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.

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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.

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

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