5% protein, the amount provided pertained to 50% of the predicted 24-hour basal metabolic rate (Mifflin et al. 1990). The HCM contained 1g glucose (Dextrose, MyProtein) per kg body mass diluted in a 4:1 solution of water to glucose. Participants need to drink the test meal within 10 minutes. It is acknowledged that the HFM and HCM are not energy matched. This is acceptable as it is between sex and not between drink comparisons that, the purpose of this pilot study.

Statistical analysis

All the variables were tested for normality by the Kolmogorov-Smirnov test and test of homogeneity of variance before any statistical comparisons were made. Descriptive statistics were reported as mean values ± SD for numerical variables and frequency and percentage for categorical variables, unless otherwise indicated. A bivariate analysis of an independent t-test was used to examine the differences of participant's general characteristics between sexes. A repeated measure two-way ANOVA was used to assess the effect of gender and time with Benferoni post-hoc. Data analyses were performed using the SPSS for windows version 21.0 (SPSS Inc. Chicago, IL). A *P* value of less than 0.05 was considered to be significant.

Results

There was a significant difference in body composition profiles between men and women which men had significantly higher levels of LBM ($p<0.05$) compared with women (Table 1). In contrast, women exhibited a significantly higher percent body fat ($p<0.01$) than that of the men.

In response to HCM, RQ and carbohydrate oxidation had increased significantly by 1 hour and remained elevated above baseline levels for at least 3 hours in men and women. This suggesting that longer measurement period is required to characterize the responses to carbohydrate feeding (Figure 1a and 1b). No significant sex differences were observed, however carbohydrate oxidation appeared to peak earlier and be of greater magnitude in the women as compared to men with the HCM (Figure 1b), suggesting that there is might be potential differences between sexes that need to explore further. Fat oxidation was reduced significantly at 1 and 2 hours but not after 3 hours as compared to baseline, with no sex differences observed (Figure 1c). In contrast, no substantial metabolic changes were observed in response to HFM in either sex (Figure 2a, 2b and 2c).

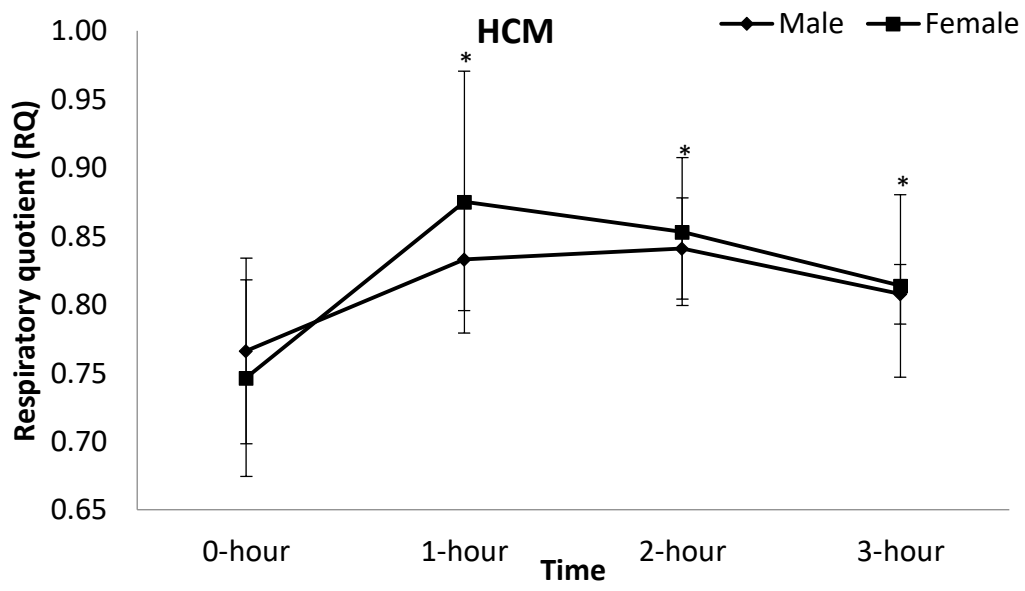

Figure 1a Mean ± SD of RQ in men and women after ingestion of HCM. *Significant difference from baseline.

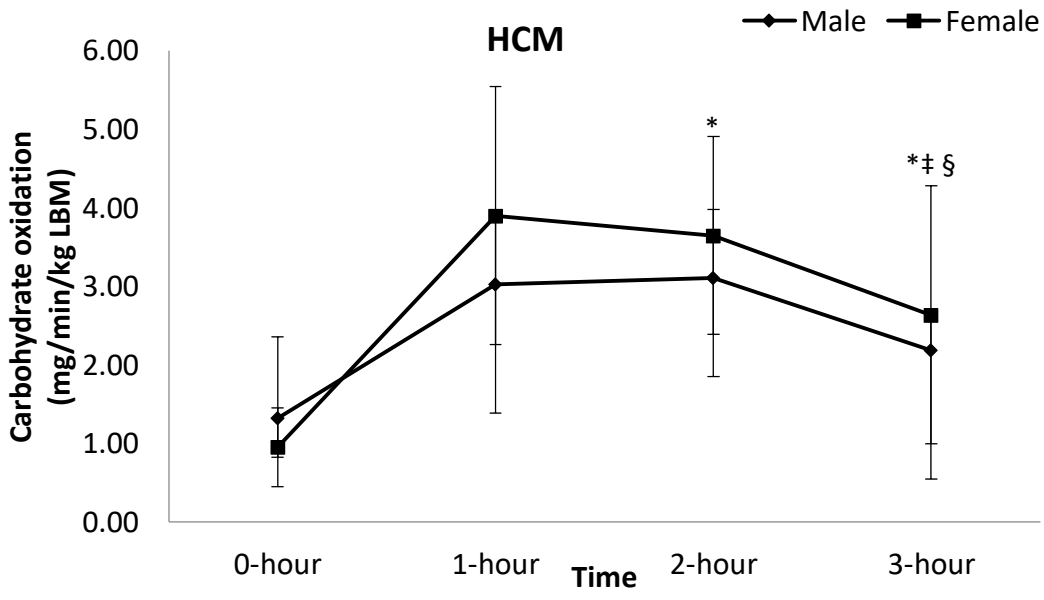

Figure 1b Mean ± SD of carbohydrate oxidation in men and women after ingestion of HCM. *Significant difference from baseline. ‡Significant difference from 1-hour. §Significant difference from 2-hour.

