EFFECTS OF EXERCISE ON POSTPRANDIAL METABOLISM, APPETITE RESPONSES, AND FEEDING BEHAVIOUR

by

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Abstract

Exaggerated metabolic perturbations during the postprandial period are likely to play a role in the development of vascular and metabolic diseases. Elevated levels of postprandial triglycerides (TG) are associated with increased risk for atherosclerosis independently of other cardiovascular risk factors, and exaggerated postprandial insulin excursions are known to contribute to lipid dysmetabolism and chronic insulin resistance. This, together with the fact that free-living humans spend most of their time in the postprandial state, suggests that interventions focusing on the improvement of postprandial metabolism could play a role in the prevention and management of cardiovascular and metabolic diseases. Exercise has a potent role in improving postprandial metabolism, by effectively attenuating postprandial lipaemia and insulinemia, as well as increasing fat oxidation, all of which providing positive outcomes for the prevention and treatment of metabolic disorders. It is however unclear the extent to which these beneficial effects of exercise persist when food is consumed *ad libitum*. In addition, the effects of exercise on appetite regulation and food intake require further elucidation. It is possible that exercise may provoke compensatory adaptations in food intake in an effort to restore energy balance, through physiological and/or behavioural responses. This has implications for the efficacy of exercise in the regulation of a healthy body weight. Therefore, the overall aim of this thesis is to describe the effects of exercise on postprandial metabolism, appetite responses and feeding behaviour in overweight/obese men.

The first two experimental chapters of this thesis (Chapters 3 and 4) aimed to investigate the effects of single *vs.* repeated exercise sessions (~700 kcal per session) on postprandial metabolism, energy intake, appetite and gut peptide responses in response to *ad libitum* feeding. Ten sedentary, overweight/obese men underwent: i) no-exercise control; ii) one exercise session (Day 3); and iii) three exercise sessions over three consecutive days (Days 1-3); prior to a 7-h metabolic assessment day (Day 4). Energy substrate utilisation, postprandial TG, insulin, acylated ghrelin, PYY_{3-36} as well as appetite responses and *ad-libitum* energy intake (breakfast, lunch, dinner) were determined. The findings of this study showed that the beneficial effects of a single exercise session on postprandial metabolism on postprandial metabolic responses persisted when meals were consumed *ad libitum*, but were not augmented by inducing a larger energy deficit by exercising on

consecutive days. Furthermore, while a single exercise session did not elicit compensatory responses in appetite and energy intake, exercising on consecutive days led to a partial compensation (~24%) in energy intake as well as increased hunger sensation. Gut peptide responses were unaltered by exercise.

The next chapter (Chapter 5) aimed to determine the effects of exercise timing relative to meal ingestion on postprandial metabolism, appetite responses, and *ad libitum* energy intake. Ten, sedentary overweight men exercised for an hour (~400 kcal) before or after consuming a standardised breakfast meal, followed by an 8.5 h metabolic assessment period. Energy substrate utilisation, postprandial TG, insulin, as well as appetite responses and *ad-libitum* energy intake (lunch, dinner) were determined. The findings indicated that exercise performed prior to a breakfast meal and exercise performed after a breakfast meal waas similarly beneficial in improving postprandial metabolism. Exercise timing relative to meal ingestion also did not influence appetite responses and *ad libitum* energy intake.

In the final experimental chapter (Chapter 6), a pilot study was designed to examine the effects of acute exercise on non-metabolic factors related to appetite using a computer-based assessment. Twenty-seven men and women walked for an hour on the treadmill or rested on a control day. Appetite-related measures were assessed before and immediately after exercise, and hourly for 2 hours post exercise. The findings showed that an acute bout of moderate intensity exercise had an anorexigenic effect; characterised by diminished hunger and lower prospective food intake (ideal portion size) compared to no exercise. Although not a primary aim, this study discovered a novel association between loss aversion and prospective food intake and food liking.

The collective findings of this thesis suggest that exercise attenuates postprandial TG and enhances fat oxidation in response to *ad libitum* feeding, indicating that exercise's benefits can be extended into the 'real world' setting. The beneficial effects of exercise on postprandial metabolism are also independent of its timing relative to meal ingestion. In line with evidence in the literature, an acute bout of aerobic exercise does not induce compensatory responses in terms of energy intake and increased appetite, supporting the role of exercise in weight management. Other than physiological factors, the behavioural and cognitive aspects related to feeding can play a role in mediating compensatory responses to exercise and this requires further investigation.

Table of Contents

List of Figures

List of Tables

Acknowledgement

"When eating bamboo sprouts, remember the man who planted them.."

- *Chinese Proverb*

Autumn 2007. From the warm, sunny skies of Kuala Lumpur, to the cold-wet and overcast Glasgow. Back then, completing this PhD journey seemed beyond the bounds of any possibility. And yet, 3 years and 6 months later, here I am. During the time, I have worked with a great number of people whose contributions in various ways to the research and the making of this thesis deserved special mention.

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Author's Declaration

Unless otherwise stated by acknowledgment or reference to published literature, the presented work in this thesis is the author's own, as approved by the Thesis committee and the Graduate Office and has not been submitted for a degree at another institution.

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List of Abbreviations

AgRP	agouti-related peptide
AMPK	5' adenosine monophosphate-activated protein
Apo	apolipoprotein
ARC	arcuate nucleus
AUC	area under curve
CCK	cholecystokinin
CHD	coronary heart disease
CVD	cardiovascular diseases
DDP-IV	dipeptidyl-peptidase IV
DEBQ	Dutch Eating Behaviour Questionnaire
EE	energy expenditure
ELISA	enzyme-linked immunosorbent assay
GHSR-1a	growth hormone secretagogue receptor 1a
GLP-1	glucagon-like peptide-1
GLUT	glucose transporter
HDL	high density lipoprotein
HSL	hormone-sensitive lipase
$HOMA_{IR}$	homeostasis model of assessment - insulin resistance
IMTG	intramuscular triglyceride
KIU	kallikrein unit
LDL	low density lipoprotein
LPL	lipoprotein lipase
NEFA	non-esterified fatty acids
NPY	neuropeptide-Y
PMSF	phenylmethylsulfonyl fluoride
POMC	pro-opiomelanocortin
PYY	peptide YY
PVN	paraventricular nucleus
RER	respiratory exchange ration
RIA	radioimmunoassay
RMR	resting metabolic rate
RPE	ratings of perceived exertion

SD	standard deviation
SEM	standard error of mean
STPD	standard temperature pressure dry gas
TAUC	time-averaged area under curve
TFEQ	Three Factor Eating Questionnaire
TG	triglyceride
TRL	triglyceride-rich lipoprotein
T2D	type 2 diabetes
VLDL	very low density lipoprotein
$\dot{V}O_2$	rate of oxygen consumption
$\dot{V}CO_2$	rate of carbon dioxide production
$\dot{V}O_2max$	maximal oxygen consumption

“Acquire knowledge. It enables its possessor to distinguish right from wrong; it lightens the path to Heavens; it is our friend in the desert, our society in solitude, our companion when friendless; it guides us to happiness; it sustains us in misery; it is an ornament among friends, and an armour against enemies..”

- Prophet Muhammad PBUH

"If we could give every individual the right amount of nourishment and exercise, not too little and not too much, we would have found the safest way to health…"

- Hippocrates

CHAPTER 1

General Introduction

The following introductory chapter has been divided into several main sections. The first section primarily focuses on vascular and metabolic diseases, and how physical activity plays a role in minimising the risks. The next section addresses lipoprotein metabolism and associated implications of the postprandial state in the development and progression of cardiovascular diseases and other metabolic disorders. This section concludes by focussing on the beneficial role of exercise in postprandial lipid metabolism. The next section introduces the concept of energy balance and factors which influence it, with a focus on fat balance and exercise. Regulatory mechanisms for appetite and feeding behaviour are then discussed, including how exercise affects these elements. The final section addresses issues relating to experimental methods used in the appetite-related investigations.

1.1 Vascular and Metabolic Diseases

Cardiovascular and metabolic diseases are one of the leading causes of chronic disease morbidity and mortality in developed countries and are becoming increasingly prevalent in developing countries. Although these conditions are often considered to be diseases of affluence, the burdens of hypertension, diabetes mellitus, obesity, and dyslipidaemia are now becoming more common among the poorest citizens in the industrialised countries (Dahlöf 2010). Recent statistics from the British Heart Foundation stated that cardiovascular diseases (CVD) are the main causes of death in the UK, almost 191,000 deaths each year, with majority are from coronary heart disease (CHD), followed by stroke, and further deaths from other circulatory diseases (Scarborough *et al.* 2010). Apart from CVD, diabetes is also becoming the one of the biggest health challenges facing the UK today. Since 1996, the number of people diagnosed with diabetes has increased from 1.4 million to 2.6 million cases. According to forecasts, it is estimated that over 4 million people in the UK will be diagnosed with diabetes by 2025, with the majority of these cases being type 2 diabetes (T2D), due to the increasing ageing population and rapidly rising numbers of overweight and obese individuals (Diabetes UK 2010).

1.1.1 Physical Activity, CVD and Diabetes

Exercise, either alone or in combination with diet, is fundamental in the prevention and management of cardiovascular and metabolic diseases. The link between physical activity and the protection against heart disease was first studied by Morris and his colleagues in the 1940s, who found that male conductors of London buses had lower annual total incidence of CHD compared to their driver colleagues (Morris *et al.* 1953). Since the initial observations of Morris *et al*, there has been substantial evidence, particularly from epidemiological studies (*e.g.* Li *et al.* 2006; Oguma & Shinoda-Tagawa 2004; Davey et al. 2000; Sesso *et al.* 2000), to support an inverse relationship between physical activity and CHD/CVD risk. A comprehensive meta-analysis of 33 prospective cohort studies with a total of 883 372 participants, with follow-ups ranging from 4 to 20 years have shown that physical activity is associated with risk reductions of 30-50% for cardiovascular mortality and 20-50% for all-cause mortality in both men and women, with pooled risk reductions of 35% for the former and 33% for the latter, even after adjusting for important risk factors such as hypertension, hypercholesterolaemia, and diabetes (Nocon *et al.* 2008). In addition, numerous studies have documented the importance of engaging in exercise-based interventions to attenuate or reverse the disease process in patients with CVD. For instance, a systematic review and meta-analysis of 48 clinical trials revealed that exercise-based cardiac rehabilitation significantly reduced the incidence of all-cause and cardiovascular mortality, compared with usual care (Taylor *et al.* 2004). Improvements in functional capacity and quality of life such as increment in maximal oxygen consumption have also been observed in patients with heart failure who participated in exercise training (Smart & Marwick (2004).

Apart from reducing cardiovascular risks, there is ample evidence from prospective cohorts reporting a consistent link between the protective effect of physical activity and the development of T2D (*e.g.* Villegas *et al.* 2006; Hsia *et al.* 2005; Perry *et al.* 1995; Manson *et al.* 1992; Manson *et al.* 1991), with regular physical activity presenting a 20-30% reduction in risk after adjustment for confounding factors including age, health status including family history of diabetes and BMI (Gill & Cooper 2008). In addition to epidemiological data, at least five major clinical trials have demonstrated that lifestyle interventions can significantly delay or possibly prevent the onset of T2D (Ramachandran *et al.* 2006; Knowler *et al.* 2002; Tuomilehto *et al.* 2001; Pan *et al.* 1997; Eriksson & Lindgärde 1991). The Da Qing study, which involved over 110,000 men and women with IGT in China, showed a 31% reduction in risk of developing diabetes by diet intervention,

a 46% reduction by exercise intervention, and a 41% reduction for the combined diet and exercise intervention after 6 years (Pan *et al.* 1997). In the Diabetes Prevention Program which studied 3234 subjects of various races, lifestyle intervention (diet and physical activity) had a 58% reduction in risk of developing diabetes, compared to 31% reduction in the medication group, regardless of race or age (Knowler *et al.* 2002). For improvement of cardiovascular risk factors and weight management, at least 150 minutes of moderate intensity (approximately 40 – 60% of $\dot{V}O_2$ max) of physical activity per week or at least 75 minutes of vigorous aerobic exercise per week, has been recommended by the American College of Sports Medicine (Haskell *et al.* 2007) and the American Heart Association (Buse *et al.* 2007), as well as by the panel of experts of the British Association of Sport and Exercise Sciences (BASES) (O'Donovan *et al.* 2010). The basis for this recommendation is supported by data from diabetes prevention trials (Ramachandran *et al.* 2006; Knowler *et al.* 2002; Tuomilehto *et al.* 2001) which showed that increasing moderate physical activity by approximately 150 minutes per week, reduced incidence of diabetes in men and women with impaired glucose tolerance (IGT), with this effect being greater if accompanied by weight loss (Gill & Cooper 2008). Accumulated bouts of physical activity over the day as opposed to one continuous session may also be an effective way to achieve recommended guidelines of a 30-min activity per day, as a meta-analysis revealed that active commuting in daily living which incorporates walking and cycling is associated with an overall 11% reduction in cardiovascular risk (Hamer & Chida 2008). Furthermore, evidence also suggest that this recommendation does not represent a minimum threshold level for risk reduction, especially among those with very low levels of physical activity or who are unfit, even smaller amounts of physical activity may be associated reductions in with CHD/CVD risk, thus conveying a "some is good; more is better" message (Shiroma & Lee 2010).

1.1.2 Mechanisms of Physical Activity on Cardiovascular Diseases and Type 2 Diabetes Risks

Physical activity and fitness clearly reduces the risk of CVD. However, the precise mechanisms through which physical activity lowers CVD risk are not fully understood. Even after traditional cardiovascular risk factors such as hypertension (Paffenbarger *et al.* 1986), body weight (Bijnen *et al.* 1998), and diabetes (Mora *et al.* 2007) are accounted for, the inverse relation between physical activity and CVD risk persists. The magnitude of the exercise effect is influenced by characteristics of the exercise intervention, individual variation, and whether exercise produces concomitant reductions in body

weight. In general, the effect of exercise on atherosclerotic risk factors is substantially less than that achieved by pharmacological therapies, and can be significantly augmented by other lifestyle changes such as changes in dietary habit and weight loss (Thompson *et al.* 2003). One of the potential mechanisms mediating the cardioprotective effects of exercise is improved endothelium-dependent vasodilation that is believed to be the result of increased shear stress over the endothelium during exercise training bouts (Whyte & Laughlin 2010). The increase laminar flow brought on by regular exercise stimulated release of vasoactive substances such as nitric oxide and prostacyclin which decreases endothelium permeability to plasma lipoproteins as well as adhesion of leukocytes, and inhibits endothelial smooth muscle cell proliferation and migration, thus playing a role in the prevention of atherogenesis (Pan 2009). Regular exercise has also been shown to exert anti-inflammatory effects, with reductions in inflammatory markers such as C-reactive protein (CRP) and soluble intercellular adhesion molecules (ICAM) (Kasapis & Thompson 2005; Adamopoulos *et al.* 2001). Some of the protective effects of exercise are due to autonomic nervous system adaptations such as enhanced peripheral baroreflex function, such as reciprocal reduction in sympathetic activity and increased parasympathetic activity (Joyner & Green 2009). Many randomised controlled trials have also reported significant reduction on resting blood pressure with exercise training (Fagard 2001). With regards to lipid changes, exercise interventions have been associated with reductions in TG, particularly in the postprandial state (Petit & Cureton 2003) and increase in HDL-C concentrations (Halverstadt *et al.* 2007; Kodama *et al.* 2007; Durstine *et al.* 2001). Although the effect of exercise on LDL concentrations are somewhat inconsistent (Kelley & Kelley 2006; Kelley *et al.* 2005), the favourable effects are rather related to changes in LDL size and compositional characteristics. Exercise training has been associated with increase in peak LDL particle size which promotes a shift in the distribution of cholesterol carried by LDL from a smaller, denser particle ($\leq$25.5 nm) to a larger (>25.5 nm), more cholesterol-rich LDL particle, thereby rendering the particles less atherogenic (Halverstadt *et al.* 2007; Beard *et al.* 1996). Details of these mechanisms will be discussed in a separate section.

Physical activity also reduces insulin resistance, improves insulin sensitivity and reduces postprandial hyperglycaemia (Thompson *et al.* 2001). Evidence has shown that both acute and chronic exercise enhanced glucose uptake and utilisation (Dela *et al.* 2006; Giacca *et al.* 1998; Rogers *et al.* 1988) and glycogen storage (Praet *et al.* 2008; Dela *et al.* 2006; Christ-Roberts *et al.* 2004; Perseghin *et al.* 1996) in both normoglycaemic and diabetic subjects. This effect may be related to evidence showing that exercise acutely promotes

the translocation of GLUT4, the insulin-regulated glucose transporter, to the plasma membrane in skeletal muscle in both healthy and diabetic individuals (Taniguchi *et al.* 2000; Kennedy *et al.* 1999). Furthermore, upregulation of GLUT4 protein expression content in the skeletal muscle has been shown to be induced by training (O'Gorman *et al.* 2006; Christ-Roberts *et al.* 2004; Hughes *et al.* 1993). Whole-body insulin sensitivity during euglycaemic-hyperinsulinaemic conditions (Kirwan *et al.* 2009; Winnick *et al.* 2008) and reduced secretion of hepatic glucose production have also been observed with chronic exercise in T2D patients (Kirwan *et al.* 2009, Segal *et al.* 1991). Enhanced insulin-mediated glucose metabolism with exercise training can be further attributed to the increased expression/activity of key signalling proteins involved in insulin signal transduction in skeletal muscle (Hawley & Lessard 2008), such as 5'-AMP-activated protein kinase (AMPK) (Sriwijitkamol *et al.* 2007) and the protein kinase B (Akt) substrate AS160 (Treebak *et al.* 2009; Frøsig *et al.* 2007) in diabetic individuals. Additionally, oxidative metabolism in skeletal muscles is improved in the obese and insulin-resistant individuals with regular exercise due to increase in muscle fibre size (Wang *et al.* 2009), mitochondrial content (Röckl *et al.* 2008; Bruce *et al.* 2006), and fatty acid transporters such as FAT/CD36 (Scheck & Horowitz 2006) and carnitine palmitoyltransferase complexes (Bruce *et al.* 2006), all of which are consequently associated with increased fatty acid oxidation in the skeletal muscle.