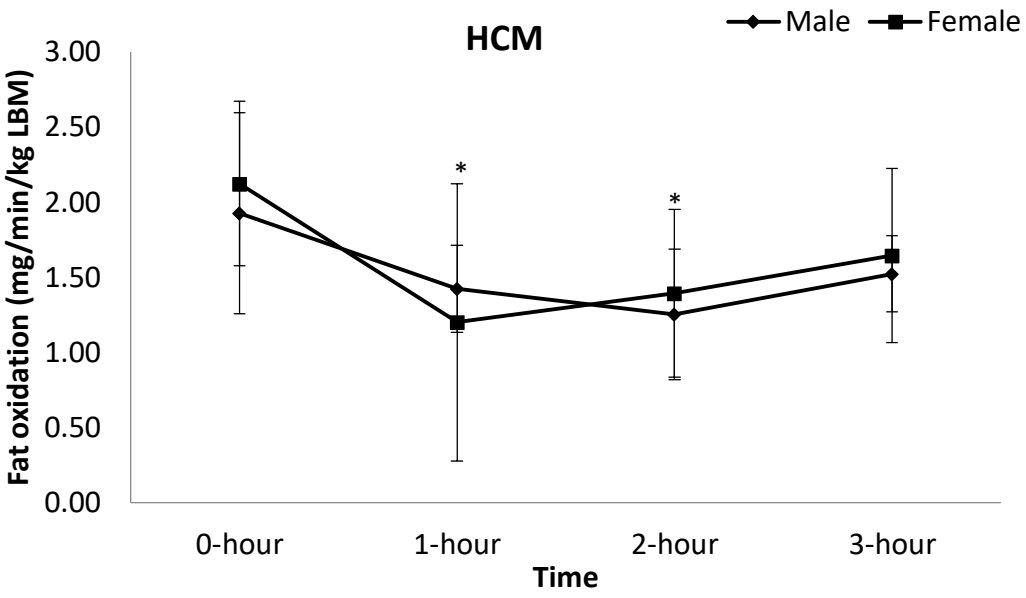

Figure 1c Mean ± SD of fat oxidation in men and women after ingestion of HCM.

*Significant difference from baseline.

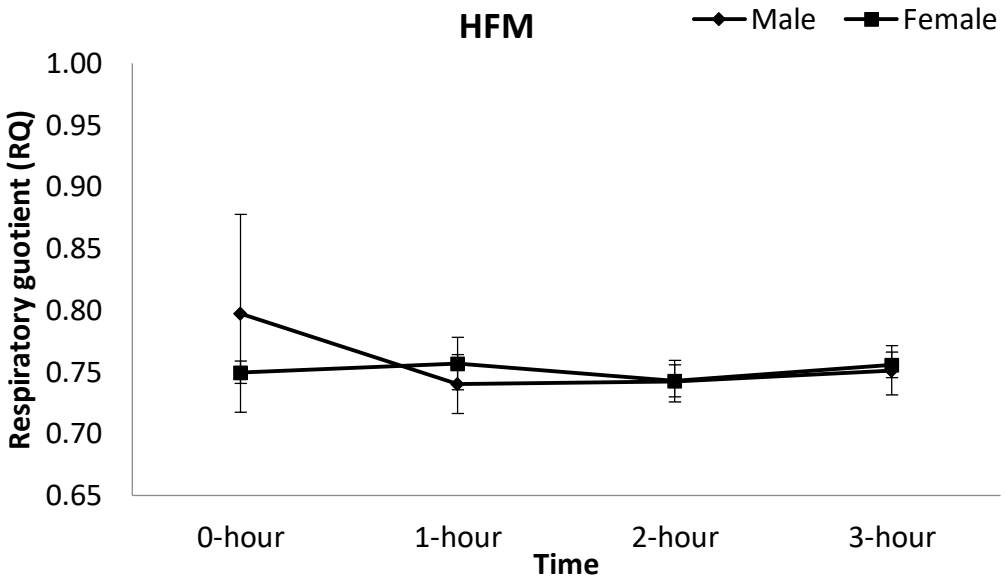

Figure 2a Mean ± SD of RQ in men and women after ingestion of HFM

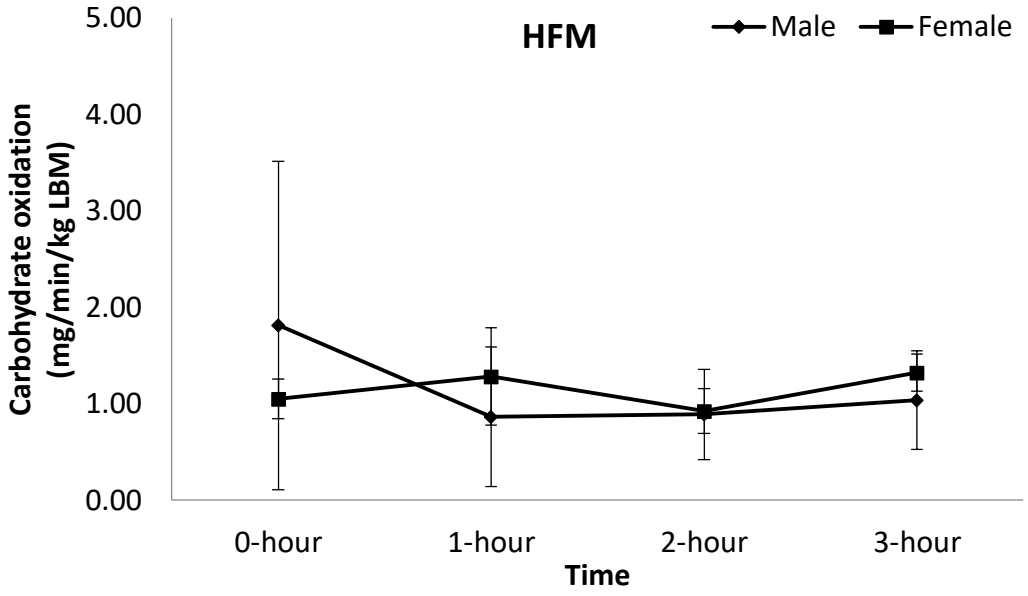

Figure 2b Mean ± SD of carbohydrate oxidation in men and women after ingestion of HFM

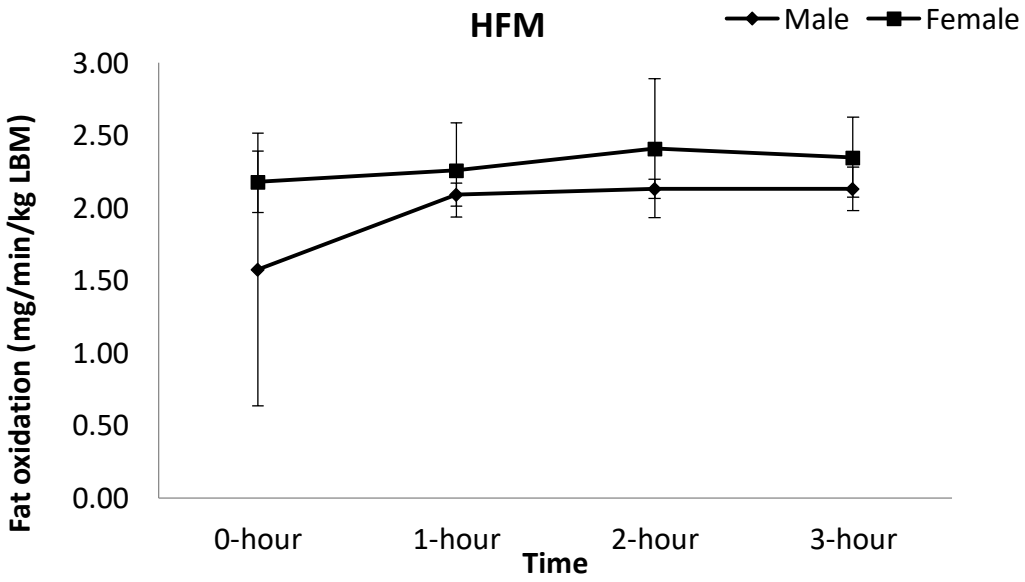

Figure 2b Mean ± SD of fat oxidation in men and women after ingestion of HFM

Conclusion

This preliminary study has revealed the potential for sex-difference in the magnitude and pattern of the carbohydrate oxidation response to carbohydrate feeding but not for fat feeding. However, women received higher dietary carbohydrate relative to LBM in this preliminary study and it might explain the quick respond by women as compare to men. In addition, neither sex showed a return to baseline substrate oxidation levels after 3 hours postprandial. Therefore, in order to fully characterise potential sex-differences further pilot work will explore longer term (5 hours) responses to carbohydrate feeding using doses relative to lean body mass.

Study 2: Comparison of Substrate Oxidation between Men and Women after High-CHO Feeding

Summary

The first preliminary study has revealed the potential for sex-difference in the size and pattern of the carbohydrate oxidation response to carbohydrate feeding. However, women received higher dietary carbohydrate relative to lean body mass and neither sex showed a return to baseline substrate oxidation levels. Therefore, in order to fully characterize potential sex-differences, longer term (5 hours) responses to carbohydrate feeding using doses relative to lean body mass is needed. Therefore, the purpose of this second preliminary study was to investigate the influence of biological sex on metabolic flexibility (substrate oxidation responses) in response to acute provision of carbohydrate relative to lean body mass during 5

hours of postprandial. The RQ and substrate oxidation rate were measured in 6 well-matched young men and women who reported low habitual physical activity for over a 5 hour period in response to HCM (1 g/kg LBM). Similar results were seen in this study, in response to HCM, RQ and carbohydrate oxidation had increased significantly by 1 hour and remained elevated above baseline levels for at least 3 hours in men and women and return to baseline by 5 hours, with no statistically significant sex-differences. Fat oxidation was reduced significantly at 1 and 2 hours as compared to baseline and return to baseline by 4 hour in women and 5 hours in men, with no sex differences observed. In women, the RQ and carbohydrate oxidation were peak earlier (at 1 hour) as compared to men (at 2 hour). In conclusion, this preliminary study has revealed the potential for sex-difference in the size and pattern of the carbohydrate oxidation response to carbohydrate feeding, which women responded quicker than men. Therefore, a comprehensive study with larger sample size is needed to explore the potential mechanism on gender differences in metabolic flexibility.

Introduction

It has become increasingly apparent that sex-difference has profound effect on substrate metabolism. Established evidences has shown higher glucose uptake in female skeletal muscle when stimulated by physiologic insulin concentrations (Høeg et al. 2011; Høeg et al. 2009). Indeed, in our previous preliminary study, results showed, even not statistically significant which might duet to small sample size, that women tend to response quicker (at 1 hour of postprandial) than men (at 2 hour of

postprandial) after digestion of high carbohydrate meal (HCM). However, in that study, women received higher dietary carbohydrate relative to lean body mass as compared to men. Hence, the difference might be due to higher glucose uptake in women skeletal muscle as they received more than men did. In addition, in our previous preliminary study, neither sex showed a return to baseline levels and respiratory quotient (RQ) even after 3 hours of high carbohydrate meal provision. Therefore, the purpose of this preliminary study was to investigate the influence of sex on longer-term (5 hours) responses to acute carbohydrate feeding by feeding doses relative to lean body mass.

Methodology

Study participant

Similar study protocol was carried out for this second preliminary study. In brief, 13 men and 14 women were screened and only 7 men and 9 women were eligible for this study based on inclusion criteria of the study Based on the matched criteria (age, ethnicity, BMI and VO2 max relative to LBM), 6 men and 6 women were matched and included in the experimental visit and their general characteristics were presented in **Table 2**. The Safety and Ethics Subcommittee of School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham approved this study. Written informed consent was obtained from participants prior to the study.

Table 2 General characteristic of participants

	Men (N=6)	Women (N=6)
Age (years)	19.0 ± 0.6	20.8 ± 1.2**
BMI (kg/m^2)	22.1 ± 1.1	21.7 ± 2.1
Lean body mass, LBM (kg)	61.0 ± 4.9	43.2 ± 4.9***
Body fat (%)	12.0 ± 3.8	26.0 ± 2.6***
$\dot{V}O_{2peak}$ /body weight (ml/kg/min)	37.7 ± 6.0	30.8 ± 2.7*
$\dot{V}O_{2peak}$ /LBM (ml/kg/min)	42.8 ± 5.5	42.5 ± 3.5

Mean ± SD. Significant difference from men at **p<0.01, ***p<0.001

Study design

This second preliminary study involved of two visits to the Human Performance Laboratory in the School of Sport, Exercise and Rehabilitation Sciences. Similar screening procedures were carried out during the first Visit 1 in this second preliminary study that involved of informed consent, health screening, familiarisation with resting measurement of substrate oxidation and estimation of $\dot{V}O_{2peak}$ through a sub maximal predictive test. For the experimental visit, participants arrived to the laboratory with overnight fasting. Then, they consumed HCM (glucose drink) and their postprandial substrate oxidation will be determined 5 times for a period of 20-25 minutes at 1-hour intervals that took about ~6 hours for the whole visit.

Test meal

The HCM contained 1 g glucose (Dextrose, MyProtein) per kg LBM diluted in a 4:1 solution of water to glucose. Participants need to drink the test meal within 10 minutes.

Statistical analysis

All the variables were tested for normality by the Kolmogorov-Smirnov test and test of homogeneity of variance before any statistical comparisons were made. Descriptive statistics were reported as mean values ± SD for numerical variables and frequency and percentage for categorical variables, unless otherwise indicated. A bivariate analysis of an independent t-test was used to examine the differences of participant's general characteristics between sexes. A repeated measure two-way ANOVA was used to assess the effect of gender and time with Benferoni post-hoc and adjustment for energy balance as a covariate. Further, a paired-sample t-test was used to test the difference from baseline in men and women separately. Data analyses were performed using the SPSS for windows version 22.0 (SPSS Inc. Chicago, IL). A *P* value of less than 0.05 was considered to be significant.