1.2 Obesity: a Risk Factor for Cardiovascular Diseases and Type 2 Diabetes

The epidemic of obesity took off from the 1980s and is now treated as a major public health problem around the world (James 2008). According to the recent health survey in the UK, the average prevalence of overweight and obesity (BMI > 25) is 66.1% in men and 57.5% in women, while the prevalence of obesity (BMI > 30) alone is 24.8% in men and 25.3% in women (Scottish Health Survey 2010). The UK's Foresight analysis projected that 60% and 40% of men and women respectively will be clinically obese by year 2050 (James 2008). Obesity is clearly associated with increased mortality and adverse health outcomes, especially CVD and T2D (Poirier *et al.* 2006; Yusuf *et al.* 2006). In a study of 5,881 Framingham Heart Study participants, Kenchaiah *et al.* (2002) showed that during a 14-year follow-up, for every 1 $kg \cdot m^2$ increment in BMI, the risk of heart failure increased 5% in men and 7% in women. An integrated analysis of 33 cohorts from the Asia-Pacific region, followed for an average of 7 years, showed that the risk of

CVD, particularly ischaemic stroke and ischaemic heart disease, increases progressively with higher BMI (Ni Mhurchu *et al.* 2004). The British Whitehall study, with over 18,000 men followed up for up to 35 years, showed that all cause and ischaemic heart disease mortality rates were increased in the overweight/obese group (Batty *et al.* 2006). The Nurses' Health Study of 88 393 women followed for 20 years, reported that even a modest weight gain (4 - 10 kg) during adulthood was associated with 27% increased risk of CHD compared with women with a stable weight after adjusting for physical activity and other cardiovascular risk factors (Li *et al.* 2006). In a large meta-analysis involving 302,296 participants worldwide, adverse effects of overweight on blood pressure and cholesterol levels account for about 45% of the increased risk of CHD, and 16% increased risk after adjustments of both factors (Bogers *et al.* 2007).

Large cohort studies in men and women have shown that the development of T2D is strongly associated with increased BMI (Oguma *et al.* 2005; Wang *et al.* 2005; Weinstein *et al.* 2004; Folsom *et al.* 2000; Carey *et al.* 1997; Chan *et al.* 1994). For example, men with BMI > 35 $kg \cdot m^{-2}$ had an age-adjusted risk of 42 times greater than men with BMI between 23 $kg \cdot m^{-2}$ - 35 $kg \cdot m^{-2}$ for developing diabetes (Chan *et al.* 1994). A similar trend was observed in female nurses with a BMI > 35 $kg \cdot m^{-2}$, with the risk increasing to a staggering 93% of developing T2D (Carey *et al.* 1997). Moreover, it was found that those who have been at a of BMI > 30 $kg \cdot m^{-2}$ for more than 10 years possessed twice the risk of T2D compared with those who have been obese for less than 5 years (Chan *et al.* 1994). A prospective study from the Asia Pacific regions including 154,989 participants with an average of 8 years follow-up, found an association between baseline BMI and risk of diabetes, with each 2 $kg \cdot m^{-2}$ lower body mass index associated with 23-30% lower risk of diabetes (Ni Mhurchu *et al.* 2006). In most of these studies, the impact of waist circumference and waist-to-hip ratio were independent of BMI, suggesting that abdominal obesity may play a role in the pathogenesis of insulin resistance (Guh *et al.* 2009). Besides being the cornerstone of diabetes management, weight control can serve to lower the risk of CVD among diabetic individuals. Other cohort studies have also reported strong positive associations between increasing BMI and CVD and mortality in patients with IGT/T2D (Eeg-Olofsson *et al.* 2009; Ridderstråle *et al.* 2006; Batty *et al.* 2007; Cho *et al.* 2002). Interventions aimed at reducing obesity therefore appear to be a primary goal in the prevention of CVD and T2D.

1.2.1 Mechanisms by which Obesity Influences Risk Factors

Adipose tissue plays a major role in maintaining metabolic functions by buffering the postprandial influx of fatty acids, a function which is often disturbed in obesity (Rogge 2009; Goossens 2008). For a given degree of obesity, abdominal obesity confers a greater risk of insulin resistance, T2D, and CVD than gluteofemoral obesity (Zoeller 2007). Upper body adipocytes, as in abdominal obesity, have been shown to respond more readily to stimulation of lipolysis compared to lower body adipocytes (Jensen 1997), in part due to their complement of adrenergic receptors (Kahn & Flier 2000). This would increase intraportal free fatty acids (FFA) levels and flux, promotes FFA uptake by the liver and consequently, increased hepatic secretion of apo B-100 and accelerates synthesis of hepatic very low density lipoproteins (VLDL) levels, leading to hypertriglyceridaemia (Bamba & Rader 2007). Increased availability and uptake of fatty acids, together with diminished mitochondrial oxidative enzyme capacity in obese individuals (Kelley *et al.* 2002), as well as chronic imbalances between uptake and oxidation of fatty acids ultimately result in excess intracellular lipid accumulation, both at the whole body level and in individual organs or tissues (Shulman 2000). Obesity is also associated with diminished responsiveness of lipoprotein lipase (LPL), a key enzyme in the regulation of lipid metabolism (Wang & Eckel 2009). Abnormalities in LPL function have been found to be associated with several pathophysiological conditions, including atherosclerosis and dyslipidaemia, associated with obesity and insulin resistance (Mead *et al.* 2002).

Impaired responsiveness of skeletal muscle to insulin is a primary condition in obesity and a precondition for the onset of T2D. An important mediator in contributing to the pathogenesis of insulin resistance in obesity is elevated circulating FFA (Boden 1997). There have been a strong association of obesity and insulin resistance with high circulating FFA levels (Savage *et al.* 2007; Boden & Shulman 2002). Conversely, lowering plasma fatty acids for 1 week with acipimox in subjects with T2D reduced intramuscular long chain acyl-CoA and improved insulin sensitivity (Bajaj *et al.* 2005). Furthermore, failure of adipose tissue to respond to the antilipolytic effect of insulin in insulin resistant state causes non-adipose tissues such as skeletal muscle, liver and the pancreatic ß-cell to be subjected to an increased influx of FFA (Savage *et al.* 2007), leading to triglycerides (TG) accumulation and diacylglycerols, an intermediate product from the synthesis of TG (Itani *et al.* 2002), as well as other lipotoxic fatty acid derivatives such as ceramides and long chain acyl-CoA (Adams *et al.* 2004; Yu *et al.*

2002). These metabolites can interfere with insulin-signalling pathways, consequently reducing insulin-stimulated glucose uptake in tissues (Venables & Jeukendrup 2009). Lipotoxicity in pancreatic β-cell can contribute to mechanisms underlying β-cell dysfunction and apoptosis, further leading to insulin resistance and eventual failure of insulin secretion (Mcgarry 2002). In addition, enhanced inflammatory and thrombotic cytokines expression and secretion by intra-abdominal adipocytes such as tumor necrosis factor-alpha (TNF-alpha) (Rydén & Arner 2007), monocyte chemoattractant protein-1 (MCP-1) (Sell *et al.* 2006) and C-reactive protein (Brooks *et al.* 2010; Lemieux *et al.* 2001) are implicated in the impairment of insulin signalling pathways, thus contributing to insulin resistance as well as vascular inflammation (Kahn & Flier 2000).

1.2.2 Atherogenic Lipoprotein Phenotype

Abdominal obesity and insulin resistance are inter-related risk factors for atherosclerosis (Frayn 2002), and dyslipidaemia is one of the common mechanisms by which obesity and insulin resistance relates to CVD. The dyslipidaemia associated with obesity and insulin resistance is termed the '*atherogenic lipoprotein phenotype*' (Rizzo & Bernais 2005; Austin *et al.* 1990). Atherogenic lipoprotein phenotype or 'lipid triad' is characterised by elevated plasma concentrations of TG in the fasted state, an exaggerated postprandial rise in plasma TG, low HDL concentrations, and the predominance of small, cholesterol ester-depleted, dense LDL (Hardman 1999). Evidence from epidemiologic studies suggests that the co-occurrence of low HDL-C and elevated TG levels is a strong risk factor for CHD (Jeppesen *et al.* 1997; Assmann & Schulte 1992). Furthermore, individuals with small, dense LDL particles, designated as LDL subclass pattern B, exhibit higher risk for CHD relative to individuals with predominantly large and buoyant LDL particles (subclass pattern A) (Krauss 2001). It has been suggested that the clinical importance of the atherogenic lipoprotein phenotype (ALP) outweighs that of LDL-cholesterol, because many patients with CHD are found to have the ALP trait than hypercholesterolaemia (Sattar *et al.* 1998; Superko 1996). The small and dense LDL phenotype can also be an additional fasting marker of an exaggerated postprandial lipeamia and of an impaired clearance of triglyceride-rich lipoproteins (Lemieux *et al.* 2000). Many clinical studies have shown that the magnitude and duration of postprandial lipaemia is positively related to the pathogenesis and progression of CHD and CVD (Roche & Gibney 2000), and is a prominent feature in obesity and insulin resistance (Hardman 1999). The mechanisms and adverse effects of elevated postprandial lipaemic response will be discussed in detail in section 1.4.

1.2.3 Obesity and Physical Activity

Obesity and physical inactivity are established risk factors for cardiovascular and T2D comorbidities (Warburton *et al.* 2006; Sullivan *et al.* 2005). Findings from epidemiological studies have consistently shown that regular physical activity prevents unhealthy weight gain and obesity (Lee *et al.* 2010; Saris *et al.* 2003; Fogelholm & Kukkonen-Harjula 2000). In addition, randomised controlled trials lasting 4 – 25 months and consisting of 3 – 5 exercise sessions of 30 – 60 min per week have shown that body weight and fat mass are reduced with exercise training in overweight/obese individuals, without dietary restrictions (Ross *et al.* 2004; Slentz *et al.* 2004; Jeffery *et al.* 2003; Andersen *et al.* 1999). Because obesity is strongly associated with CVD and T2D, weight loss is therefore recommended in the management of the risk factors. Much evidence have demonstrated that lifestyle modifications focusing on weight loss through dietary modification and increased physical activity are effective in reducing the progression from IGT to T2D and in reducing CVD risk factors (Horton 2009). However, it is also important to acknowledge that overweight and obese adults may benefit from significant improvements in health-related outcomes through physical activity independent of weight loss. Even in the absence of changes in body weight, the benefits of physical activity in improving CVD and T2D risk factors in the overweight/obese can be observed, such as lower blood pressure (King *et al.* 2009; Fagard 1999), increased HDL and decreased TG levels (Zois *et al.* 2009), increased insulin sensitivity (Boulé *et al.* 2005; Tokmakidis *et al.* 2004), and reduced hepatic and visceral fat (van der Heijden *et al.* 2010; Johnson *et al.* 2009).

There is a current interest in whether higher levels of physical fitness can ameliorate the increased risk for premature mortality or CHD/CVD associated with being overweight or obese (Fogelhom 2010). In an 8-year follow-up of 21 925 men in the Aerobics Center Longitudinal Study, Lee *et al.* (1999) found that men with low physical fitness (maximal aerobic power ~ 8.7 METs) had a higher risk of all-cause and CVD mortality than did fit men in all body fatness and fat-free mass categories. Moreover, unfit, lean men also had a higher risk of all-cause and CVD mortality than did men who were fit and obese (Lee *et al.* 1999). Contrary to Lee *et al.*'s earlier finding, data from the Nurses' Health Study found that the relative risks of CHD were greater for women who were active but obese, than normal-weight, sedentary women (Li *et al.* 2006). Based on evidence to date, higher levels of physical fitness appear to be able to offset the increased risk of CVD associated with being overweight or obese, with the highest risks were observed among subjects who

were both inactive and overweight/obese (Fogelholm 2010; Gill & Malkova 2006). However, in T2D, the data are more consistent in showing that being overweight or obese is associated with far greater increases in risk of developing T2D than being unfit or being inactive, and that higher levels of physical fitness or activity do not fully ameliorate the increase in risk of diabetes associated obesity (Fogelholm 2010; Siegel *et al.* 2009; Weinstein *et al.* 2004). One of the acute benefits of physical activity in obesity is by improving the lipid and lipoprotein profile. Given that perturbations in postprandial lipid metabolism can influence the atherogenic disease process by a number of different mechanisms (described in section 1.4 below) (Karpe 1999), interventions focused on reducing exaggerations postprandial lipaemia are justified. The following section of this thesis will focus on lipoprotein metabolism and how exercise influences this.

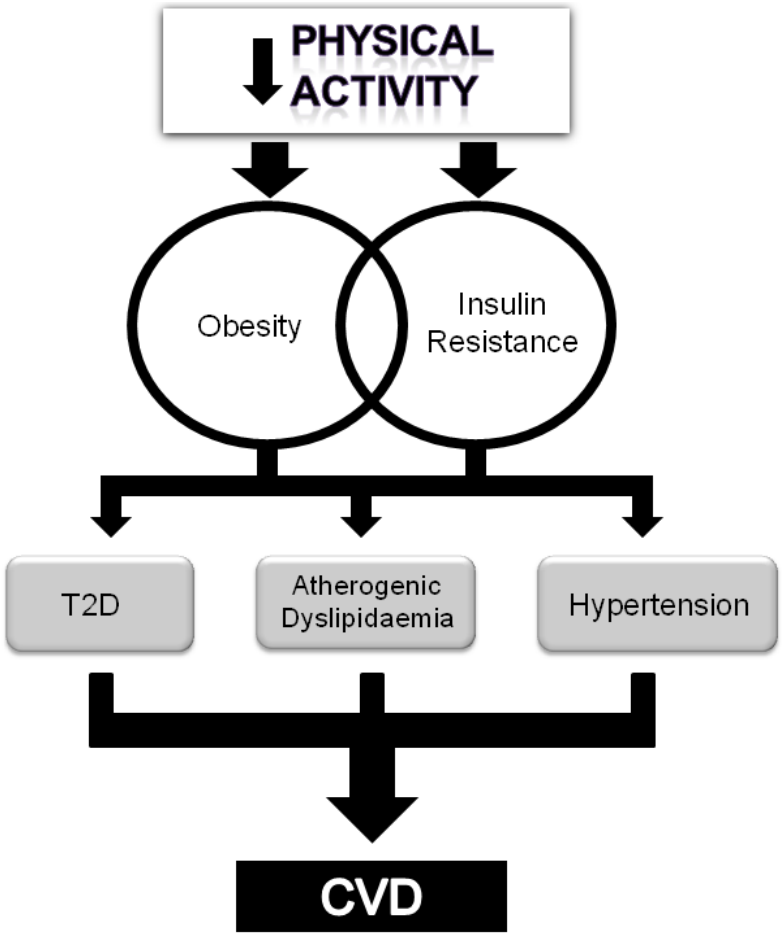


Figure 1.1. **Model describing the relationship between low levels of physical activity and increased risks for obesity, insulin resistance and cardiovascular diseases.**
Abbreviations: T2D: type2 diabetes; CVD: cardiovascular diseases

1.3 Lipoprotein Metabolism

1.3.1 Structures and Functions

Lipids are essential for energy homeostasis, reproductive and organ physiology, and numerous aspects of cellular biology (Lee *et al.* 2003). Lipids, which are water-insoluble, are transported in plasma as lipoprotein complexes, composed of several classes of lipids (*i.e.* cholesterol, triglycerides, and phospholipids) and proteins known as apolipoproteins. Triglycerides (TG) and cholesterol esters form the core of the lipoprotein molecule, while the phospholipids and apolipoproteins surround the surface of the molecule (Roheim 1986). According to major lipid composition, lipoproteins can be classified into TG-rich lipoproteins (TRL), which are the chylomicrons and very low density lipoproteins (VLDL); and cholesterol-rich lipoproteins, which include LDL and HDL (Lee *et al.* 2003). The major function of lipoproteins is to transport TG and cholesterol in the circulation (Frayn 2003). In the forward transport system, chylomicrons and VLDL, both TG-rich lipoproteins; transport dietary and hepatic TG to tissues, while LDL delivers cholesterol to peripheral tissues for steroidogenesis. Conversely, in the reverse transport system, HDL is involved in the transport of cholesterol from peripheral tissues to the liver for catabolism (Lee *et al.* 2003). Apolipoproteins (apo) play critical roles in the regulation of plasma lipid and lipoprotein transport. Among the major human apolipoprotein classes, apo B-100 is required for the generation of hepatic-derived VLDL, intermediate density lipoproteins (IDL), and LDL (Ginsberg *at el.* 2005). Apo B-48 is a truncated form of apo B-100 that is required for secretion of chylomicrons from the small intestine, while apo A-I is the major structural protein in HDL (Frayn 2003). The metabolism of lipoprotein can be divided into the exogenous and endogenous pathways.