Results

Similarly with previous preliminary study, there were significant differences in body composition profiles between men and women whereby men had higher lean body mass ($P<0.001$) than women, while women had higher percentage of body fat ($P<0.001$) than men (Table 2). In addition, there was significant difference in $\dot{V}O_{2peak}$

relative to body weight between men and women (P<0.05) with mean were 37.7 ± 6.0 and 30.8 ± 2.7, respectively. However, when $\dot{V}O_{2peak}$ was express relative to lean body mass, no significant difference was found between sexes.

In this second pilot study, in response to HCM, the result from repeated measure two-way ANOVA showed that there is significant effect of time and gender on RQ and substrates (carbohydrate and fat) oxidation rate. The RQ (Figure 3) and carbohydrate oxidation (Figure 4) had increased significantly by 1 hour, remained elevated above baseline levels for at least 3 hours, and returned to the baseline by 4 hours in women. However, the RQ and carbohydrate oxidation rate was significant increase by 2 hours and returned to baseline at 5 hours in men. For fat oxidation rate, it was reduced significantly at 1 and 2 hours as compared to baseline and return to baseline by 3 hour in women (Figure 5), while it was reduces significantly at 2 and 3 and 4 hours in men. Further analysis by separate sex using paired t-test showed that, for RQ and carbohydrate oxidation rate, significant difference from baseline was found at 1-hour and 2-hour of postprandial in women, while in men, the significant difference from baseline was shown at 2-hour and 3 hour. This result suggesting that, women were peak earlier (at 1 hour) as compared to men (at 2 hour) after digestion of high carbohydrate meal.

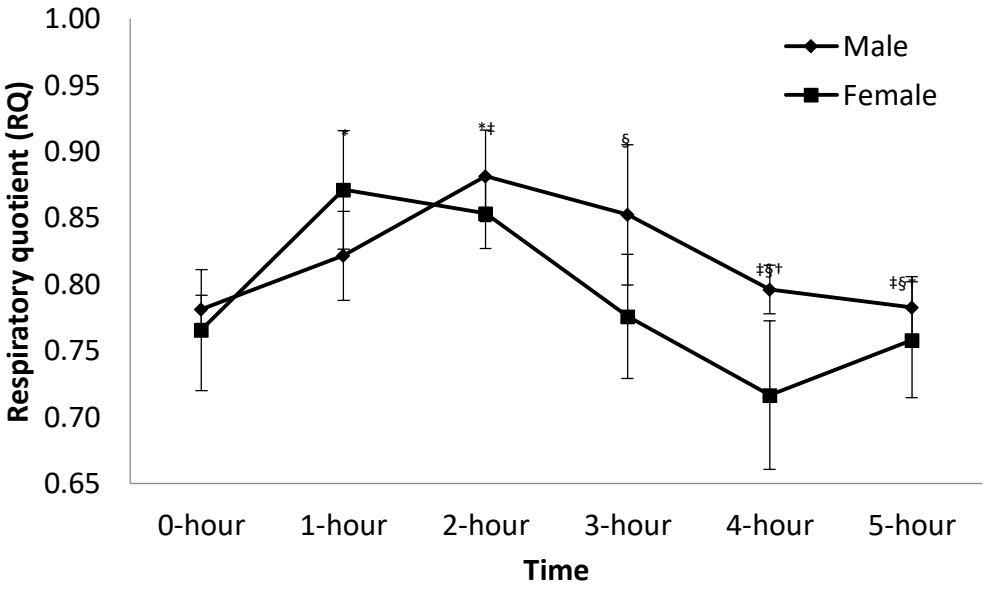

Figure 3 Mean ± SD of RQ in men and women after ingestion of HCM. *Significant difference from baseline. ‡Significant difference from 1-hour, §Significant difference from 2-hour. †Significant difference from 3-hour.

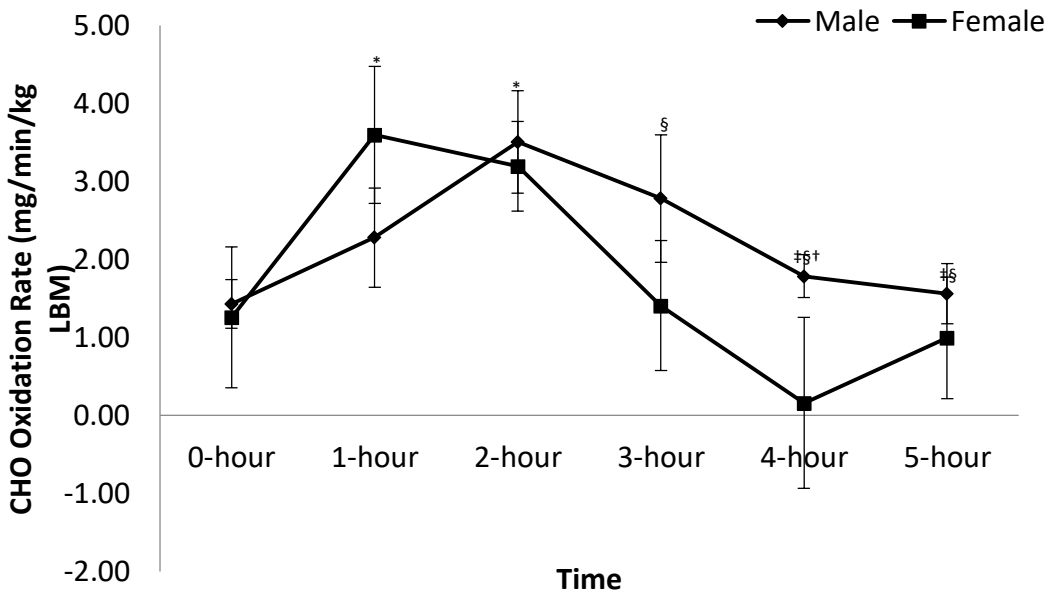

Figure 4 Mean ± SD of carbohydrate oxidation in men and women after ingestion of HCM. *Significant difference from baseline. ‡Significant difference from 1-hour. §Significant difference from 2-hour, †Significant difference from 3-hour.