1.3.2 Chylomicron Metabolism : The Exogenous Pathway

Dietary TG and esterifed cholesterol are absorbed and processed in the intestine and incorporated into the core of nascent chylomicrons (Ginsberg *et al.* 2005). The lipid composition of chylomicrons comprises ~90% TG, with the remainder comprising cholesterol ester, free cholesterol, phospholipids, and protein (Green & Glickman 1981). The key to the assembly of chylomicrons requires apo B-48 (van Greevenbroek & de Bruin 1998), after which the chylomicron complexes are liberated into the circulation via the lymphatic system (Frayn 2003). Apart from apo B-48, chylomicrons also carry apo A-1 and A-IV (Frayn 2003) and acquire apo CII, apo CIII, and apo E from the circulation

(Ginsberg *et al.* 2005). While in the bloodstream, the TG within the chylomicrons is hydrolysed by lipoprotein lipase (LPL), an enzyme synthesised mainly in the parenchymal cells of adipocytes, skeletal and cardiac muscle before being translocated to functional sites at the luminal surface of endothelial cells (Wang & Eckel 2009).

LPL plays a major role in the metabolism of lipids. One of the essential co-factors for LPL activity is apo C-II, as evidenced by patients exhibiting chylomicronaemia and hypertriglyceridaemia due to defects in apo C-II (Franssen *et al.* 2008; Fojo & Brewer 1992). Apo C-III can inhibit LPL actions (Wang *et al.* 1985), and high levels of apo C-III are strongly associated with hypertriglyceridaemia and the progression of CVD (Ooi *et al.* 2008). It is the balance between apo CII and apo CIII that determines, in part, the efficiency with which LPL hydrolyses TRL (Ginsberg *et al.* 2005). Hydrolysis of TG liberates glycerol and non-esterified fatty acids (NEFA), the latter can either enter the adipocytes to be esterified with glycerol-3-phosphate into new TG for storage, or released into the systemic NEFA pool for the uptake of peripheral tissues (Frayn 2003). The hydolysis process results in smaller chylomicron remnants, with altered composition of surface phospholipids and enriched in dietary-derived, and HDL-derived cholesterol ester, as well as apo E (Ginsberg *et al.* 2005). The remnant particles are carried to the liver where are cleared directly by the LDL receptor (Crawford & Borensztajn 1999) or further lipolysed by hepatic lipase (HL) (Sultan *et al.* 1990). Evidence indicates that the acquisition of apo E allows the chylomicron remnants to be recognised and removed by the LDL receptor-related protein in the liver (Cooper 1997), mediated by binding to cell-surface proteoglycans in the space of Disse (Mahley & Ji 1999; Ji *et al.* 1993).

1.3.3 VLDL Metabolism: The Endogenous Pathway

VLDL is assembled and secreted by the liver, with apo B-100, phospholipids, and a small amount of free cholesterol forming the surface of VLDL, whereas TG and esterified cholesterol make up the core of the particle (Davis *et al.* 1982). Some apo C and apo E are present on the nascent VLDL particles as they are secreted from the hepatocytes, but the majority of these apolipoproteins are transferred to VLDL after their entry into circulation from other lipoproteins, mainly HDL (Ginsberg *et al.* 2003; Frayn 2003). Plasma chylomicrons and VLDL share common catabolic pathways, known as the ‘common saturable removable process’ (Frayn 2003). Being a substrate for LPL in the capillary beds, the TG in VLDL is hydrolysed into NEFA and glycerol, resulting in

smaller and denser VLDL and, subsequently, IDL (Kwiterovich 2000). Some of the IDL particles are cleared through the interaction of apo E and apo B-100 with the LDL receptor and LDL receptor-related protein in the liver (Mahley & Ji 1999). Alternatively, TG in IDL can be further hydrolysed by hepatic lipase to produce cholesterol-enriched LDL (Frayn 2003; Ji *et al.* 1993). LDL particles are removed by uptake into tissues via the LDL receptor (Frayn 2003).

1.3.4 HDL Metabolism and Reverse Cholesterol Transport

Reverse cholesterol transport is a series of metabolic events resulting in the transport of cholesterol from peripheral tissues to the liver and plays a major role in maintaining cholesterol homeostasis in the body. HDL particles are central to this mechanism, which is inversely associated with atherosclerotic events (Hersberger & Eckardstein 2003; Franceschini *et al.* 1991). Nascent HDL or pre-β HDL particles are secreted predominantly by the liver (Castle *et al.* 1991) and intestine (Danielsen *et al.* 1993), containing mainly apo A-I and phospholipids (Frayn 2003). Deficiency in apo A-1 has been associated with low levels of HDL and coronary heart diseases (Ikewaki *et al.* 2004; Pisciotta *et al.* 2003). The subsequent acquisition of cholesterol and phospholipids by nascent HDL occurs mainly via ABCA-1 transporter protein-mediated efflux from extrahepatic cells (Oram & Vaughan 2000) and LPL-mediated lipolysis of TG-rich lipoproteins (Kwiterovich 2000) to form HDL_3 particles. The cholesterol in the nascent HDL is then esterified by lecithin cholesterol acyl transferase (LCAT), carried on HDL_3 particles, and activated by apo A-1. Hydrophobic cholesterol esters are retained in the HDL core forming spherical, and mature α-migrating HDL_2 particles (Lewis & Rader 2005). The activity of LCAT is critical to normal HDL metabolism, as it has been shown that genetic LCAT deficiency syndromes are associated with markedly reduced HDL and apo A-I levels (Kuivenhoven *et al.* 1997). The cholesterol ester in the core of HDL_2 is then returned to the liver for secretion into the bile via interaction with SR-B1 receptors expressed in the liver (Trigatti *et al.* 2003; Silver *et al.* 2001), after which the resultant lipid-poor apo A-I particles are recycled into the circulation to accept further cholesterol from peripheral tissues (Frayn 2003).

Other indirect pathways that facilitate the removal of cholesterol ester and phospholipids from HDL involve cholesterol ester transfer protein (CETP) (Masson *et al.* 2009; Barter *et al.* 2003), phospholipid transfer protein (Settasatian *et al.* 2001) and hepatic lipase (Barrans *et al.* 1994). CETP catalyses the exchange of cholesterol esters of HDL_2 with

TG of apo B–containing lipoproteins (*i.e.* chylomicrons, VLDL, IDL and LDL), producing TG-enriched but cholesteryl-ester–depleted HDL particles (Lewis & Rader 2005). Subsequent hydrolysis of TG in HDL by hepatic lipase promotes HDL remodelling with the generation of smaller HDL together with the release of lipid poor apo A-I (Tall 1995). The removal of lipids from HDL regenerates pre-β HDL or lipid-free apo A-I, these small apolipoproteins can diffuse into the extravascular space where they serve as acceptors of cellular lipids and again initiate the generation of HDL, or can be catabolised rapidly by the kidney (Hersberger & Eckardstein 2003). In the steady state, CETP activity appears not to change the overall efficiency of reverse cholesterol transport, though by transferring cholesterol esters from HDL to apo B-containing lipoproteins, CETP potentially decreases the concentration of HDL and apoA-I and increases the concentration of cholesterol ester-enriched lipoprotein remnants (Masson *et al.* 2009). The adverse effects of this will be discussed further in the coming section.

1.3.5 Regulation of Lipoprotein Metabolism

Insulin plays a key role in the tight control of lipoprotein metabolism during postabsorptive and postprandial periods (Sparks & Sparks 1994). The fall in insulin levels during postabsorptive state upregulates the lipolytic activity within the adipocytes to liberate NEFA and TG from the adipose tissue and into the circulation (Frayn 2003). The major lipase integral to this process is hormone-sensitive lipase (HSL) (Holm 2003; Haemmerle *et al.* 2002). Catecholamines, released by the sympathetic innervations within the adipose tissue are also important stimulators of lipolysis, and HSL is one of the major targets of this regulation (Holm 2003). Other lipases involved in the adipose tissue lipolysis are triacylglycerol hydrolase (TGH) (Soni *et al.* 2004) and adipose triglyceride lipase (ATGL) (Schweiger *et al.* 2006). NEFA released into the circulation are predominantly taken up by skeletal muscles as substrates for oxidative fuel, and by the liver to produce VLDL-TG or used as substrates for gluconeogenesis (Frayn 2003).

In the postprandial state, insulinaemia is responsible for the upregulation of LPL in adipose tissue, triggering the hydrolysis of chylomicron-TG particles, thus clearing them from the circulation (Frayn 2003). However, the removal of chylomicrons is a saturable process, reflecting the limited activity of LPL (Goldberg 1996). Evidence indicates that both chylomicrons and VLDL compete for the hydrolysis by LPL (Bjorkegren *et al.* 1996; Karpe *et al.* 1993), although chylomicrons appear to be the favoured substrate for LPL due to their larger size (Karpe *et al.* 2007). LPL-mediated lipolysis causes the

release of NEFA that are then taken up by receptors such as FAT/CD-36 located on the plasma membrane of adipocytes and myocytes (Goldberg *et al.* 2009). Within these cells, the fatty acids are re-esterified and used for storage or they provide direct energy through mitochondrial oxidation (Dallinga-Thie *et al.* 2010; Preiss-Landl *et al.* 2002). Meanwhile, insulin inhibits lipolysis in adipocytes, through the inhibition of adipose tissue lipases including HSL, which then suppresses the release of NEFA from adipocytes into the systemic circulation (Frayn 2003). In addition, any NEFA produced are re-esterified within the adipose tissue, a process stimulated by insulin (Frayn 2002).

Evidence has shown that insulin also acutely suppresses hepatic apoB-100-containing VLDL production both *in vitro* (Taniguchi *et al.* 2000; Adeli & Theriault 1992) and *in vivo* (Malmström *et al.* 1998; Lewis *et al.* 1995), making it likely that VLDL-TG secretion is inhibited in the postprandial period. This is in part, due to the suppression of NEFA flux to the liver which acts as the substrate for hepatic VLDL-TG secretion (Frayn 2003; Lewis *et al.* 1995). This is supported by findings by which elevations in intralipid/heparin-induced NEFA have been shown to stimulate hepatic and intestinally-derived TRL particles production in healthy humans in the fed state (Pavlic *et al.* 2010; Duez *et al.* 2008). All these metabolic events emphasise the important role of insulin in regulating substrate metabolism in the postprandial state. In the presence of a dysregulation of insulin secretion or insulin resistance, these metabolic interactions will be interrupted and consequently contributing to perturbations in postprandial lipid metabolism (DeFronzo & Ferrannini 1991).

1.4 Postprandial Lipid Metabolism

Humans spend a considerable amount of time in the postprandial state. Based on typical Western food intake, most people consume three or more meals a day, each containing 30–40 g fat, while the amount of TG in the circulation is typically around 3 g. Compared to glucose, there is a relatively slow rise in plasma TG, peaking 3 – 5 hours following a meal, before declining to baseline. The rise in the plasma TG following a meal is defined as ‘postprandial lipaemia’ (Frayn 2002). Except at breakfast, each of the subsequent meal in daily living is most likely consumed before plasma lipid levels have returned to baseline from the lipaemic conditions resulting from the previous intake. Therefore, the majority of time in a daily life is spent in a postprandial (fed) state, with a continual fluctuation in the degree of lipaemia throughout the day (Lopez-Miranda *et al.* 2007).

Insulin plays a major role in coordinating the mechanisms that minimise plasma TG excursions in the postprandial period by upregulating LPL activity in the adipose tissue, and suppression of NEFA from adipose tissue and hepatic VLDL-TG (Frayn 2002). Thus in conditions where insulin function is compromised (*i.e.* insulin resistance), impairment in postprandial lipid metabolism will ensue.

In 1979, Donald Zilversmit proposed that atherogenesis was a postprandial phenomenon. He implied that remnants of lipoprotein particles in response to a meal might exert atherogenic effects on the vasculature, based on his observations in cholesterol-fed rabbits, that the predominant cholesterol-containing lipoproteins in the plasma of rabbits which developed atherosclerosis consisted of chylomicron remnants (Zilversmit 1979). Decades later, subsequent studies provided further support to this earlier hypothesis by confirming that patients with CHD have increased postprandial levels of TG and intestinally-derived TRL in response to a fat load challenge, compared to healthy controls (Carstensen *et al.* 2004; Weintraub *et al.* 1996; Patsch *et al.* 1992; Groot *et al.* 1991; Simons *et al.* 1987). Many more studies then showed that postprandial, but not fasting, TG concentration is an independent cardiovascular risk factor (Nordestgaard *et al.* 2007; Bansal *et al.* 2007; Iso *et al.* 2001; Austin *et al.* 1998). A meta-analysis of 17 prospective population-based studies highlighted the role of hypertriglyceridaemia as an independent risk factor for CVD (Cullen *et al.* 2000). Rather than being an actual atherogenic agent, elevated TG levels can reflect impaired postprandial lipid metabolism and thus a poor ability in TRL clearance from the circulation (Ginsberg 2002). The elevated TRL and their prolonged residence time in the circulation may increase the exchange rate of esterified cholesterol from HDL and LDL to TRL, with TG being transported in the opposite direction, mediated by CETP. This consequently leads to TG-enrichment of LDL particles, rendering them better substrates for hepatic lipase, which hydrolyses TG from the core of LDL and turning them into smaller and denser LDL particles. Similarly, hydrolysis of TG-enriched HDL particles resulted in smaller and more rapidly catabolised HDL particles (Sposito *et al.* 2004; Frayn 2003; Weinthrop *et al.* 1996). Small, dense LDL and remnant lipoprotein particles can enter the subendothelial space and be trapped inside the arterial wall, where they can be oxidised and taken up by macrophages and smooth muscle cells, leading to the development and progression of atherosclerosis (Twickler *et al.* 2005; Carmena 2004). Thus, repeated episodes of exaggerated postprandial lipaemia can result in disturbances of the lipoprotein profile, characterised by elevated TG levels, increased hepatic VLDL-TG production and a decrease in their

clearance, a predominance of small, dense LDL particles, and reduced levels of HDL (Hardman 1999).

1.4.1 Obesity and Postprandial Lipid Metabolism

Abdominal obesity, especially when accompanied by an excess of visceral adipose tissue, has been associated with numerous metabolic disturbances including hypertriglyceridaemia and hyperinsulinaemia, which prematurely increase the risk of CVD (Gruson *et al.* 2010; Fox *et al.* 2009; Yusuf *et al*. 2006; Lamarche *et al.* 1998). Consistent with this, postprandial lipaemia is exacerbated in abdominal obesity (Blackburn *et al.* 2003; Couillard *et al.* 1998; Roust & Jensen 1993), a condition typically associated with insulin resistance (Frayn 2002). Even in the absence of the fasting hypertriglyceridaemia, abdominally-obese individuals may have higher postprandial hyperlipidemia compared to non-obese control patients (Nabeno-Kaeriyama *et al.* 2010; Lopez-Miranda *et al*. 2007; Castro-Cabezas *et al.* 2001; Mekki *et al.* 1999). The exaggerated postprandial lipaemic response commonly observed in such subjects is due to increased concentrations of both hepatic- and intestinally-derived lipoproteins (Couillard *et al.* 2002; Couillard *et al.* 1998). Due to the effects of insulin resistance, exaggerated lipolysis during the postprandial period results in an increased portal flux of fatty acids which has been shown to stimulate hepatic secretion of VLDL (Chan *et al.* 2004; Lewis 1997). At the peripheral level, blunted LPL activity, commonly observed in visceral obesity (Kobayshi *et al.* 2007), decreases catabolism of VLDL, thereby exacerbating postprandial lipaemia. It is also evident that in insulin-resistant obese individuals, the occurrence of delayed clearance pathways of exogenous lipoproteins contributes to postprandial hyperlipaemia (Chan *et al.* 2002; Taira *et al.* 1999), through increasing competition between chylomicrons and VLDL for LPL lipolysis (Bjorkegren *et al.* 1996; Karpe *et al.* 1993) and between chylomicron remnants and VLDL remnants for LDL receptor-mediated clearance (Mamo *et al.* 2001). Thus, the inefficient metabolism of postprandial lipoproteins leading to exaggerations in postprandial lipaemia is a common feature in obesity presented with insulin resistance (Frayn 2002) Although insulin resistance is also associated with the adverse lipid profile in obesity, it would appear that this association is largely based on the fact obesity and insulin resistance are closely related (Nieves *et al.* 2003). Thus, interventions aimed at reducing obesity and improving insulin sensitivity would have a beneficial effect on atherogenic dyslipidaemia. One such intervention is exercise and this will be discussed in the following section.