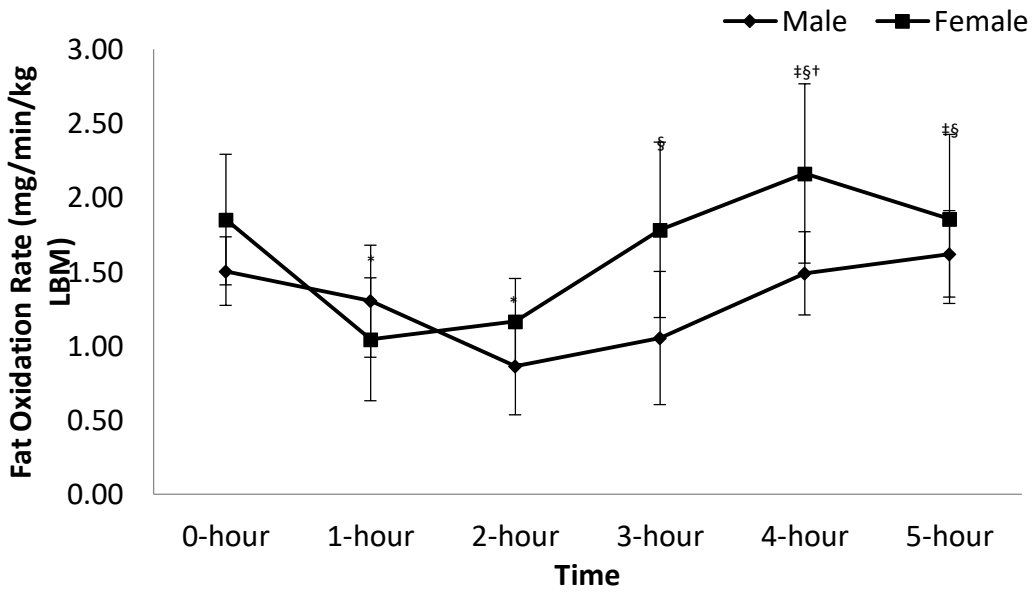

Figure 5 Mean ± SD of fat oxidation in men and women after ingestion of HCM. *Significant difference from baseline. ‡Significant difference from 1-hour. §Significant difference from 2-hour, †Significant difference from 3-hour.

Conclusion

This second preliminary study has revealed that there is for sex-difference in the timing of the carbohydrate oxidation and fat oxidation responses to carbohydrate feeding, which women responded quicker than men. However, this result only showed the difference of responses timing to carbohydrate feeding but the mechanism explaining the differences is not clear. Therefore, a comprehensive study with larger sample size will carry out in order to explain the mechanism of this sex difference.

References

BLAAK, E. E., VAN AGGEL-LEIJSSEN, D. P., WAGENMAKERS, A. J., SARIS, W. H. & VAN BAAK, M. A. 2000. Impaired oxidation of plasma-derived fatty acids in type 2 diabetic subjects during moderate-intensity exercise. *Diabetes,* 49**,** 2102.

BORG, G. 1982. Ratings of Perceived Exertion and Heart-Rates during Short-Term Cycle Exercise and Their Use in a New Cycling Strength Test. *International Journal of Sports Medicine,* 3, 153-58.

BROOKS, G. A., BUTTE, N. F., RAND, W. M., FLATT, J. P. & CABALLERO, B. 2004. Chronicle of the Institute of Medicine physical activity recommendation: how a physical activity recommendation came to be among dietary recommendations. *The American Journal of Clinical Nutrition*, *79*(5), 921S-930S.

CLAUSEN, J. O., BORCH-JOHNSEN, K., IBSEN, H., BERGMAN, R. N., HOUGAARD, P., WINTHER, K. & PEDERSEN, O. 1996. Insulin sensitivity index, acute insulin response, and glucose effectiveness in a population-based sample of 380 young healthy Caucasians. Analysis of the impact of gender, body fat, physical fitness, and life-style factors. *The Journal of Clinical Investigation*, *98*(5), 1195-1209.

CORPELEIJN, E., SARIS, W. & BLAAK, E. 2009. Metabolic flexibility in the development of insulin resistance and type 2 diabetes; effects of lifestyle. *Obesity Reviews,* 10**,** 16.

CRAIG, C. L., MarshaLL, A. L., SJÖSTRÖM, M., BAUMAN, A. E., BOOTH, M. L., AINSWORTH, B. E., PRATT, M., EKELUND, U. L. F., YNGVE, A., SALLIS, J. F. & OJA, P. 2003. International physical activity questionnaire: 12-country reliability and validity. *Medicine and Science In Sports and Exercise,* 35(8), 1381-1395.

DAVIDSON, L. E., WANG, J., THORNTON, J. C., KALEEM, Z., SILVA-PALACIOS, F., PIERSON, R. N., HEYMSFIELD, S. B. & GALLAGHER, D. 2011. Predicting fat percent by skinfolds in racial groups: Durnin and Womersley revisited. *Medicine and Science in Sports and Exercise*, *43*(3), 542-549.

FRAYN, K. N. 1983. Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology,* 55, 628-634.

GALGANI, J. E., MORO, C. & RAVUSSIN, E. 2008. Metabolic flexibility and insulin resistance. *American Journal of Physiology. Endocrinology and Metabolism*, 295(5), E1009–E1017.

GERSH, B. J., SLIWA, K., MAYOSI, B. M. & YUSUF, S. 2010. Novel therapeutic concepts the epidemic of cardiovascular disease in the developing world: global implications. *European Heart Journal*, *31*(6), 642-648.

HENDERSON, G.C., 2014. Sexual dimorphism in the effects of exercise on metabolism of lipids to support resting metabolism. *Frontiers in Endocrinology*, 5, 1–8.

HØEG, L., ROEPSTORFF, C., THIELE, M., RICHTER, E. A., WOJTASZEWSKI, J. F. P. & KIENS, B. 2009. Higher intramuscular triacylglycerol in women does not impair insulin sensitivity and proximal insulin signaling. *Journal of Applied Physiology,* 107**,** 824-831.

HØEG, L.D., SJØBERG, K.A., JEPPESEN, J., JENSEN, T. E., FRØSIG, C., BIRK, J.B., BISIANI, B., HISCOCK, N., PILEGAARD, H., WOJTASZEWSKI, J. F. P., RICHTER, E. A. & KIENS, B. 2011. Lipid-induced insulin resistance affects women less than men and is not accompanied by inflammation or impaired proximal insulin signalling. *Diabetes,* 60, 64-73.

KHAN, B. B. & FLIER, J. S. 2000. Obesity and insulin resistance. *Clinics in Dermatology*, 42(4), .473–481.

KRSSAK, M., PETERSEN, K. F., DRESNER, A., DIPIETRO, L., VOGEL, S. M., ROTHMAN, D. L., SHULMAN, G. I. & RODEN, M. 1999. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a ^{1}H NMR spectroscopy study. *Diabetologia,* 42, 113-116.

KUHL, J., HILDING, A., OSTENSON, C. G., GRILL, V., EFENDIC, S. & BAVENHOLM, P. 2005. Characterisation of subjects with early abnormalities of glucose tolerance in the Stockholm Diabetes Prevention Programme: The impact of sex and Type 2 diabetes heredity. Diabetologia, 48(1), 35–40.

LUNDSGAARDS, A. M. & KIENS, B. 2014. Gender differences in skeletal muscle substrate metabolism – molecular mechanism and insulin sensitivity. Frontiers in Endocrinology, 5(195), 1-16.

MIFFLIN, M. D., ST JEOR, S. T., HILL, L. A., SCOTT, B. J., DAUGHERTY, S. A. & KOH, Y. O. 1990. A new predictive equation for resting energy expenditure in healthy individuals. *The American Journal of Clinical Nutrition*, *51*(2), 241-247.

POPKIN, B. M. 2010. Recent dynamics suggest selected countries catching up to US obesity. *American Journal of Clinical Nutrition*, 91(1), 284–288.

STORLIEN, L., OAKES, N. D. & KELLEY, D. E. 2004. Metabolic flexibility. *The Proceedings of the Nutrition Society*, 63(2), 363–368.

WANG, Y. C., MCPHERSON, K., MARSH, T., GORTMAKER, S. L. & BROWN, M. 2011. Health and economic burden of the projected obesity trends in the USA and the UK. *The Lancet*, 378(9793), 815-825.

APPENDIX 2: GENERAL HEALTH QUESTIONNAIRE (CHAPTER 2)

UNIVERSITY OF BIRMINGHAM

Title of Project: **Influence of pre- or post-exercised food intake on muscle metabolism in obesity**

Please answer the following questions. If you have any doubts or difficulty with the questions, please ask the investigator for guidance. Your answers will be kept strictly confidential.

1.	You are a.....	Man	Woman
2.	What is your exact date of birth? Day..... Month.....Year..19..... So your age is..... Years		
3.	How would you describe your ethnicity? …….. Please refer to ethnicity codes and enter a code.		
4.	When did you last see your doctor? In the: Last week..... Last month..... Last six months..... Year..... More than a year.....		
5.	Are you currently taking any prescription or non-prescription medication?	YES	NO
6.	Has your doctor ever advised you not to perform vigorous exercise?	YES	NO
7.	Has your doctor ever said you have “heart trouble”?	YES	NO
8.	Has your doctor ever said you have high blood pressure?	YES	NO
9.	Have you ever taken medication for blood pressure or your heart?	YES	NO
10.	Do you feel pain in your chest when you undertake physical activity?	YES	NO
11.	In the last month, have you had pains in your chest when not doing any physical activity?	YES	NO
12.	Has your doctor (or anyone else) said that you have raised blood cholesterol?	YES	NO
13.	Have you had a cold or feverish illness in the last month?	YES	NO
14.	Do you ever lose balance because of dizziness, or do you ever lose consciousness?	YES	NO

15.	a) Do you suffer from back pain b) If so, does it ever prevent you from exercising?	YES YES	NO NO
16.	Do you suffer from asthma?	YES	NO
17.	Do you have any joint or bone problems that may be made worse by exercise?	YES	NO
18.	Has your doctor ever said you have diabetes?	YES	NO
19.	Have you ever had viral hepatitis?	YES	NO
20.	Are you currently participating in another clinical study?	YES	NO
21.	Do you have any bleeding disorders?	YES	NO
22.	Are you aware of any known allergies to local anaesthetic (such as lidocaine)?	YES	NO
23.	Are you a current or recent smoker (last 30 days)	YES	NO
24.	Do you have a past history of substance abuse or engagement in uncommon eating practices (e.g., sustained periods of fasting)?	YES	NO
25.	Have you previously (within 5 years of the present study) had 4 or more muscle biopsies obtained from the thigh quadriceps region?	YES	NO
26.	Have you previously donated blood (last 12 weeks)?	YES	NO
27.	If you are a woman, are you pregnant?	YES	NO
29.	If you are woman, are you breastfeeding?	YES	NO
28.	Have you gained/lost ≥2kg's body mass in the past 3 months?	YES	NO
29.	Do you know of any reason, not mentioned above, why you should not exercise?	YES	NO
30	Do you perform regular physical activity?	YES	NO

APPENDIX 3: GENERAL HEALTH QUESTIONNAIRE (CHAPTER 3)

UNIVERSITY OF BIRMINGHAM

Title of Project: **Influence of pre- or post-exercise food intake on fat oxidation in obese women**

Please answer the following questions. If you have any doubts or difficulty with the questions, please ask the investigator for guidance. Your answers will be kept strictly confidential.

1	What is your exact date of birth? Day..... Month.....Year 19..... So your age is..... Years		
2	How would you describe your ethnicity? …….. *Please refer to ethnicity codes and enter a code*		
3	When did you last see your doctor? In the: Last week..... Last month..... Last six months..... Year..... More than a year.....		
4	Are you currently taking any prescription or non-prescription medication or nutritional supplements?	YES	NO
5	Has your doctor ever advised you not to perform vigorous exercise?	YES	NO
6	Has your doctor ever said you have “heart trouble”?	YES	NO
7	Has your doctor ever said you have high blood pressure?	YES	NO
8	Have you ever taken medication for blood pressure or your heart?	YES	NO
9	Do you feel pain in your chest when you undertake physical activity?	YES	NO
10	In the last month, have you had pains in your chest when not doing any physical activity?	YES	NO
11	Has your doctor (or anyone else) said that you have raised blood cholesterol?	YES	NO
12	Have you had a cold or feverish illness in the last month?	YES	NO
13	Do you ever lose balance because of dizziness, or do you ever lose consciousness?	YES	NO
14	a) Do you suffer from back pain b) If so, does it ever prevent you from exercising?	YES YES	NO NO
15	Do you suffer from asthma?	YES	NO
16	Do you have any joint or bone problems that may be made worse by exercise?	YES	NO

17	Has your doctor ever said you have diabetes?	YES	NO
18	Have you ever had viral hepatitis?	YES	NO
19	Are you currently participating in another clinical study?	YES	NO
20	Do you have any bleeding disorders?	YES	NO
21	Are you aware of any known allergies to local anaesthetic (such as lidocaine)?	YES	NO
22	Are you a current or recent smoker (last 30 days)	YES	NO
23	Do you have a past history of substance abuse or engagement in uncommon eating practices (e.g., sustained periods of fasting)?	YES	NO
24	Have you previously donated blood (last 12 weeks)?	YES	NO
25	Do you have any known food allergies or intolerances?	YES	NO
26	Are you pregnant?	YES	NO
27	Are you breastfeeding?	YES	NO
28	Have you had regular periods in the last 3 months?	YES	NO
29	Typically, how long is your menstrual cycle, from day 1 of menses/period to day 1 of the next period? _____Days		
30	Is the above time the same between periods? If no please indicate the irregularity	YES	NO
31	How many days does your menstrual (blood) flow last? ____days		
32	Number of days since the start of your last period? ____days		
33	If you take oral contraception, a) what brand and dose are you prescribed _____ _____ b) How long have you been prescribed this type of contraception? _____months		
34	Have you gained/lost ≥2kg's body mass in the past 3 months?	YES	NO
35	Do you know of any reason, not mentioned above, why you should not exercise?	YES	NO
36	Do you perform regular physical activity?	YES	NO

Ethnicity codes

1. White – British
2. White – Irish
3. Other – White background
4. Black or Black British-Caribbean
5. Black or Black British-African
6. Other black background
7. Asian or Asian British Indian
8. Asian or Asian British Pakistani
9. Asian or Asian British-Bangladeshi
10. Chinese
11. Other Asian background
12. Mixed – White and Black Caribbean
13. Mixed – White and Black African
14. Mixed – White and Asian
15. Other mixed background
16. Other ethnic background
17. Other – please specify

……

APPENDIX 4: GENERAL HEALTH QUESTIONNAIRE (CHAPTER 4)

UNIVERSITY OF BIRMINGHAM

Title of Project: **Metabolic Flexibility in Men and Women**

Please answer the following questions. If you have any doubts or difficulty with the questions, please ask the investigator for guidance. Your answers will be kept strictly confidential.