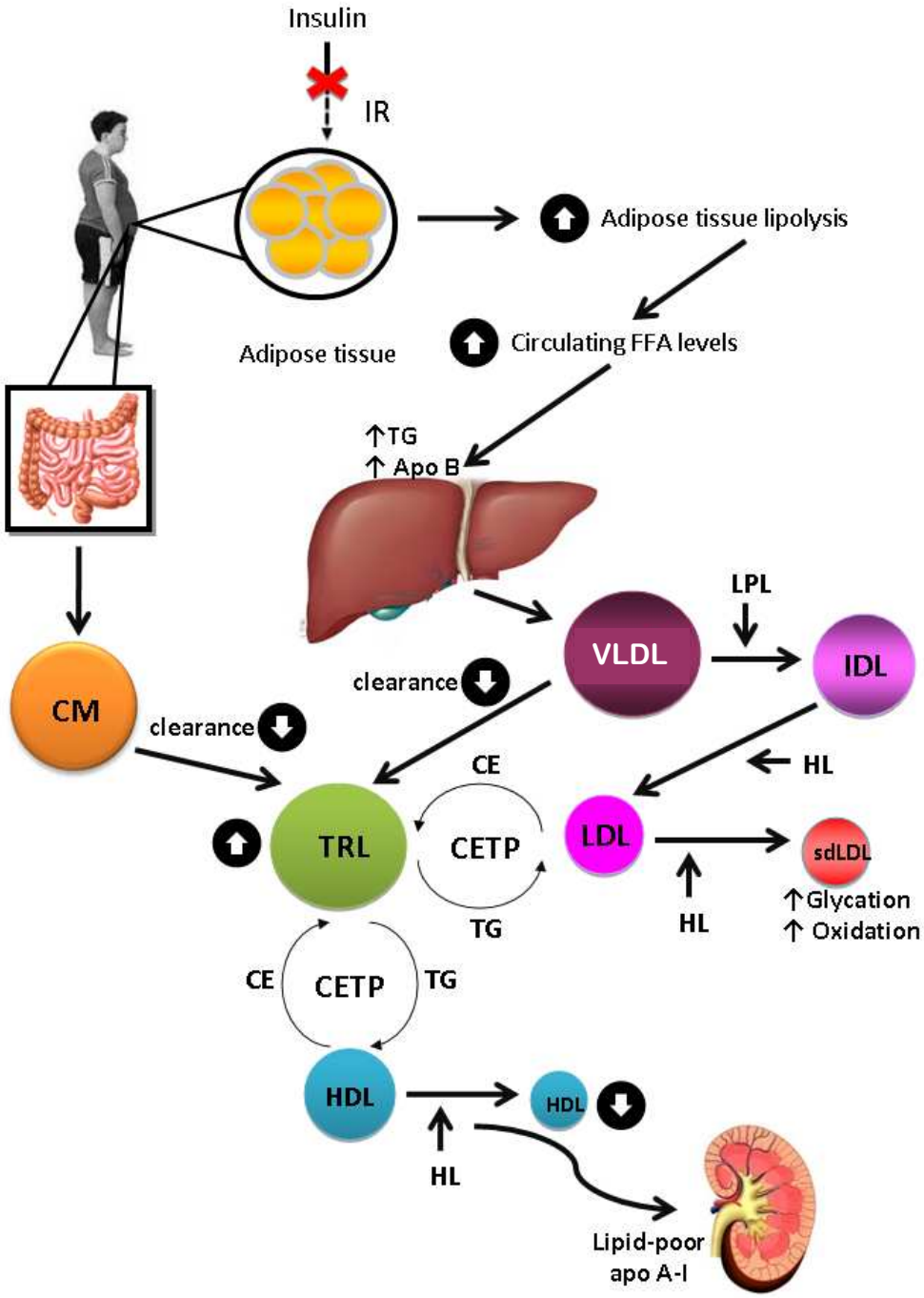


Figure 1.2. Pathophysiology of atherogenic dyslipidaemia associated with insulin resistance. Dotted arrow represents resistant effect. Insulin resistance leads to enhanced lipolysis and increased FFA flux from adipose tissue to the liver. *De novo* hepatic lipogenesis in the liver increased TG levels and apo B secretions as VLDL. Delayed clearance of CM and VLDL remnants results in the increased presence of TRL in the plasma. Mediated by CETP, TG from TRL is exchanged for CE from LDL and HDL particles, producing TG-enriched HDL and LDL, which are rapidly hydrolysed by HL, producing smaller, denser LDL and HDL particles. Small and dense LDL particles are susceptible to glycation and oxidative modifications, rendering them atherogenic. TG-rich HDL particles can undergo further modification, leading to dissociation of the structurally important protein apo A-I. The unbound, lipid-poor apo A-I in plasma is cleared rapidly by the kidney, leading to decreased number of HDL particles. *Abbreviations:* Apo: Apolipoprotein; CE: cholesterol ester; CETP: cholesterol ester transfer protein; CM: chylomicron; FFA: free fatty acids; HL: hepatic lipase; IR: insulin resistance; LPL: lipoprotein lipase; sd: small and dense; TG: triglyceride; TRL: triglyceride-rich lipoproteins.

1.4.2 Exercise and Postprandial Lipid Metabolism

Exercise has long been well-documented as a therapeutic approach in preventing and ameliorating lipid dysmetabolism in both postabsorptive (Durstine *et al.* 2001) and postprandial states (Hardman & Herd 1998). The mechanisms of TG-lowering effect of exercise have been of interest for the past decades and causal evidence has been established. The potential mechanisms that may result in reduction of postprandial lipaemia following an acute exercise bout are enhanced clearance rate of TG-rich particles from the circulation and/or a reduced rate of appearance.

Studies have shown that exercise acutely increases the expression and activity of LPL in skeletal muscles and post-heparin plasma (Perreault *et al.* 2004; Zhang *et al.* 2002; Kantor *et al.* 1984), which can be responsible for clearing TG-rich particles from the circulation, with the magnitude of increase being greater following prolonged exercise than exercise of shorter duration (Katsanos *et al.* 2004; Ferguson *et al.* 1998). Post-heparin LPL activity is indeed higher in endurance-trained individuals compared with previously untrained condition (Miyashita *et al.* 2010; Duncan *et al.* 2003; Bergeron *et al.* 2001; Tikkanen *et al.* 1999) and untrained counterparts (Podl *et al.* 1994). It is also conceivable that increased LPL mass and activity induced by exercise would increase VLDL-TG hydrolysis, in addition to chylomicron-TG hydrolysis (Kiens *et al.* 1998). However, reductions in postprandial TG have been documented without accompanying increases in LPL activity (Miyashita & Tokuyama 2008; Gill *et al.* 2003; Herd *et al.* 2001), suggesting that besides LPL, there are other factors mediating the increased clearance rate of TG from the circulation. On the other hand, studies have also shown that reductions in TG following exercise can occur in the absence of increased TG clearance and uptake (Tsekouras *et al.* 2008; Gill *et al.* 2001b; Malkova *et al.* 2000), indicating that other mechanisms other than enhanced TG clearance are likely to contribute to the hypotriglyceridaemic effect of exercise.

Decreased secretion of hepatic VLDL-TG has also been proposed to be responsible for the reduced appearance of TG in the circulation following acute exercise (Tsekouras *et al.* 2008; Magkos *et al.* 2006; Gill *et al.* 2001a; Malkova *et al.* 2000). A possible cause for the attenuation in hepatic secretion of VLDL-TG could be increased in hepatic sensitivity to insulin, thereby suppressing hepatic VLDL production (Malmström *et al.* 1998). Furthermore, reduced secretion rate of VLDL-apoB-100 by the liver after 2-h of exercise at 60% of $\dot{V}O_2$max has been reported, which consequently resulted in fewer, but TG-

richer VLDL particles (Magkos *et al.* 2006). Evidence has also suggested that increasing TG content and the size of the TG-rich VLDL particles enhance their susceptibility to hydrolysis by LPL (Karpe *et al.* 2007; Fischer *et al.* 1995). Following on from their earlier study, Magkos *et al.* had reported exercise at 60% of $\dot{V}O_2$max for 1 hour had no effect on plasma VLDL-TG and VLDL-apoB-100 concentrations, hepatic VLDL-TG and VLDL-apoB-100 secretion rates, and VLDL-TG and VLDL-apoB-100 plasma clearance rates in untrained lean men compared to rest (Magkos *et al.* 2007). Other studies demonstrated that whole-body resistance exercise at 80% of maximum peak torque production (Tsekouras *et al*. 2009) and brisk-walking for 90 min at 60% of $\dot{V}O_2$max (Tsekouras *et al*. 2007) reduced plasma VLDL-TG concentrations by augmenting VLDL-TG removal from plasma, but without altering the secretion rate. Recently however, reduced VLDL-TG levels and secretion rate have been demonstrated in exercise training studies in animals (Barsalani *et al.* 2010) and humans (Yoshida *et al.* 2010; Tsekouras *et al.* 2008), suggesting that training may be effective in lowering hepatic VLDL-TG secretion rates than does acute exercise (Magkos 2009). The mechanisms responsible for the exercise-induced attenuation in plasma TG requires further elucidation but it is very likely that both LPL-mediated clearance and reduced hepatic VLDL secretion contribute to the effectiveness of exercise in modulating postprandial TG metabolism.

1.4.2.1 Effects of Acute Exercise

Cohen and Goldberg (1960) first reported that walking 6 miles after the ingestion of a 75-g oral fat load reduced postprandial lipaemia, indicated by a decrease in plasma turbidity, for up to 7 hours. In the subsequent years, a large number of evidence have shown that a single bout of aerobic exercise performed prior to meal ingestion (high-fat or moderate-fat content) attenuates postprandial lipaemia in lean, normolipidaemic subjects (Harrison *et al.* 2009; Miyashita & Tokuyama 2008; Gill *et al.* 2006; Katsanos *et al.* 2004; Kolifa *et al.* 2004; Gill *et al.* 2003; Herd *et al*. 2001; Gill *et al.* 2001a; Gill *et al.* 2001b; Tsetsonis *et al.* 1997; Tsetsonis & Hardman 1996a), overweight and obese (Dekker *et al.* 2010; Hurren *et al*. 2011; MacEneaney *et al.* 2009; Burton *et al.* 2008; Mitchell *et al.* 2008; Miyashita 2008; Gill *et al.* 2004; Zhang *et al.* 2004), individuals with metabolic syndrome (Mestek *et al.* 2008; Zhang *et al.* 2007), as well as T2D (Tobin *et al.* 2008), although some reported no change in diabetic patients (Gill *et al.* 2007; Dalgaard *et al.* 2004). In studies investigating the effects of exercise after meal ingestion on postprandial lipaemia, less clear-cut evidence have been found, with some reporting exercise-induced reduction (Katsanos & Moffatt 2004; Hardman & Aldred 1995; Klein *et al.* 1992; Schlierf *et al.*

1987) while others had found no change (Zhang *et al.* 1998; Welle 1984). Specifically, with regards to exercise timing relative to meal ingestion on postprandial lipaemia, only two studies have compared the direct effects of pre-meal and post-meal exercise, and with contrasting results. Zhang *et al.* (1998) reported that although 1-h exercise at 60% of $\dot{V}O_2$max prior to a high-fat meal attenuated postprandial lipaemia, exercise in the postprandial period did not. On the other hand, Katsanos & Moffatt (2004) found that walking at 50% of $\dot{V}O_2$max for 90 min reduced postprandial lipaemia irrespective of exercise timing relative to meal ingestion. It remains a topic of debate if exercise performed in the postprandial period is as effective as exercise prior to meal ingestion, therefore this warrants further investigation.

Intermittent exercise, rather than continuous bout, has been shown to be similarly effective in reducing postprandial lipaemia in both lean (Miyashita *et al.* 2008; Barrett *et al.* 2006; Gill *et al.* 1998) and obese subjects (Miyashita 2008). Altena *et al.* (2004) however, reported that intermittent exercise (3 x 10 minutes at 60% of $\dot{V}O_2$max) was more effective than a 30-min continuous bout in lowering postprandial lipaemia. Conversely, intermittent exercise of 250 kcal (2 x 60-70% $\dot{V}O_2$max) did not seem to induce the hypotriglyceridaemic effect compared to continuous exercise bout at the same intensity in men with metabolic syndrome (Mestek *et al.* 2008). It is speculated that exercise may need to be distributed over more than two sessions to produce an effect (Miyashita & Stensel 2009). Regarding the duration of attenuation of postprandial lipaemia in response to exercise, there is clear evidence showing that postprandial lipaemia can be attenuated for up to 15 - 24 hours following acute exercise (Burton *et al.* 2008; Miyashita 2008; Katsanos 2006; Gill *et al.* 2003; Herd *et al*. 2001).

1.4.2.2 Effects of Energy Expenditure

It has become apparent that the size of the exercise-induced energy expenditure is a critical factor in the manifestation of hypotriglyceridaemic effect, with higher the energy expenditures leading to greater reductions in postprandial lipaemia (Gill *et al.* 2002; Petitt & Cureton 2003). For example, doubling exercise energy expenditure by either doubling exercise intensity for the same duration (Tsetsonis & Hardman 1996a) or duration at the same intensity (Gill *et al.* 2002) doubles the magnitude of hypotriglyceridaemia. Furthermore, Tsetsonis & Hardman (1996b) reported that expending the same amount of

energy at different relative intensity (*i.e.* 30% $\dot{V}O_2$max for 180 min and 60% $\dot{V}O_2$max for 90 min) reduced postprandial lipaemia to the same degree compared to control.

Therefore, the optimal benefits of exercise on postprandial lipemia are thought to likely be achieved with sufficient energy expenditure. According to a recent systematic review, the energy deficit threshold (prior to a high-fat meal) for the aerobic exercise-induced hyportriglyceridaemic effects to occur seems to lie around 500 kcal per session in a healthy population, but lower energy expenditures (~350 kcal per session) in obese individuals or those with metabolic syndrome (Maraki & Sidossis 2010). On the other hand, a few studies have demonstrated that lower energy deficit is required (~250 kcal) to reduce postprandial lipaemic response when a moderate-fat meal is given in both lean and overweight subjects (Miyashita 2008; Miyashita & Tokuyama 2008; Miyashita *et al.* 2006; Kolifa *et al.* 2004), compared to studies giving high-fat meals. A similar finding was also noted in a group of overweight women consuming a high-carbohydrate meal prior to exercise (Mitchell *et al.* 2008). Therefore, these findings seem to suggest that, smaller exercise-induced energy deficit can still be effective in reducing postprandial lipaemia, especially in the obese, for whom performing exercise can be an arduous task.

1.4.2.3 Effects of Energy Deficit

Although it was earlier shown that the exercise-induced hypotriglyceridaemia cannot be replicated with an equivalent energy deficit induced by dietary intake restriction (Gill & Hardman 2000), recent evidence highlighted the importance of dietary energy intake in modulating the hypotriglyceridaemic effects of exercise. A single bout of exercise expending ~250 kcal, which was not likely to affect plasma TG by itself, was shown to exhibit attenuation in postprandial TG when the exercise was accompanied by superimposing a mild caloric restriction of ~ 350 kcal, compared to no-exercise control (Maraki *et al.* 2009). In another recent study, Maraki *et al.* (2010) demonstrated that moderate energy deficit (~500 kcal) independently of its method (*i.e.* diet or exercise, or combination of both) reduced fasting and postprandial lipaemia, although exercise elicited a somewhat greater effect than caloric restriction. These observations seem to suggest that both exercise-induced and diet-induced energy deficit may induce the TG-lowering effects, though the effect may be greater achieved with exercise. To further highlight the importance of negative energy balance in mediating the attenuation in postprandial lipaemia, replacing energy deficit created by exercise with increased energy intake to maintain zero energy balance was shown to markedly attenuate the TG-lowering effect of

exercise (Harrison *et al.* 2009; Burton *et al.* 2008). Thus, it appears that when individuals increase their energy intake post exercise, replacing exercise-induced energy deficit, the beneficial effects of exercise on postprandial lipaemia are blunted. This is of particular importance in daily life situations where energy intake is often uncontrolled. It is therefore important to understand the real magnitude of the effects of exercise on postprandial metabolism in the real world, however, there is a paucity of evidence in the literature with regards to postprandial lipid metabolism and feeding, hence, further research is warranted.

1.4.2.4 Effects of Training

Foger and Patsch (1995) indicated that exercise training decreases postprandial lipaemia and, in turn, increases HDL levels. The inverse relationship between the magnitude of postprandial lipaemia and the plasma levels of HDL (Patsch *et al.* 1983) described HDL cholesterol as an integrative marker for efficient TG metabolism (Patsch *et al.* 1992). Thus, high HDL levels in physically active individuals are likely to be contributed by the effects of habitual exercise on the improvement in TG clearance and reverse cholesterol transport (Hardman 1999). Having said that, the long term effects of exercise on postprandial lipaemia however, is still debatable. While some studies showed lower postprandial lipaemia (Tsetsonis *et al.* 1997; Ziogas *et al.* 1997; Hartung *et al.* 1993; Cohen *et al.* 1989; Merrill *et al.* 1989; Wirth *et al.* 1985) and enhanced TG clearance (Podl *et al.* 1994; Cohen *et al.* 1989; Sady *et al.* 1988; Wirth *et al.* 1985), in trained subjects compared to sedentary controls, it can be argued that these favourable changes were likely to be contributed by the recent exercise bout since exercise-induced attenuation in lipaemia persists for up to 24 hours (Burton *et al.* 2008; Miyashita 2008; Katsanos 2006; Gill *et al.* 2003; Herd *et al*. 2001). This is further supported by studies in which trained subjects refrained from exercise at least 2 days prior to test and showed no differences in postprandial lipaemic response compared to control (Bloomer *et al.* 2010; Herd *et al.* 2000; Tsetsonis *et al.* 1997; Aldred *et al.* 1995).

Further evidence is illustrated in de-training studies where endurance athletes reportedly experienced ~37-41% increase in postprandial chylomicron and chylomicron remnant concentrations with 14-22 days of detraining (Mankowitz *et al.* 1992) and 40% increase in postprandial lipaemia after detraining for 6 days (Hardman *et al.* 1998). Similarly, in untrained individuals who underwent 13 weeks of training, postprandial lipaemia was increased by 37% and 46% within 60 hours and 9 days respectively after detraining (Herd

et al. 1998). Collectively, evidence is suggesting that the TG-lowering effects of exercise seem to be elicited by individual exercise sessions rather than adaptation from chronic training, however exercise training *per se* is likely to have beneficial, indirect effects on postprandial lipid metabolism through body weight loss and larger energy expenditure expended by trained individuals compared to untrained peers (Gill 2004).

1.5 Energy Balance

An individual's body weight and composition represent the cumulative balance between all previous energy intake and energy expenditure. The classic equation of energy balance states that $E_{stores} = E_{intake} - E_{expenditure}$ (Spiegelman & Flier 2001). E_{intake} consists of all ingested food and beverages with energy value. It is the foundation of energy, where nutrients are provided by the metabolism of proteins, carbohydrates, and fats to fulfil the energy requirement (Woo *et al.* 1985b). $E_{expenditure}$ is represented by three major components, which, when added together provide an accurate measure of an individual's daily caloric requirement: the basal metabolic rate (BMR), activity energy expenditure, and the thermic effect of food (Woo *et al.* 1985b). When energy intake (E_{in}) equals energy expenditure (E_{out}), energy balance is achieved and hence, body adiposity is stable (Spiegelman & Flier 2001). However, when considering the regulation of body adiposity, the traditional concept of the energy balance equation, which describes weight gain as an excessive positive energy imbalance, might be too simplistic a concept, and that the concept of macronutrient balance should be taken into consideration.

1.5.1 Macronutrient Balance

The concept of macronutrient balance has been accepted as the physiological basis for determining body composition changes (Schutz 2004; Flatt 1988). The basis for this is that the composition of macronutrient intake tends to differ from their metabolic effects, in such a way that the oxidative hierarchy is inversely proportionate to the size of available stores for each macronutrient (Astrup 1999). Alcohol, amino acid, and glucose oxidation corresponds readily to alcohol, protein, and carbohydrate intakes, therefore carbohydrate and protein stores are tightly regulated by adjusting oxidation to intake (Frayn 1995). In contrast, there is virtually no acute feedback between fat intake and fat oxidation, resulting in much less accurately maintained fat stores (Schutz 2004; Abbot *et al.* 1988). Thus, for stability of body weight and body composition, not only does energy

intake have to match energy expenditure, but also macronutrient intake must balance macronutrient oxidation. Since fat is the only nutrient capable of causing a chronic imbalance between intake and oxidation, a positive energy balance is therefore equivalent to a positive fat balance, which subsequently leads to body fat gain (Galgani & Ravussin 2008).

1.5.2 Fat Balance

Fat balance indicates the balance of all metabolic activity resulting in storage and utilisation of exogenous and endogenous lipid (Shah & Garg 1996). Negative fat balance is achieved when fat oxidation exceeds fat intake, which ultimately results in body fat loss (Schutz 2004). Indeed, studies have shown that low rates of fat oxidation and increased reliance on carbohydrate oxidation have been associated with accelerated weight gain in the Pima Indians, a population with a high prevalence of obesity. This effect was independent of 24-h metabolic rate (Zurlo *et al.* 1990). Similar findings have been reported in Caucasians (Seidell *et al.* 1992). In addition, others have shown that post-obese individuals have low rates of fat oxidation (Larson *et al*. 1995; Astrup *et al.* 1994), and those successful at maintaining weight loss have higher fat oxidation rates than those experiencing weight relapse (Froidevaux *et al.* 1993). Unlike carbohydrate intake, increasing fat intake does not markedly lead to increase in fat oxidation (Roy *et al.* 1998; Bennett *et al.* 1992; Schutz *et al.* 1989; Flatt *et al.* 1985). Because increasing fat intake does not stimulate fat oxidation, therefore the maintenance of negative fat balance generally requires a reduction in fat intake and/or increase in fat oxidation via changes in physical activity.

1.5.3 Physical Activity, Body Weight, and Fat Balance

There is a large body of scientific evidence to support the importance of physical activity for the primary prevention of weight gain, successful weight loss, and the prevention of weight regain (Chaput *et al.* 2011; Jakicic 2009). Findings from prospective cohort studies investigating the relationship between obesity and levels of physical activity over time consistently reported that regular exercisers are less likely to gain weight compared to their sedentary peers (Hankinson *et al.* 2010; Drøyvold *et al.* 2004). Furthermore, physical activity may reduce health risks associated with body weight through changes in body composition, such as by decreasing fat-to-lean mass ratio and also by decreasing visceral-to-subcutaneous fat ratio (Fogelhom 2010). Evidence from prospective cohorts

(Meisinger *et al.* 2005; Nakanishi *et al.* 2004; Weinstein *et al.* 2004; Hu *et al.* 2003; Folsom *et al.* 2000; Okada *et al.* 2000; Hu *et al.* 1999; Burchfiel *et al.* 1995; Manson *et al.* 1992) with a total number of 201 653 participants suggests that the relative risk of developing T2D in physically active individuals is attenuated when adjusted for BMI or other markers of adiposity (Gill & Cooper 2008). Physical activity influences the energy expenditure side of the energy balance equation and effectively promotes negative fat balance by increasing whole-body fat oxidation, thereby helping to maintain fat balance at lower levels of body fatness (Hill & Commerford 1996).

1.5.4 Exercise and Fat Oxidation

The increase in fat oxidation in response to exercise results mainly from the increased in fatty acid availability, as a consequence of increased lipolysis (Romijn *et al.* 1993) and a reduced rate of re-esterification of fatty acids (Wolfe *et al.* 1990). One of the pathways involved in the stimulation of lipolysis with exercise is through activation of AMP-activated protein kinase (AMPK) in adipocytes (Steinberg 2009). In skeletal muscle, exercise-induced activation of AMPK is associated with increases in fatty acid uptake and oxidation (Thomson & Winder 2009). In addition, increased blood flow to the skeletal muscles transports the fatty acids away from the adipose tissue and toward the exercising muscle (Romijn *et al.* 1993; Bulow & Madsen 1981) and can enhance partitioning of excess fatty acids toward IMTG synthesis (Schenk & Horowitz 2007). In addition to acute exercise, exercise training can induce adaptations that increase the capacity of skeletal muscle fat oxidation during submaximal exercise. Several factors contribute to these adaptive responses: increased fatty acid transport protein content and localisation, which regulates transport of fatty acids within the muscle (Talanian *et al.* 2010); enhanced proliferation of capillaries within skeletal muscle, which enhances fatty acid delivery to muscle (Prior *et al.* 2004); increased density of skeletal muscles mitochondria, which increases the capacity for fat oxidation (Toledo *et al.* 2006); and increased carnitine palmitoyl transferase (CPT) complexes which facilitates fatty acid transport across the mitochondria membrane (Melanson *et al.* 2009b).

1.5.4.1 Effects of Intensity and Duration

It is well documented that the beneficial effects of aerobic exercise on maximising fat oxidation are best achieved with low to moderate intensity exercise (45–65% of $\dot{V}O_2$max) (Achten & Jeukendrup 2004). High intensity exercise however, results in lower

contribution of fat to energy expenditure during exercise (Romijn *et al.* 1993). Despite the increased rate of lipolysis, the mobilisation of NEFA from adipocytes during high intensity exercise may be compromised due to restricted blood flow to adipose tissue (Coyle 1995) and suppressed mitochondrial uptake of fatty acids (Romijn *et al.* 1995). There is also evidence that IMTG oxidation contributes significantly to energy production during moderate intensity compared to high intensity exercise (van Loon *et al.* 2003; Watt *et al.* 2002; Romijn *et al.* 1993), which may partially explain the enhanced fat oxidation rate at moderate intensity. Prolonged exercise of moderate intensity is associated with a time-dependent decrease in carbohydrate oxidation and greater fat oxidation (Romijn *et al.* 1993; Ahlborg *et al.* 1974). The enhanced fat oxidation in prolonged exercise is a function of decreased muscle glycogen utilisation which results from reduced muscle glycogen availability (Vøllestad & Blom 1985) while the relative contribution from NEFA increases progressively with exercise duration (Ahlborg *et al.* 1974).

1.5.4.2 Effects of Post-Exercise Fat Oxidation

Studies have shown that prolonged, moderate intensity exercise stimulates fat oxidation in favour of carbohydrate for several hours in the post-exercise period (Kuo *et al.* 2005; Kimber *et al.* 2003; Marion-Latard *et al.* 2003; Kiens & Richter 1998; Bielinski *et al.* 1985), extending the benefit of exercise on fat oxidation beyond the exercise period. These authors suggested that following glycogen-depleting exercise, muscle glycogen resynthesis is of high metabolic priority, resulting in the preferential utilisation of circulating NEFA and other lipids for oxidation by the recovering skeletal muscle. Meanwhile, elevations in catecholamines in the circulation postexercise, and increased blood flow to adipose tissue stimulate lipolysis, resulting in increased plasma NEFA concentrations and elevation in fat oxidation (Coyle 1995). Furthermore, studies have found that exercise-induced increase in NEFA mobilisation can persists for 12 - 24 hours after exercise, with a progressive decline over time; and dependent on the duration as well as the intensity of exercise (Magkos *et al.* 2009; Mulla *et al.* 2000), although the rate of fat oxidation is not strictly dependent on NEFA availability (Bennard *et al.* 2005). Regarding exercise intensities, the rates of post-exercise fat oxidation appear to be greater at higher exercise intensities compared to lower intensities when matched for energy expenditure (Pillard *et al.* 2010; Warren *et al.* 2009; Kuo *et al.* 2005). Thus, apart from the exercise period, there is also a great potential for the increase in post-exercise fat oxidation in inducing negative fat balance.

1.5.4.3 Effects of Pre-Exercise Meal Ingestion

Carbohydrate ingestion prior to exercise has been shown to increase carbohydrate oxidation and concomitantly decreases fat oxidation during the exercise period, compared to exercise in the fasting or postabsorptive period (Wallis *et al.* 2006; Wu *et al.* 2003; Bergman & Brooks 1999; Coyle *et al.* 1997; Horowitz *et al.* 1997; Montain *et al.* 1991). The glycaemic and insulinaemic perturbations accompanying the consumption of carbohydrate stimulate carbohydrate oxidation while suppressing lipolysis and fatty acid oxidation during the exercise period (Coyle *et al.* 1997; Sidossis & Wolfe 1996). Furthermore, Montain *et al.* (1991) observed that increase in carbohydrate oxidation and decrease in plasma NEFA can persist for up to 6 hours after a pre-exercise carbohydrate meal, although this might be dependent on the size of the meal, thereby indicating that the effect of pre-exercise meal is not limited to the exercise period only but also during the post-exercise recovery periods. Interestingly, many studies have shown that unlike carbohydrate, ingestion of a high-fat meal prior to exercise does not increase fat oxidation during the exercise period (Paul *et al.* 2003; Bergman & Brooks 1999; Whitley *et al.* 1998; Flatt 1988), although this is not unequivocal (Ainslie *et al.* 2002; Hawley *et al.* 2000). These evidence collectively show that, while exercise intensity predominantly influences substrate utilisation during exercise, pre-exercise meal ingestion can alter this, although the dietary effect can depend on the amount of carbohydrate or fat ingested, timing of meal and duration of dietary treatment (Hansen *et al.* 2005).

1.5.4.4 Effects of Post-Exercise Meal Ingestion

Another consideration in the influence of exercise on fat oxidation is the effects of meal ingestion in the post-exercise period. A study has shown that post-exercise carbohydrate-rich meal ingestion led to a decrease in fat oxidation for several hours, compared to exercise with placebo (Long *et al.* 2008). However, when comparing the effects of exercise on fat oxidation relative to a no-exercise control, others studies reported elevations in fat oxidation despite meal ingestions in the post-exercise period, and this effect appear to be evident for up to 24 hours (Votruba *et al.* 2002; Folch *et al.* 2001; Gill *et al.* 2001a). It could be that the energy content of a post-exercise meal influences the magnitude of fat oxidation thereafter, as it has been demonstrated that neither post-exercise energy expenditure nor substrate metabolism differed over 24-h when sub-maximal exercise was performed preceding a caloric-equivalent meal, compared to rest (Dionne *et al.* 1999). In support of this, Melanson *et al.* (2009b) recently reported that

exercise when performed with energy replacement (*i.e.* energy balance is maintained), does not increase 24-h fat oxidation. On the contrary, Burton *et al.* (2008) demonstrated that exercise with isocaloric energy replacement increased postprandial fat oxidation compared to control in the following day, albeit to a lesser degree than exercise with an energy deficit. Therefore it appears that there is some discrepancy between findings on subsequent meal ingestion on post-exercise fat oxidation, and much less is known on how this can translate into daily living with *ad libitum* feeding patterns. In addition, although meal ingestion influences fat oxidation, it is still unclear whether pre-exercise or post-exercise food ingestion is more effective in creating a negative fat balance over the course of the day. Addressing this issue will help to plan exercise and dietary strategies in maximising fat oxidation, specifically by manipulating the temporal sequence of meal ingestion and exercise.

1.5.4.5 Effects of Obesity

There is strong evidence showing a blunted lipolytic response to catecholamines (Jocken & Blaak 2008; Horowitz & Klein 2000b) and beta-adrenergic stimulation (Jocken *et al.* 2008), as well as to exercise stimulus (Mittendorfer *et al.* 2004; Pérez-Martin *et al.* 2001) in obese individuals. However, many other studies have demonstrated that whole-body fat oxidation during moderate exercise is similar across lean, overweight and obese subjects (Mittendorfer *et al.* 2004; Horowitz & Klein 2000a; Ezell *et al.* 1999), although the source of fatty acids for oxidation during exercise may differ between these groups. Fatty acid oxidation of non-plasma sources is quite common in obesity due to the blunted lipolytic response (Mittendorfer *et al.* 2004; Horowitz & Klein 2000a; Goodpaster *et al.* 2002). Regardless, this shows that exercise is just as beneficial in improving fat oxidation in the overweight/obese populations as in the lean. However, it is unclear how subsequent ingestion of food, whether in the pre-exercise or post-exercise period can affect fat balance in this population, therefore further investigation is needed.

1.5.5 Exercise and Fat Balance: Loss or Gain?

The effect of exercise on 24-hour fat balance is most important in understanding the role of exercise in the prevention of fat accumulation and obesity. Although exercise increases energy expenditure and induces fat balance, long-term exercise studies have however, consistently shown that weight loss achieved with exercise alone appears to be modest and is typically ~3% of initial body weight (Jakicic 2009). Given that physical activity

and energy intake are the two major behavioural determinants of body weight, their independent and combined compensatory responses could serve barriers to exercise-induced weight loss (King *et al.* 2007). The concept of compensatory adjustments to exercise-induced changes in energy expenditure was highlighted over 30 years ago by Epstein and Wing (1980) who stated that *'…exercise may stimulate the appetite so that persons who exercise increase their eating and do not lose as much weight as expected'* and *'…a person who exercises in the early evening may go to sleep earlier or require more rest in the evening…'*. Speakman *et al.* (2002) in their review explained that alterations in the energy balance system will activate compensatory mechanisms to 'defend' body mass at its existing level, or to restore it to that level once conditions allow, thus providing reason why some individuals fail to lose weight with exercise. Increased energy intake is commonly assumed to be the compensatory mechanism responsible for a lack of, or lower than expected, exercise-induced weight loss (King *et al.* 2007). Fundamental to feeding behaviour is the appetite system, which modulates the energy intake side of the energy balance equation. The balance between energy intake and energy expenditure is maintained via a complex homeostatic system, involving both the brain and the peripheral nervous system (Spiegelman & Flier 2001). Under stable conditions, equilibrium exists between anabolic signals that stimulate feeding behaviour, as well as decrease energy expenditure and lipid utilisation in favour of energy storage, and catabolic signals that attenuate food intake, while stimulating sympathetic nervous system activity and restricting energy storage by increasing lipid metabolism (Blundell *et al.* 2008). This section henceforth will focus on the energy intake component of the energy balance system.

1.6 The Appetite System

Appetite is the internal driving force for the ingestion of food and is divided into three components: hunger, satiation, and satiety (Mattes *et al.* 2005). Hunger describes the motivational state that promotes food consumption and also reflects a physiologic state in which the metabolic fuels such as glucose and free fatty acids are low (Wardle 1987). Satiation is a process that occurs while foods are being eaten which govern meal size and duration (Blundell & MacDiarmid 1997). Following the initiation of a meal, hunger subsides while satiation becomes increasingly dominant. Eventually, feelings of satiation will contribute to the cessation of eating and begins a period of abstinence from eating.

The sensation that determines the intermeal period of fasting is termed satiety (Mattes *et al.* 2005). The control of appetite is a complex process, involving the interactions of central and peripheral organs to influence feeding behaviour in the short term, and also an adaptive process responding to energy input and energy expenditure in the long term (Delzenne *et al.* 2010).

1.6.1 Control of Appetite: Role of the Hypothalamus

The hypothalamus plays a pivotal role in the control of body weight by regulating energy intake and energy balance. Two main regions in the hypothalamus that are involved in feeding and satiety are the lateral hypothalamic nuclei which serve as a feeding or 'hunger' centre while the ventromedial nuclei serving as a 'satiety' centre (Wynne *et al.* 2005). The role of hypothalamus in regulating food intake was first established when it was observed that lesions to the ventromedial nuclei resulted in hyperphagia and obesity, while lesions to the lateral hypothalamic nuclei caused aphagia and weight loss (Anand & Brobeck 1951). The lateral hypothalamic nuclei contain glucose-sensitive neurons that are sensitive to hypoglycemia and it is crucial in mediating hypoglycemia-induced hyperphagia. The ventromedical nuclei mainly acts as a satiety centre and is a key target for leptin and insulin, which act on the hypothalamus to inhibit feeding, and stimulate energy expenditure (Bernadis & Bellinger 1996). In addition, the paraventricular nuclei (PVN) structure contains neurosecretory neurons that project into the arcuate nucleus (ARC), at the base of the hypothalamus, where the neuron terminals release peptides neuropeptide Y (NPY), agouti-related peptide (AgRP) and the melanocortin precursor, proopiomelanocortin (POMC), as well as being the main site of corticotrophin-releasing hormone (CRH) and thyrotropin releasing hormone (TRH) secretions (Neary *et al.* 2004).