1	What is your exact date of birth? Day..... Month.....Year 19..... So your age is..... Years		
2	How would you describe your ethnicity? …….. *Please refer to ethnicity codes and enter a code*		
3	When did you last see your doctor? In the: Last week..... Last month..... Last six months..... Year..... More than a year.....		
4	Are you currently taking any prescription or non-prescription medication or nutritional supplements?	YES	NO
5	Has your doctor ever advised you not to perform vigorous exercise?	YES	NO
6	Has your doctor ever said you have “heart trouble”?	YES	NO
7	Has your doctor ever said you have high blood pressure?	YES	NO
8	Have you ever taken medication for blood pressure or your heart?	YES	NO
9	Do you feel pain in your chest when you undertake physical activity?	YES	NO
10	In the last month, have you had pains in your chest when not doing any physical activity?	YES	NO
11	Has your doctor (or anyone else) said that you have raised blood cholesterol?	YES	NO
12	Have you had a cold or feverish illness in the last month?	YES	NO
13	Do you ever lose balance because of dizziness, or do you ever lose consciousness?	YES	NO
14	a) Do you suffer from back pain b) If so, does it ever prevent you from exercising?	YES YES	NO NO
15	Do you suffer from asthma?	YES	NO
16	Do you have any joint or bone problems that may be made worse by exercise?	YES	NO

17	Has your doctor ever said you have diabetes or do you have a family history (parents) of diabetes?	YES	NO
18	Have you ever had viral hepatitis?	YES	NO
19	Are you currently participating in another clinical study?	YES	NO
20	Are you a current or recent smoker (last 30 days)	YES	NO
21	Do you have a past history of substance abuse or engagement in uncommon eating practices (e.g., sustained periods of fasting)?	YES	NO
22	Have you previously donated blood (last 12 weeks)?	YES	NO
23	Do you have any known food allergies or intolerances?	YES	NO
24	Have you gained/lost ≥2kg's body mass in the past 3 months?	YES	NO
25	Do you know of any reason, not mentioned above, why you should not exercise?	YES	NO

Women only:			
26	Are you pregnant or planning to become pregnant during 2018?	YES	NO
27	Are you breastfeeding?	YES	NO
28	Have you had regular periods in the last 3 months?	YES	NO
29	Typically, how long is your menstrual cycle, from day 1 of menses/period to day 1 of the next period? _____Days		
30	Is the above time the same between periods? If no please indicate the irregularity	YES	NO
31	How many days does your menstrual (blood) flow last? ____days		
32	Number of days since the start of your last period? ____days		
33	If you take oral contraception, a) what brand and dose are you prescribed _____ _____ b) How long have you been prescribed this type of contraception? _____months		

Ethnicity codes

18. White – British
19. White – Irish
20. Other – White background
21. Black or Black British-Caribbean
22. Black or Black British-African
23. Other black background
24. Asian or Asian British Indian
25. Asian or Asian British Pakistani
26. Asian or Asian British-Bangladeshi
27. Chinese
28. Other Asian background
29. Mixed – White and Black Caribbean
30. Mixed – White and Black African
31. Mixed – White and Asian
32. Other mixed background
33. Other ethnic background
34. Other – please specify

……

APPENDIX 5: APPETITE QUESTIONNAIRE

UNIVERSITY OF BIRMINGHAM

Please answer the following questions. If you have any doubts or difficulty with the questions, please ask the investigator for guidance. Your answers will be kept strictly confidential

How hungry do you feel?

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

How satisfied do you feel?

I am completely empty _____ I cannot eat another bite

How full do you feel?

Not at all full _____ Totally full

How much do you think you can eat?

Nothing at all _____ A lot

Would you like to eat something sweet?

Yes, very much _____ No, not at all

Would you like to eat something salty?

Yes, very much _____ No, not at all

Would you like to eat something savoury?

Yes, very much _____ No, not at all

Would you like to eat something fatty?

Yes, very much _____ No, not at all

APPENDIX 6: PHYSICAL ACTIVITY QUESTIONNAIRE (IPAQ-SF)

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ **days per week**

☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing vigorous **physical activities on one of those days?**

_____ hours per day

_____ minutes per day

☐ **Don't know/Not sure**

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week**

☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing moderate **physical activities on one of those days?**

_____ hours per day

_____ minutes per day

☐ **Don't know/Not sure**

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend walking **on one of those days?**

_____ hours per day

_____ minutes per day

☐ **Don't know/Not sure**

The last question is about the time you spent sitting **on weekdays during the** last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.**

7. During the last 7 days**, how much time did you spend** sitting **on a** week day**?**

_____ hours per day

_____ minutes per day

☐ **Don't know/Not sure**

APPENDIX 7: FOOD DIARY

PLEASE READ THROUGH THESE PAGES BEFORE STARTING YOUR DIARY

We would like you to keep this diary of everything you eat and drink over 2 weekdays and 1 weekend days (these need not be consecutive).
Please include all food consumed at home and outside the home e.g. work, college or restaurants. It is very important that you do not change what you normally eat and drink just because you are keeping this record. Please eat as you normally would do.

Time Slots
Please note the time of each eating occasion into the space provided.

What did you eat?
Please describe the foods you eat in as much detail as possible. Be as specific as you can. The example day shows the level of detail needed such as –

- Cooking methods: (fried, grilled, baked, microwaved etc.).
- Any additions (sugar/sweeteners, sauces, pepper, salt, mustard, etc.).
- Type and amount of fat / oil used for cooking, e.g. teaspoon peanut oil, 15g butter.
- What brands, e.g. Kellogg's corn flakes, places food bought, fresh, frozen, tinned, and raw.
- What variety – e.g. semi skimmed milk, low fat pro-biotic yogurt, reduced salt, reduced sugar?
- Whether soft drinks were low calorie (diet) or decaffeinated?
- Were fruit juices UHT, pasteurised or freshly squeezed, made from concentrate
- Products such as cheese, fish and meat were they smoked or not
- Meats – what part of the animal? Chicken breast? Legs? Wings? back bacon, streaky bacon, extra lean beef mince, value beef mince.

E.g. a cheese sandwich is really 3 foods – Bread, Margarine and Cheese. What type / amount of bread? Type / amount of spread? Type / amount of cheese?

Remember to record all snacks and drinks throughout the day.

Portion sizes

- Please use the kitchen weighing scales provided to weigh every item of food / drink consumed – Remember to Zero after each item of food, and check it's in grams. Eat the amount you would normally eat – don't use the scales to determine when to stop!
- When weighing is not possible, food quantities can be described using:
  - household measures, e.g. one teaspoon (tsp) of sugar, two thick slices of bread, 4 tablespoons (tbsp) of peas, ½ cup of gravy, large portion of takeaway chips. Be careful when describing amounts in spoons that you are referring to the correct spoon size.