The ARC, on the other hand, contains receptors for hormones and neuropeptides that regulate feeding (Arora & Anubhuti 2006). It is an area where the blood-brain barrier is modified to allow entry of various gut peptides and proteins including insulin and leptin, both of which are signals for adiposity (Banks 2008). The gut peptides act on the hypothalamus via the ARC to mediate appetite stimulation through activation of NPY and AgRP (Chen *et al.* 2004) or appetite inhibitory effects via POMC (Kristensen *et al.* 1998). NPY and AgRP act to stimulate feeding predominantly through activation of Y1 and Y5 receptors (Kanatani *et al.* 2000) and antagonism of the melanocortin MC3 and MC4 receptors. Stimulation of MC3 and MC4 receptors by POMC in turn, inhibits feeding

(Raffin-Sanson & Bertherat 2001). Afferent fibres of the vagus nerve also provide a gateway for neural signals (mainly satiety) from the gut to the brain stem (Williams *et al.* 2001). The brain stem is linked to the hypothalamus via projections from nucleus tractus solitarius neurones to the PVN and lateral hypothalamus (Neary *et al.* 2004).

1.6.2 Control of Appetite: Role of Gut Peptides

Episodic signals arising from the gastrointestinal tract such as peptide YY (PYY), ghrelin, cholecystokinin (CCK), and glucagon-like peptide 1 (GLP-1) regulate meal initiation and termination. Tonic signals such as leptin and insulin are responsible for maintaining energy balance and fat stores over weeks and months rather than meal-to-meal basis (Blundell *et al.* 2008). Together, the integration of episodic and tonic signals provides information to the hypothalamus that will further regulate feeding behavior to promote energy balance.

1.6.2.1 Peripheral Orexigenic Peptide: Ghrelin

Ghrelin is the only gastrointestinal hormone with potent orexigenic properties. It is a 28-amino acid peptide, primarily released by the oxyntic glands of the human gastric mucosa (Sakata *et al.* 2002), but is also isolated from other tissues such as the hypothalamus (Cowley *et al.* 2003), pancreas (Volante *et al.* 2002a), lungs (Volante *et al.* 2002b), and anterior pituitary gland (Korbonits *et al.* 2001). The majority of ghrelin actions are produced through its binding with receptor growth hormone secretagogue receptors 1a (GHSR-1a) which are found distributed in the brain, stomach, pancreas, kidney (Ueno *et al.* 2005) and expressed abundantly in the hypothalamus (Guan *et al.* 1997). Modification of its hydroxyl group at the third residue, a serine, with *n*-octonoic acid produces acylated form of ghrelin, which is essential for binding to the GHSR-1a, therefore deemed to be the biologically active form of ghrelin (Castaneda *et al.* 2010). Due to its widespread expression in various tissues, ghrelin exerts multiple physiological effects in the human body. Ghrelin is a potent releasing-stimulator of growth hormone from the anterior pituitary via activation of the GHSR-1a (Kojima *et al.* 1999). Apart from GH, ghrelin has also been reported to influence other endocrine secretions such as insulin (Tong *et al.* 2010) and cortisol (Schmid *et al.* 2005).

Perhaps the most widely discussed ghrelin function is its role in the regulation of food intake and energy balance. The effects of ghrelin on food intake are by a large part

mediated by the hypothalamic arcuate nucleus (ARC). Upon binding to its receptors, ghrelin stimulates the activity of neurons expressing NPY and AgRP, both of which are appetite-stimulating (orexigenic) peptides (Morton & Schwartz 2001). The secretion of ghrelin by the stomach depends largely on the nutritional state. Plasma concentration of ghrelin peaks under fasting conditions before a meal, suggesting a role in meal initiation (Cummings *et al.* 2001) and levels off to a nadir after a meal and increase again after gastric emptying before next meal (Cummings *et al.* 2005). Many studies have shown that intravenous infusion of ghrelin increases energy intake from buffet meals by in both lean and obese humans (Druce *et al.* 2006; Druce *et al.* 2005; Wren *et al.* 2001). Ghrelin concentrations have been shown to correlate with hunger scores (Cummings *et al.* 2004; Wren *et al.* 2001) and inversely associated with intermeal interval (Cummings *et al.* 2001). However, there is also strong evidence that ghrelin levels rise in anticipation of a meal, rather than eliciting a meal, and can be conditioned by habitual meal patterns (Frecka & Mattes 2008). Magnitude of ghrelin suppression in the postprandial period is dose-dependently related to amount of ingested calories (Callahan *et al.* 2004), and carbohydrate and protein suppress ghrelin more effectively than fat at equals loads (Foster-Schubert *et al.* 2008; Thorner *et al.* 2008).

Besides playing a role in the short-term regulation of food intake, ghrelin might also play a role in the long-term regulation of energy balance. Peripheral chronic administration of ghrelin in rodents results in prolonged hyperphagia and increased adiposity (Tschop *et al.* 2000). Supporting the role of ghrelin in inducing positive energy balance, some studies have shown that the pharmacologic blockade of ghrelin decreases food intake and body weight (Wortley *et al.* 2005) and mice lacking ghrelin signalling are protected against diet-induced obesity (Zigman *et al.* 2005). The putative adipogenic effects of ghrelin in humans however, remain to be shown because it is possible that ghrelin has different effects on energy balance in humans and rodents (Horvath *et al.* 2001). In humans, plasma ghrelin levels are inversely correlated with body adiposity (Tschop *et al.* 2001), being low in the obese, higher in lean subjects, and these levels increase with weight loss in obesity (Hansen *et al.* 2002). Whether low levels of ghrelin in obesity represent an adaptation to the state of positive energy balance or an increased sensitivity is open to debate.

1.6.2.2 Peripheral Anorexigenic Peptides: Peptide YY (PYY)

PYY, or peptide tyrosine-tyrosine, is a 36-amino acid peptide produced by the entero-endocrine cells of the ileum and colon (Adrian *et al.* 1985a). It is also found in the upper gastrointestinal tract, pancreas, and hypothalamus (Ekblad & Sundler 2002). There are two endogenous forms of PYY: PYY_{1-36} and PYY_{3-36}, the latter is produced by the removal of two N-terminal Tyr-Pro residues by the action of dipeptidyl peptidase IV (DDP-IV) in the colon (Grandt *et al.* 1994). Removal of the N terminal amino acids changes the receptor affinity of PYY_{3-36} to Y2 receptors, in contrast to PYY_{1-36} which binds to Y1, Y2, Y4 and Y5 receptors (Blomqvist & Herzog 1997). PYY regulates a wide range of gastrointestinal functions that are mainly inhibitory such as inhibiting gastric and pancreatic secretion (Adrian *et al.* 1985b), delaying gastric emptying (Allen *et al.* 1984) and increases the absorption of fluids and electrolytes from the ileum after a meal (Hoentjen *et al.* 2001). Such PYY-mediated effects on digestion processes, by essentially increasing absorption time lead to another well-known function of PYY as a potent satiety signal. In humans, PYY_{3-36} is the main form produced postprandially, with levels peaking to a plateau 1 – 2 hours following food intake and can remain elevated for up to 6 hours (Adrian *et al.* 1985a). PYY concentrations are proportional to caloric load (Degen *et al.* 2005), with progressively larger increases being seen after ingestion of fat as compared to carbohydrate and proteins (Adrian *et al.* 1985a). Helou *et al.* (2008) demonstrated that a high-fat meal induced immediate increase in postprandial PYY_{3-36}, whereas the postprandial increase in PYY_{3-36} following a high-protein meal was delayed, concluding that increasing both fat and protein content of a meal may induce an immediate and prolonged satiety effect in humans.

The role of PYY_{3-36} in satiation demonstrated in many studies makes this peptide a promising anti-obesity therapy (Batterham *et al.* 2002). Peripheral administration of PYY_{3-36} at doses generating postprandial physiological concentrations has been shown to inhibit food intake (Neary *et al.* 2004). PYY_{3-36} crosses the blood brain barrier freely to bind to the Y2 receptors in the hypothalamus (Ballantyne 2006), which leads to an inhibition of the NPY neurons in the arcuate nucleus and a possible reciprocal stimulation of the anorexigenic POMC neurons to decrease food intake through stimulation of satiety (Batterham *et al.* 2002). Consistent with this model, the satiating effects of $PYY_{3–36}$ are abolished by pharmacologic blockade of Y2 receptors (Scott *et al.* 2005). Peripheral administrations of $PYY_{3–36}$ demonstrate dose-dependent decreases in energy intake (le Roux *et al.* 2006), and is capable to inhibit food intake for several hours (Halatchev *et al.*

2004; Batterham *et al.* 2002). A study by Batterham *et al.* (2003) demonstrated a 30% reduction in caloric intake and subjective hunger during a buffet lunch offered two hours after the infusion of $PYY_{3–36}$ in the obese subjects, also suggesting that obesity is not associated with resistance to PYY, in contrast to obesity-related leptin resistance (Bjørbaek 2009). Interestingly, obese subjects have been shown to have relatively lower postprandial PYY levels compared to lean subjects (le Roux *et al.* 2006; Stock *et al.* 2005). However, PYY levels are reported to be elevated in obese patients following bariatric surgery (Reinehr *et al.* 2007) or vertical-banded gastroplasty (Alvarez *et al.* 2002), which may have contributed to reduced food intake and consequently weight loss post-surgery. Taken together, all these findings suggest a role of PYY in satiety regulation by reducing the energy intake side of the energy balance equation.

1.6.3 Control of Appetite: Role of Adiposity Signalling

Apart from the neural stimulation and gut peptide signals that help regulate food intake on a meal-to-meal basis, satiety processes can be induced by changes in body adiposity (*i.e.* adiposity signals). Adiposity signalling involves circulating hormones which are relatively constant and proportional to adipose tissue mass and act as tonic signals to the brain for regulating food intake and body weight over long periods of time (Trayhurn & Bing 2006). Critical elements of this control system are hormones secreted in proportion to body adiposity, which are leptin and insulin. Insulin was the first hormone to be implicated in the hypothalamic control of food intake and long term stability of body weight (Kennedy 1953). Central administration of insulin to the hypothalamus caused a reduction in food intake in dogs (Baura *et al.* 1993). The *ob/ob* mouse, completely deficient in leptin, is characterised as hyperphagic, hyperinsulinaemic, and obese, while chronic administration of leptin to the *ob/ob* mouse results in sustained reduction in body weight, and reduced food intake (Chua *et al.* 1996). During states of negative energy balance (*i.e.* fasting, starvation), less insulin and leptin are secreted. As a result, anabolic pathways are stimulated which leads to conditions that favour increased food intake and energy storage. Conversely, during states of positive energy balance, the adipose tissue expands and increases concentrations of leptin and insulin. The resulting output from the brain favours reduced food intake and a reduction of the size of adipose mass. These key negative feedback pathways help maintain stability of the size of body adiposity over time (Trayhurn & Bing 2006).

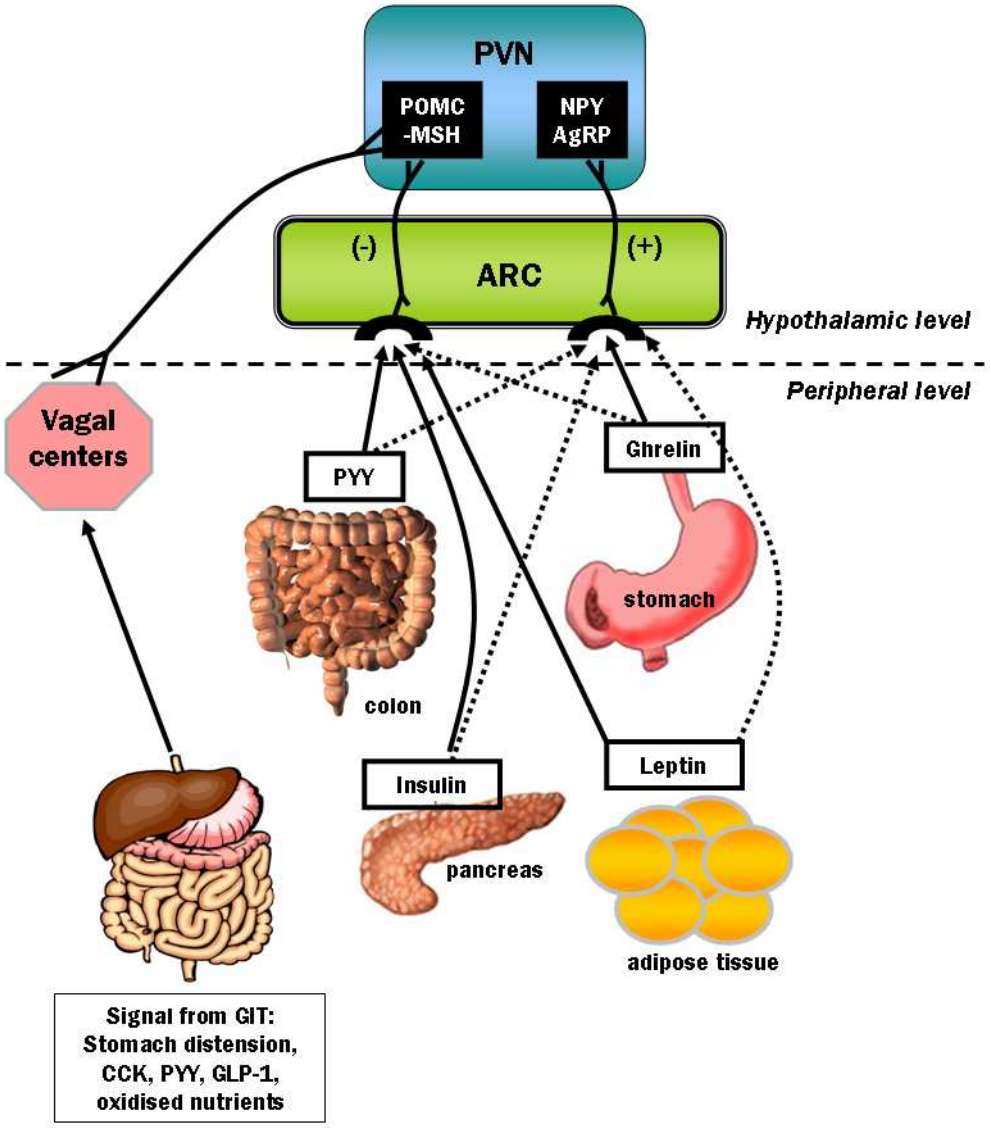


Figure 1.3. **Model of central and peripheral regulation of appetite and food intake**. Solid arrows represent stimulatory action while dotted arrows represent inhibitory action. Plus signs indicate hunger stimulation, and minus signs indicate satiety stimulation. In the short-term regulation of food intake, ghrelin, released from empty stomach, binds to the receptors in the ARC to activate NPY and AgRP containing neurons to stimulate hunger, while inhibiting POMC-MSH neurons. In contrast, following a meal, PYY from colon binds to the receptors in the ARC, stimulating POMC-MSH neurons to activate satiety, while inhibiting the ghrelin-NPY/AgRP pathway. Signals from the gastrointestinal tract and various other gut hormones act on the hypothalamic centers via vagus nerve to reduce food intake. Long-term (tonic) signals such as insulin and leptin, released in proportion to body adiposity, positively regulate POMC-MSH neuronal pathways, stimulating satiety center and reducing activity of NPY/AgRP neurons driving the appetite behaviour. *Abbreviations*: AgRP: agouti-related peptide; ARC: arcuate nuclei; CCK: cholecystokinin; GLP-1: glucagon-like peptide 1; GIT: gastrointestinal tract; NPY: neuropeptide Y; PVN: paraventricular nuclei; POMC-MSH: melanocortin-derived proopiomelanocortin; PYY: peptide YY.

1.6.4 Control of Appetite: Role of Non-Metabolic Factors

Traditional views of the regulation of energy homeostasis pointed strongly to the hypothalamus as the key area for the physiological controls of appetite and energy balance. Recently however, it was also considered that the regulation of appetite is not limited to the hypothalamus, but engage other parts of the brain, particularly the caudal brainstem and limbic forebrain circuit, which include the hippocampus, amygdala and the substantia nigra; areas that are implicated in mediating motivation and reward aspects of feeding behaviour (Figlewicz & Benoit 2009). This led to the expanded view of the control of energy homeostasis which includes neural integration of metabolic and behavioural drives in the regulation of food intake. It has been identified that adiposity signals and other metabolic signals can modulate brain areas that are involved in the processing of external food cues (*e.g.* sight and smell of palatable food) and reward functions (*e.g.* liking, wanting) (Blundell *et al.* 2010; Figlewicz *et al.* 2007). Liking reflects the immediate experience or anticipation of pleasure from the orosensory stimulation of eating a food of tasting a particular food (Mela 2006). Neural circuits in the hindbrain, as well as areas in the ventral striatum and amygdala are involved in the expression of liking (Shin *et al.* 2009). Intracerebroventricular (ICV) infusion of insulin in rats decreased activity in a 5-min sucrose 'lick-rate' task, which represents a 'pure hedonic' response of the animal to a solution (Figlewicz *et al.* 2007).

Wanting, or incentive salience, refers to the desire to actually ingest a particular food, and is usually, but not always, follows liking (Blundell *et al.* 2010). While liking is a sensory process, wanting is linked to the mesolimbic dopamine system, which is crucial for the orchestration of motor action to obtain rewards (Shin *et al.* 2009). Leptin and ghrelin can act directly on the mesolimbic dopamine neurons to modulate 'wanting' of food; leptin administration decreased firing rate of dopamine neurons and food intake (Hommel *et al.* 2006) while ghrelin ICV in rats increases progressive ratio performance to obtain food reward (Jewet *et al.* 2006). Thus, metabolic feedback signals involved in appetite do not act exclusively on hypothalamus, but also on sensory pathways and cortico-limbic structures, indicating that hedonic processes and cognitive functions are also important for the control of food intake and the regulation of energy balance (Shin *et al.* 2009). This concept is especially important for the study of feeding behaviour in the obesogenic, modern environment, where food is easily-accesible, energy-dense, and physical activity is being reduced to a luxury afforded by environment and lifestyle (Finlayson *et al.* 2007).