    - use weights from labels, e.g. 4oz steak, 420g tin of baked beans, 125g pot of Yoghurt – but only if everything is eaten – did you eat the fat /skin / bones?
    - number of items, e.g. 4 fish fingers, 1 Rich Tea biscuit, 1 king size mars bar

- With foods such as fruit, remember to record the weight of the skin/core/stone in the left-overs column

For drinks, quantity can be described using:

- weight (grams), the size of glass, cup etc (e.g. large glass) or the volume (e.g. 300ml, 1 pint).
- volumes from labels (e.g. 330ml can of fizzy drink, 500ml strawberry Innocent smoothie).

We would like to know the amount that was actually eaten which means taking into account leftovers. You can do this in two ways:

1. Record what was served and note what was not eaten e.g. 30g of peas, only 12g eaten; 1 Weetabix, ate ½
2. Only record the amount actually eaten i.e. 18g of peas; ½ Weetabix

Homemade dishes

If you have eaten any homemade dishes e.g. chicken casserole, please record the name of the recipe, ingredients with amounts (including water or other fluids) for the whole recipe, the number of people the recipe serves, and the cooking methods used. Write this down in the Recipe/Takeaways section at the end of the main diary. Record how much of the whole recipe you have eaten in the amounts column of the main diary.

Take-aways and eating out

If you have eaten a take-away or eaten dishes not prepared at home such as at a restaurant or a friend's house, please record as much detail about the ingredients as you can in the Recipes/Takeaways section at the end of the main diary e.g. vegetable curry containing chickpeas, aubergine, onion and tomato. Please also record the name of the restaurant, takeaway, and the name of the meal, e.g. Domino's pizza, 16inch meat feast, thin base.

Brand name

Please note the brand name (if known). Most packed foods will list a brand name, e.g. Bird's eye, Hovis, or Supermarket own brands. For ready-made meals or for less well known brands, please keep the packet's nutritional information in the bag provided.

Supplements / Medications

Please also provide information about any supplements you took. Please record the brand name, full name of supplement, strength and the amount taken should be recorded, e.g. Maximuscle cyclone powder – 40g (2 scoops), Holland and Barret Cod Liver Oil and Glucosamine Capsules (500mg) – 1 capsule.

If you take any new medicines or stop taking ones we know about please record it here.

Was it a typical day?

After each day of recording please add some notes to tell us whether this was a typical day or whether there were any reasons why you ate and drank more or less than usual. E.g. Drank 4 pints of Guinness as it was St Patricks day, day 2 ate very little as not feeling well.

When to fill in the diary
Please record you are eating as you go, not from memory at the end of the day. Use written notes if you forget to take your diary with you and fill out your diary ASAP. Each diary day covers a 24hr period, so please include any food or drinks that you may have had during the night. Remember to include foods and drinks between meals (snacks) including water.

Overleaf you can see an example day that has been filled in. These examples show you how we would like you to record your food and drink, and how to record a homemade dish and a takeaway.

Please document what you ate & drank in as much detail as possible, remember If it has passed your lips record it! ☺

It only takes a few minutes for each eating occasion!

We thank you for your efforts in filling out this diary.

Example day

Date: Tuesday 15th December 2015

Time	Item / Description	Brand / variety	Preparation	Amount	Left-overs
07.00	Coco pops	Asda		82g	
	Raisins	Tesco value		27g	
	Skimmed Milk	Asda		322g	
	Pure Orange Juice, not from concentrate	Tropicana – with the bits		1 large Glass - 457g	
09.15	Crunchy Granola Oats and Honey flavour Cereal Bar	Nature Valley		1 bar – 42g	
	Mug of strong tea	Tetley	1 bag Water Sugar Semi skimmed milk	 220g 1 teaspoon 27g	
10.30	Apple	Golden Delicious	Raw	120g	17g core
	Pear	Conference	Raw	101g	10g core
12.00	Humus Sandwich	Home made			
	Bread (Tesco the finest)	Tesco, sliced, multigrain		2 slices	
	Tomato	On the vine		84g	
	Lettuce	Iceberg		35g	
	Chicken Sandwich		Previously roasted leftover chicken breast meat, no skin	105g	
	Butter	Anchor		Thin layer	
	Cucumber	Market		47g	
	Water	Tap		1 pint	
	Mug of strong tea – Same as above	Same as above			
14.30	Can of Coca-Cola zero	Coca-Cola		1 can 330ml	
15.45	Grande Latte	Starbucks	Extra shot of coffee + vanilla syrup	Grande size	

15:45	Chocolate Brownie	Starbucks		154g	
16.30	Water	Tap		1 pint	
18.30	Pizza - meat feast	Big Joe's – see recipe	Takeaway	Half of 16 inch	
18.45	Diet Lemonade	Aldi		1.5pints	
20.00	Beer normal strength	Carlsberg (5%)		1 pints	
20.20	Beer normal strength	Carlsberg (5%)		1 pints	
20.45	Beer normal strength	Carlsberg (5%)		1 pints	
21.20	Beer normal strength	Carlsberg (5%)		1 pints	
22.00	Red-bull, normal	(not diet)		1 glass	
	Vodka	Smirnoff		Double measure	
22.30	Toast (Tesco the finest)	Tesco, sliced, multigrain	toast	2 slice	
	Baked beans – reduced sugar.	Tesco	Microwave	420g	
	Chilli Sauce	Nandos extra hot peri peri sauce		3 desert spoons	
22.45	Water	Tap water		1 pint	

*Notes: This day I drank more alcohol than normal as it was a colleagues birthday

Recipes / Takeaways
Write in recipes of ingredients of homemade dishes or take-aways

Name of Dish Fairy Cakes	Serves: makes 20 cakes
Ingredients – Description, Brand,	Amounts
Tate & Lyle caster sugar	175g
Anchor butter, unsalted	175g
ASDA, free range eggs	3 eggs, 172g (no shell)
ASDA Self raising flour	175g
Co-op Baking powder	1 teaspoon
Silver Spoon Icing Sugar	140g
Water	10ml
Description of cooking method Mix all ingredients (1-5) together, then separate into 20 equal portions in cup-cases, cook in oven for 15mins Mix ingredients 6 and 7, pour on top of individual cakes after they are cooked and cooled.	

Name of Dish Big Joe's 16" Meat Feast pizza	Serves: 2 equal portions
Ingredients	Amounts
Deep pan pizza base	16 inch, weight unknown
Tomato Sauce based	1 ladle
Green peppers	Half green bell pepper
Spicy salami	~ 12 large slices
Pepperoni	~ 20 small slices
Tandoori chicken pieces	~ half chicken breast
Beef meatballs	6 small meatballs
Onion	Half
Description of cooking method Oven cooked	