1.6.5 Control of Appetite: Role of Physical Activity

Physical activity could influence the regulation of food intake by adjusting the sensitivity of appetite control or by altering energy balance that could adjust the drive to eat (King *et al.* 1997). Many efforts recently have also looked into how exercise can modulate appetite control by investigating gut peptides in response to exercise. Ghrelin and PYY are among the short-term signals that received wide attention as they are responsible for meal-to-meal regulation and have potential correlation with subjective feelings of hunger and satiety (Benelam 2009). Leptin and insulin levels, while they are responsible for long-term regulation of energy balance and appetite, their levels are unlikely to have changed in short-term exercise interventions where no change in body composition is observed (Kraemer *et al.* 2002).

1.6.5.1 Effects of Acute Exercise on Appetite and Energy Intake

Exercise leads to an increase in energy expenditure, and therefore results in negative energy balance. It seems rather natural that the energy deficit induced by exercise would be compensated for by an increase in food intake to maintain energy balance. This assumption was first made 50 years ago by Mayer *et al.* (1956), through a study assessing the relationship between physical work and caloric intake in Indian men, implying that '*the regulation of food intake functions with such flexibility that an increase in energy output due to exercise is automatically followed by an equivalent increase in caloric intake*'. However, evidence to date is still ambiguous. The majority of studies have shown that acute aerobic-type exercise did not alter energy intake and hunger sensations (King *et al.* 2010b; Unick *et al.* 2010; Malkova *et al.* 2008; Imbeault *et al.* 1997; King *et al.* 1997b; Westerterp-Plantega *et al.* 1997; Thompson *et al.* 1988), and a few studies have reported reductions in hunger sensations without concomitant changes in food intake (King *et al.* 2010a; Borer *et al.* 2009; Lluch *et al.* 1998; King *et al.* 1997).

However, other studies have documented an increase in appetite sensations (King *et al.* 2011a; Dodd *et al.* 2008; Maraki *et al.* 2005; Verger *et al.* 1992) and absolute energy intake (Finlayson *et al.* 2009; Martins *et al.* 2007a; George & Morganstein 2003; Verger *et al.* 1994; Verger *et al.* 1992) in response to acute exercise. The lack of consistency among studies could be attributed to variations in exercise intensity (Thompson *et al.* 1988), mode of exercise (King & Blundell 1995), nutritional state (Durrant *et al.* 1982),

or composition of test meals (Hubert *et al.* 1998). In addition, exercise has been shown to improve appetite sensitivity in response to covert preload energy manipulation, where active individuals seem more able to distinguish between preloads by adequately adjusting energy intake at a subsequent meal, denoting a better short-term appetite control (Martins *et al.* 2007b; Van Walleghen *et al.* 2007; King *et al.* 1999). Overall, the evidence points to a rather weak coupling between energy expenditure and energy intake.

1.6.5.2 Effects of Chronic Exercise on Appetite and Energy Intake

In one of the earliest work of monitoring the coupling between energy intake and energy expenditure over a long term, Edholm *et al.* (1955) demonstrated that there was a significant correlation between energy expenditure and energy intake 2 days later in military cadets. This led to Edholm (1977) to remark that '*we do not eat for today but for the day before yesterday*'. In further attempts to investigate this delayed compensatory response, many studies however, have found no compensation in energy intake in response to physical activity interventions (Blundell & King 1999; McGowan *et al.* 1986; Dickson-Parnell & Amos 1985; Woo *et al.* 1982). The limitations with long-term exercise intervention studies are that most energy intakes were monitored using dietary record, which may not always be accurate, and subjects often had a preconceived goal associated with weight reduction, therefore under these conditions, energy intake can be controlled deliberately and may not be entirely *ad libitum*.

A few studies have reported a partial compensatory increase in energy intake corresponding to increases in energy expenditure in long term studies ranging from 6 – 19 days (Whybrow *et al.* 2008; Stubbs *et al*. 2002a; Woo & Pi-Sunyer 1985a; Durrant *et al.* 1982). Irrespective of gender, there is also a considerable variation in the extent of compensation, which may explain the variable success in exercise interventions on weight loss because some individuals possess adaptive mechanisms to oppose the negative energy balance resulting from the imposed exercise (Finlayson *et al.* 2011; King *et al.* 2008). Overall, there is very little evidence of complete compensation in energy intake in response to exercise-induced energy expenditure. However partial compensation is possible. Partial compensation for exercise-induced energy deficits is detectable over two weeks, and is slow and variable between individuals (Blundell *et al.* 2003). Reasons for variability in energy intake compensation for the increased energy expenditure from exercise are still unclear, but might be at least partially explained by exercise-induced changes in neural and/or hormonal stimuli that influence sensations of hunger and satiety.

1.6.5.3 Effects of Exercise on Gut Peptides: Ghrelin

Among many gut peptides, ghrelin is the only gut peptide associated with meal initiation, and as its levels correlate with energy deficit (Cummings *et al.* 2005), it is possible that exercise may influence ghrelin levels. Similar to energy intake, studies on the effects of acute exercise on plasma ghrelin are equivocal; many studies have reported no change (King *et al*. 2010b; Burns *et al.* 2007; Zoladz *et al.* 2005; Kraemer *et al.* 2004a; Schmidt *et al.* 2004), in addition to both exercise-induced suppression (Marzullo *et al*. 2008; Malkova *et al.* 2008; Toshinai *et al.* 2007; Vestergaard *et al*. 2007; Kraemer *et al.* 2004b) and augmentation (Jürimäe *et al.* 2007; Erdmann *et al.* 2007; Borer *et al.* 2005). The ambiguity in studies concerning acute exercise and ghrelin could be attributed to one important determinant: exercise energy expenditure. It is well known that energy deficit is a strong stimulus for ghrelin increase (Hagobian & Braun 2010; Ravussin et *al.* 2001), hence the likelihood for ghrelin levels to change may not occur with relatively small exercise-induced energy expenditures. Additionally, the effects of exercise on ghrelin levels can vary with subjects' gender (Hagobian *et al.* 2009), body adiposity (Marzullo *et al.* 2008), exercise intensity (Fathi *et al.* 2010), among others, which may explain why findings are inconclusive. Several studies have examined changes in ghrelin with chronic exercise and reported increase in fasting ghrelin levels (Kelishadi *et al.* 2008; Foster-Schubert *et al.* 2005; Leidy *et al.* 2004). One interesting point to note from these studies was that ghrelin levels increased commensurately with the amount of weight lost and no changes were observed in subjects who were weight stable post-intervention, thus suggesting that ghrelin levels are only altered if changes in body mass occur. Indeed, data from recent studies measuring changes in acylated ghrelin concentration with long-term exercise training in overweight adolescents paralleled with previous findings that exercise training does not affect ghrelin concentrations in the absence of weight loss (Jones *et al.* 2009; Kim *et al.* 2008). The mechanisms underlying this response remain to be elucidated.

1.6.5.4 Effects of Exercise on Gut Peptides: PYY

Less is known regarding the response of other gut hormones to exercise. Only few investigators have examined the effects of exercise on alterations in PYY. Several studies have demonstrated increases in fasting and postprandial levels of other satiety hormones,

e.g. pancreatic polypeptide (PP) and GLP-1 after exercise (Unick *et al.* 2010; O'Connor *et al.* 1995), but there is a paucity of data on PYY. Emerging evidence is suggesting that exercise may stimulate PYY levels in a direction to suppress energy intake, which may provide a physiological explanation for reduced or no changes in food intake following exercise bouts. Ueda *et al.* (2009b) and Broom *et al.* (2009) recently reported that PYY levels were increased after an acute bout of moderate exercise, followed by a concomitant reduction in energy intake (Ueda *et al.* 2009b). With regard to long-term exercise training, one study has observed an increase in PYY concentrations after an 8-week aerobic exercise (Jones *et al.* 2009). Collectively, these findings suggest that PYY may regulate appetite during and after exercise, but more research is required to establish the role of exercise in modulating PYY concentrations.

1.7 Issues Associated with Measurements of Energy Intake

The measurement of food intake is crucial to the effort in understanding how exercise can influence energy intake, however this has proved to be very difficult in practice. The optimal experimental protocol is likely to remain elusive because of the complex and multifaceted nature of eating behaviour (Blundell *et al.* 2010). Reproducibility, sensitivity, and feasibility are among the issues that are often associated with the methods in assessing energy intake in laboratory studies. In addition, behavioural components such as dietary restraint and disinhibition should be taken into account when recruiting subjects for appetite-related studies.

1.7.1 Dietary Record

The traditional way of measuring food intake in the free living setting is by using the diet-diary method, in which subjects are asked to record weight and type of food consumed on a daily basis for up to 7 days. Provided all food and leftovers are named and weighed and accurately, and the normal feeding pattern is not altered in the process, this procedure can produce a reliable and valid estimate of food intake (Bingham 1987). However, a reporting bias in measuring food intake with this method can easily affect the outcome of intervention studies (Lissner *et al.* 1998). Under-reporting is the most common bias, with discrepancies between reported energy intakes and measured energy expenditures (measured with the doubly labelled water method) by as much as 20-50% below what is normally ingested in both lean (de Castro 2000; Goris & Westerterp 1999) and obese

individuals (Goris *et al.* 2000). Thus, measurements of food intake using dietary records need to be scrutinised with care to ensure that the right conclusions are drawn.

1.7.2 Buffet-Style Meals

Laboratory-based, buffet-type meals serve as a popular alternate to the dietary record method, in which subjects are provided with a selection of food to choose from, and food weighing is done in a covert manner. Although the use of a buffet-type meal is not fully representative of free-living conditions and can lead to overfeeding, it can allow measurements of *ad libitum* energy and macronutrient intakes and can be used to assess short-term energy compensation, with subjects being blinded to real purpose of the buffet (Blundell *et al.* 2010). With little contamination from external influences, it is considered a valid and reliable method to assess feeding behaviour (Arvaniti *et al.* 2000; Stubbs *et al.* 1998).

1.7.3 Appetite Questionnaires (VAS)

Appetite is a subjective concept used as a proxy of food intake and can be defined as a range of sensations associated with food consumption (Martins *et al.* 2008a; Blundell 1991). Visual analogue scales (VAS) are the most common form of assessment to quantify subjective ratings of appetite, using appetite-related terminologies developed by Rogers and Blundell (1979). VAS exhibits a good degree of reliability and reproducibility, sensitive to exposure of food components, and is predictive of energy intake in experimental conditions (Flint *et al.* 2000; Stubbs *et al.* 2000). When used appropriately, VAS can provide useful information when combined with other aspects of feeding behavior (Stubbs *et al.* 1998).

1.7.4 Biomarkers

Biomarkers can be either indicators of appetite, or they can be proven to be causal factors of appetite (Delzenne *et al.* 2010). The use of gut peptides as biomarkers for feeding behaviour is rapidly gaining interest as these gut signals form an integral part in the mechanisms behind the regulation of food intake and energy balance in humans. For a biomarker to be useful, it should be feasible, reproducible, sensitive, and specific (de Graaf *et al.* 2004). Feasibility represents relatively obtainable results by using methods that are both ethical and minimally invasive, and can be used to assess the effects of

interventions within a reasonable time. The sensitivity and specificity reflect the strength of the relation between marker and measures of appetite (de Graaf *et al.* 2004). Biomarkers however, cannot be used on their own to quantify satiety or hunger, but together with other measurements they can be indicators of appetite and food intake (Delzenne *et al.* 2010).

1.7.5 Computer-Based Procedure

Recently new to the assessment of food intake is a computer-based procedure. Several investigators have employed this innovative approach (Brunstrom & Rogers 2009; Finlayson *et al*. 2009), which can be designed to assess various indexes associated with food intake and feeding behaviour. Traditionally, methods such as test meals or buffet meals and visual analogue scales are used to assess the acute effects of exercise on food intake or preference. However, these methods are not designed and may not be sensitive enough to detect more subtle exercise-induced alterations in the hedonic measures as well as behavioural and cognitive processes that influence food intake (Finlayson *et al.* 2009). Furthermore, computer-based procedures allow for assessments using a wide array of food items that is usually impractical with buffet meals, in a relatively short amount of time.

1.7.6 Restraint and Disinhibition

Appetite is not only regulated by physiological processes, but also from external stimuli arising from food and the surrounding environment such as hedonic, psychological, social and cultural stimuli. In the face of current obesity pandemic, restricting food intake in order to maintain or lose weight is becoming an important behavioural concept (Martins *et al.* 2008b). Dietary restraint refers to the extent to which individuals are concerned with their body weight and it characterised by self-imposed resistance to internal and external cues that regulate feeding behaviour, *e.g.* dieting (Herman & Mack 1975). It is therefore a common practice to exclude restrained eaters from appetite-related studies on the basis that they have tendencies to exhibit atypical eating behaviour, as well as showing altered metabolic (Westerterp-Plantega *et al.* 1992) and endocrine functions (Pirke *et al.* 1990). Disinhibition reflects a tendency towards over-eating and failure to maintain dietary restriction. It includes eating in response to negative emotions (*e.g.* distressed, upset), over-eating when others are eating, not being able to resist stimulation to eat and over-eating in response to the palatability of food (Bryant *et al.* 2008). In relation to appetite,

individuals with high disinhibition scores are associated with a higher liking for and consumption of high-fat foods, sweet foods and alcohol, and negatively associated with the consumption of vegetables, fruit and high-fibre bread (Contento *et al.* 2005). Disinhibition has also been linked to an increased tendency to eat when people are subjected to various challenges or interventions that threaten to disturb energy balance (*i.e.* exercise) (Bryant *et al.* 2008). This raises the likelihood of exercise-induced compensation in energy intake in exercise studies.

1.7.7 Attachment Behaviours

Attachment behaviour is a broad theory of social development that describes the representational model of close interpersonal relationships, and it reflects early-life interactions with primary caregivers. Attachment behaviours are interpersonal actions that are intended to increase an individual's sense of security, particularly in times of stress or need (Ravitz *et al.* 2010). These are assessed in terms of two dimensions (*i.e.* anxiety about abandonment and avoidance of intimacy) and a high score on one or both of these dimensions is taken as evidence of an insecure attachment orientation (Brennan 1998). Adult attachment is becoming increasingly important in psychosomatic research because attachment influences many biopsychosocial phenomena, including social functioning, stress response, psychological and well-being (Ravitz *et al.* 2010). It was recently noted that attachment anxiety is evident in eating behaviour, and is particularly associated with disinhibited eating and increase in BMI, manifested by the tendency to seek comfort through overeating (Wilkinson *et al.* 2010). Over time, this behaviour may lead to a positive energy balance and weight gain. Identifying attachment orientation as a potentially important aetiology of disinhibited eating may provide insight into the many behavioural aspects of feeding behaviour.

1.8 Aims of Thesis

A large amount of evidence has shown that exercise is effective at ameliorating metabolic perturbations during postprandial state by lowering postprandial triglyceridaemia and insulinaemia as well as enhancing fat oxidation. However, most of the effects observed have been in absence of *ad libitum* food consumption. The question of whether exercise is as potent in favourably altering postprandial metabolism when meals are consumed *ad libitum* as compared to standardised laboratory test meals, is currently unknown as no such work has been published. Furthermore, given that increase in food intake being the most common form of compensatory response to exercise-induced energy deficits (King *et al.* 2007), this could pose a potential limitation on the effectiveness of exercise in inducing negative energy and fat balance. Therefore, the first experimental chapter (Chapter 3) of this thesis was aimed to determine the effects of exercise on postprandial metabolism in response to *ad libitum* feeding, in an effort to replicate free-living conditions more closely.

Evidence from the short-term studies on exercise and energy intake is equivocal, with most studies pointing to a loose coupling between exercise and energy intake (Hopkins *et al.* 2010). It is likely that the body cannot detect small changes in energy balance, which might explain why some acute exercise studies failed to observe any compensation in energy intake. Therefore it is possible that there is a threshold for energy deficit to be achieved before the compensation drive in energy intake is kicked in. The aim of Chapter 4 is to investigate the effects of different levels of exercise-induced energy deficits on *ad libitum* food consumption, appetite behaviour and gut peptide responses (*i.e.* ghrelin, peptide YY).

The published literature present strong evidence that exercise, when undertaken in the fasted state, is very effective in inducing negative fat balance. However, as food is consumed under free-living conditions, and considering that fat oxidation can be suppressed following carbohydrate consumption, this can potentially reverse the negative fat balance induced by exercise. Thus, in determining the optimal exercise condition to maximise negative fat balance, the entire day should be evaluated, from pre-exercise, throughout post-exercise periods and subsequent meal intake. Chapter 5 was designed to address the gaps in the literature relating to maximising periods of negative fat balance

across daily meals and exercise periods, by investigating the effects of timing of meal around exercise period on postprandial metabolism as well as feeding behaviour.

In human feeding behaviour, it is well known that the stimulus to eat is can be influenced by non-metabolic/behavioural (*e.g.* hedonics, reward, restraint) factors, which can sometime outweigh internal state signals. Because of this, the measurement of energy intake using the traditional buffet-style meal method can pose limitations in appetite-related studies. Therefore, in the final experimental chapter of this thesis, a pilot study was designed to determine the factors that are associated with feeding behaviour using a novel, computer-based approach, in response to acute exercise.

And finally, because lean individuals seem to be able to maintain stable body weight better than their overweight counterparts (Hankinson *et al.* 2010), and perturbations in postprandial metabolism are a common feature in obesity (Gill *et al.* 2004; Lewis *et al.* 1990) and men (Knuth & Horowtiz 2006; Kolovou *et al.* 2006), this particular subset of the population was therefore chosen for most of the investigations.

CHAPTER 2

General Methods

This chapter provides a description of all general methods that have been implemented in the following experimental chapters. Methods specific to individual chapters will be highlighted as such. Methods used for statistical and data analyses are not reported here but rather are described separately in each experimental chapter.

2.1 Subject Recruitment and Screening

Subjects were recruited from the student population of University of Glasgow and residents in the Glasgow area via local advertising and advertisement websites. All subjects were required to attend a screening visit at the university prior to participation to ensure they met with the inclusion criteria of each study. They were provided with an information sheet describing the aim of the study, the experimental procedures involved and any potential risk or discomfort associated with these procedures. Written, informed consent was recorded for each subject. Questionnaires detailing the subject's past and present health status and family history of disease were completed **(Appendix A)**. Resting blood pressure was measured using an automated sphygmomanometer (Omron Healthcare, Inc., Illinois, USA) and fasting finger-prick blood samples were taken to determine glucose and total cholesterol levels using Reflotron® Plus instrument and Reflotron® Test reagent strips.

2.2 Anthropometric Measurements

2.2.1 Standing Height

Height was measured using the stretch stature method on a stadiometer (Seca, Hamburg, Germany). Stature is the maximum distance from the floor to the highest point of the skull when the head is held in the Frankfort plane position (Ross & Marfell-Jones 2001). Measurement was recorded to the nearest 0.1 cm.

2.2.2 Body Mass

Body mass was measured in light and minimal clothing and without shoes using a balanced-beam scale. Measurement was recorded to the nearest 0.01 kg. Body mass was measured using the same balance scale throughout all experimental studies. BMI was then calculated at weight in kilograms divided by the square of height in meters.

2.2.3 Circumference Measurement

Waist and hip circumference were measured in contact with the skin using a flexible, steel tape measure (Supralip®160, West Germany). Waist circumference was taken with subjects standing with feet shoulder-width apart and arms on the side and landmarked as the narrowest part of the torso, mid-way between the inferior margin of lowest rib and the iliac crest with the abdominal muscles relaxed. Hip circumference was taken with the subjects standing with feet together and arms the side and landmarked as the maximum circumference over the trochanters (buttocks) (Lean et al. 1995). The tape was placed horizontally directly on the skin with respect to both landmarks. All measurements were taken at the end of a normal expiration, with repeat measurements. If the two measurements disagreed by more than 1 cm, a third measurement was made.

2.2.4 Skinfold Thickness Measurement

A skinfold thickness is defined as a measure of the double thickness of the epidermis, underlying fascia and subcutaneous adipose tissue on different standard anatomical sites around the body. The following four sites were used according to Durnin and Womersley (1974) who validated the sum of four skinfold thickness against densitometry and devised sex- and age-dependent population-based linear regression equations to estimate total body density:

1) **Biceps** : vertical skinfold raised on the anterior aspect of the biceps;
2) **Triceps**: vertical skinfold raised on the posterior aspect of the triceps, mid-way between the olecranon process and the acromion process (shoulder) when the hand is supinated;

3) **Subscapular**: oblique skinfold raised 1 cm below the undermost tip of the inferior angle of the scapula at approximately 45° to the horizontal plane following the natural cleavage lines of the skin;
4) **Suprailiac**: diagonal fold raised immediately superior the crest of the ilium on a vertical line from the mid-axillary line

Skinfold sites were landmarked on the body prior to measurement so that repeat measures can be taken at the same place. The skin at each respective site was pinched up firmly between thumb and forefinger to raise a double layer of skin and the underlying adipose tissue, excluding the muscle tissue. The calipers were then applied to the fold with 1 cm between the edge of fingers and the nearest edge of the calliper and a reading in millimeters (mm) was recorded. All skinfold measures were taken on the right side of the body with skinfold callipers (Holtain Ltd., Crymych, UK). Measurements were recorded in duplicate for each site, not taken consecutively but by running through all sites once and back again as to allow the skin to regroup between measurements. If the readings for each site were more than 5% apart, a third measurement would then be taken, and the two closest measurements were taken for calculation. The sum of the four skinfolds (Σ4SF = biceps + triceps + subscapular + suprailliac) was calculated. Relative fat mass was derived from the formula of Durnin and Womersley (1974) equation for estimating body density in combination with Siri's equation for estimating body fat percentage (Siri 1961):

$$\text{Density } (g.cm^3) = c - m\,(\log \Sigma 4SF) \qquad \textbf{(Eq. 2.1)}$$

where:
c and m = standard age and sex-specific coefficients
Σ4SF = sum of all four-site skinfolds (mm)

Once the density was calculated, the Siri equation was used to estimate body fat percentage:

$$\text{Body fat percentage } (\%) = [(4.95/\text{body density}) - 4.5] \times 100 \qquad \textbf{(Eq. 2.2)}$$

where:
D = density
4.95 and 4.5 = constants

2.3 Incremental Submaximal Exercise Test

In Chapters 3, 4, and 5, a submaximal incremental exercise test was performed to predict maximum oxygen consumption ($\dot{V}O_2$max) for each subject prior to commencing main trials. The test was designed to exercise subjects through a range of intensities from moderate to vigorous but not maximum. The test consisted of four, continuous 5-min stages of walking on a treadmill to determine the relationship between gradient and oxygen consumption at self-selected walking speed of about 5 – 6 km·h^{-1} (**Figure 2.1**). The first stage of the test was performed on a level treadmill and gradient was increased by 2.5 – 3.0% at the end of every stage depending on subject's heart rate response in the previous stage: if heart rate exceeded 100 beats per minute in the first stage, a 2.5% increment was used for subsequent stages. Each stage lasted five minutes with expired air being collected into Douglas bags during the last two minutes for the determination of oxygen uptake and carbon dioxide output using the Douglas bag method (described in section 2.4.2). Five-minute stages were performed to ensure subjects were in steady state during expired air collection periods. Heart rate was recorded continuously during the test and the Borg scale was used to assess subject's perceived exertion simultaneously with the expired air collections at the end of every stage. The test was terminated if subject's heart rate reached 85% of his predicted maximum heart rate. At the end of the test, the oxygen uptake at each stage was plotted against the heart rate and gradient to estimate the gradient and speed necessary to elicit an intensity corresponding to 50% $\dot{V}O_2$max during the main trials.

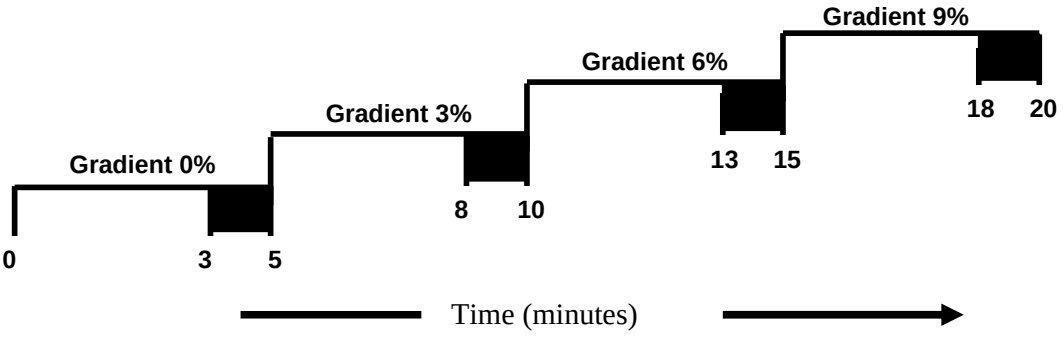


Figure 2.1: **A schematic diagram of a 4-stage submaximal incremental test** (black boxes represent expired air collection and heart rate measurements).

2.3.1 Heart Rate Monitoring

Exercise heart rates were monitored using a Polar® heart rate telemetry system which consisted of a heart rate transmitter and a wrist receiver (POLAR, Kempele, Finland) during the submaximal exercise test and all exercise trials. Exercise heart rates were obtained at 30-second intervals during the last one minute of every expired air collection stage and the average was then calculated.

2.3.2 Rating of Perceived Exertion (RPE)

RPE was determined using a 15-point category scale (from 6 to 20) introduced by Borg (1970), at the end of every expired air collection stage in all exercise bouts by presenting the subjects with the scale within easy reach and asking them to point to the number that corresponded to their respective level of effort and exertion.

2.4 Expired Air Measurements

2.4.1 Resting Metabolic Rate

Resting metabolic rate (RMR) was measured at baseline using open circuit indirect calorimetry system with a ventilated hood (Oxycon Pro, Jaeger GmbH, Hoechberg, Germany) (**Figure 2.2**). The apparatus included a high-speed differential paramagnetic O_2 sensor and an infrared absorption CO_2 analyser. Before each test, the gas analyser was calibrated using an automated calibration procedure, as provided by Jaeger, whereby a calibration gas mixture (16% O_2, 5% CO_2) was introduced to the system. A bi-directional flow-volume sensor (consisting of an amplifier, Triple V, and the pressure transducer) calibration was also performed using a calibrated 3-liter syringe connected to the Triple V assembly. A series of six complete pumps of the syringe was repeated until the percent difference between the current and the previous volume calibration was less than 1%. Further corrections were made for barometric pressure on the system.

In Chapter 3 and 4, RMR measurement was performed prior to commencing main trials to help estimate energy requirements for the 3-day standardised diet for each subject. For RMR determination, subjects arrived in the laboratory in the morning following a 12-hour

overnight fast. Before the start of each measurement, each subject was asked to lie quietly in a semi-recumbent position for 10 min in a temperature-controlled (21°C – 23 °C) environment. Next, a transparent a ventilated plastic hood connected to the gas mixing chamber by corrugated flexible plastic tubing was placed over the subject's head. Ventilation was run through the system by means of the flow volume sensor unit. Rates of oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were obtained at intervals of 1 min. Measurement was performed for 20 minutes and the last 15 minutes of steady-state values were averaged to determine RMR.

Figure 2.2. Resting metabolic rate / substrate utilisation measurement using the open-circuit indirect calorimetry system with a ventilated hood

2.4.2 Postprandial Substrate Utilisation

For measurement for substrate utilisation during the postprandial period (in Chapters 3 and 5), the same procedure described above was run through with measurements of $\dot{V}O_2$ and $\dot{V}CO_2$ taken every minute for 15 minutes and the last 10 minutes of steady-state values were averaged.

2.4.3 Measurements of Expired Air During Exercise

Expired air during exercise was collected using the Douglas bag method, which involves the collection of exhaled air in large, impermeable canvas bags and subsequent measurement of gas fractions and expired volumes. The bags should be completely

emptied using an exhaust pump prior to usage. Subjects were fitted with a rubber mouthpiece and breathed ambient air through a two-way non-rebreathing valve (Kansas City, MO) which was connected to a previously evacuated 100-liter Douglas bag (Hans Rudolph P/N 112377, Hans Rudolph Inc., Kansas City, USA) on the expired side by a 1 m corrugated flexible plastic hose of a 3.2 cm diameter. A nose clip was worn to prevent nasal breathing. After gas collection, a small quantity of air was extracted from the used Douglas bag at a constant flow rate (300 $ml \cdot min^{-1}$), measured by a flow meter and then passed into a gas analyser (Servomex, Sussex, UK) to determine fractions of O_2 and CO_2 in the bag. The analyser was calibrated prior to each test using certified reference gases (BOC Gases, Surrey, UK) of known concentration (*e.g.* 100% nitrogen, 16% O_2, 6% CO_2). The remaining volume of air in the Douglas bag was vacuumed out using a dry gas meter (Harvard Apparatus, Kent, UK). Expired air volumes and temperature were recorded. Expired gas fractions and volumes were then corrected for standard temperature (0ºC) and barometric pressure at sea level (760 mmHg) to determine $\dot{V}O_2$ (STPD), $\dot{V}CO_2$ (STPD), and respiratory exchange ratio (RER). RER was calculated as $\dot{V}CO_2$ divided by $\dot{V}O_2$.

2.4.4 Expired Air Analysis

Calculation of carbohydrate and fat oxidation rates, and energy expenditure were estimated from $\dot{V}O_2$ (STPD) and $\dot{V}CO_2$ (STPD) according to stoichiometric equations of Frayn (1983). According to the formula, O_2 uptake and CO_2 production can be assumed as:

$$\dot{V}O_2 \ (l \cdot min^{-1}) = 0.746\,c + 2.03\,f + 6.04\,n \qquad \textbf{(Eq. 2.3)}$$

$$\dot{V}CO_2 \ (l \cdot min^{-1}) = 0.746\,c + 1.43\,f + 4.89\,n \qquad \textbf{(Eq. 2.4)}$$

where;

c = carbohydrate oxidation in grams per minute

f = fat oxidation in grams per minute

n = urinary nitrogen excreted in grams per minute

For carbohydrate oxidation, a $\dot{V}O_2$ of 0.746 litres is associated with glucose oxidation but increases to 0.829 litres if glycogen is preferentially oxidised. Using equations based on glycogen as a fuel source in a situation where glucose oxidation predominates may lead to a substantial underestimation of carbohydrate oxidation (Jéquier *et al.* 1987). These equations have been derived assuming that there is an absence of net lipogenesis, gluconeogenesis, or ketogenesis or any acid-base disturbances. These equations were then solved for *c* grams of carbohydrate and *f* grams of fat oxidised per minute:

$$f\ (g \cdot min^{-1}) = 1.67\ \dot{V}O_2 - 1.67\ \dot{V}CO_2 - 1.92\ n \quad \textbf{(Eq. 2.5)}$$

$$c\ (g \cdot min^{-1}) = 4.55\ \dot{V}CO_2 - 3.21\ \dot{V}O_2 - 2.87\ n \quad \textbf{(Eq. 2.6)}$$

Nitrogen excretion rate was assumed based on data from similar studies in the literature to be 0.00011 $g \cdot kg^{-1} \cdot min^{-1}$ throughout all trials (Romijn *et al.* 1995; Flatt *et al.* 1985). Energy expenditure (EE), expressed in $kcal \cdot min^{-1}$, was calculated as the sum of each macronutrient oxidation rate ($g.min^{-1}$) multiplied by the appropriate conversion factor (glucose = 3.7 $kcal \cdot g^{-1}$; fat = 9.3 $kcal \cdot g^{-1}$) (Livesey & Elia 1998).

$$EE\ (kcal \cdot min^{-1}) = f\ (g \cdot min^{-1}) \times 9.3 + c\ (g \cdot min^{-1}) \times 3.7 \quad \textbf{(Eq. 2.7)}$$

2.4.5 Measurement of Exercise Energy Expenditure

Upon arrival for each exercise session, subjects were required to sit quietly on a chair while a resting expired air sample was collected for 10 minutes using the Douglas bag method. This baseline measurement would then be used to calculate resting energy expenditure and to determine the net energy expenditure of exercise above the resting value. The intensity at which subjects exercised was estimated individually based on the previous submaximal exercise test. Before the start of every exercise session, subjects were instructed to perform a 5-minute warm-up at a speed one step lower than their pre-determined walking speed and on a horizontal gradient. During the actual exercise, speed was adjusted to match the same walking speed that was used in the prior submaximal exercise test and a gradient predicted to elicit 50% of $\dot{V}O_2$max. Expired air was collected for 2 min at every 13 – 15 minute intervals of exercise time to estimate the energy cost of exercise. If needed, the treadmill gradient was adjusted after each expired air collection to

ensure subject was working within required intensity. The values of expired $\dot{V}O_2$, $\dot{V}CO_2$, and minute ventilation from the 2-min expired air sample were then used to calculate the rate of rates of fat and carbohydrate oxidation as well as energy expenditure during exercise. Net energy expenditure of exercise was calculated by subtracting resting energy expenditure values from the gross energy expenditure of exercise. The exercise session is called to end once the required exercise time or energy expenditure was reached, usually after the final expired air sample was collected. Once the bout of exercise was completed, the treadmill gradient was returned to horizontal before it was stopped completely. Subjects were immediately seated on a chair with mouthpiece and nose clip still in place and recovery expired air was collected continuously for 15 minutes in three sets: 0-5, 5-10 and 10-15 minutes.

2.5 Dietary Assessment

2.5.1 Dietary Restraint

In Chapters 3, 4, 5 and 6, dietary restraint in subjects was evaluated using two types of questionnaires, the Three-Factor Eating Questionnaire (Stunkard & Messick 1985) and the Dutch Eating Behaviour Questionnaire (DEBQ) (Van Strien *et al.* 1986). These questionnaires were administered to subjects during preliminary testing to determine their attitude in relation to food intake. The restraint subscales in both questionnaires have been shown to measure the actual restriction of food intake in everyday life (Laessle *et al.* 1989), therefore these two subscales were employed to establish if a subject was a restrained eater. A score in excess of the midrange for each restraint assessment is often deemed to indicate increased tendency towards dietary restraint (Stubbs *et al.* 2002).

2.5.1.1 Three-Factor Eating Questionnaire (TFEQ)

Dietary restraint was determined using the restraint scale of the TFEQ (**Appendix B)**. The 51-item instrument contained 36 items with a yes/no response format, and 15 items on a 1-4 response scale used to measure three dimensions of eating behaviour: 1) cognitive restraint of eating, 2) disinhibition, and 3) hunger. Cognitive restraint (21 items) measures dieting behaviour and restrained eating in order to influence body weight and body shape and high scores show a high restraint. Disinhibition (16 items) measures episodes of

losing control of dietary restraint and overeating. Perceived hunger (14 items) measures self-reported hunger and food cravings. The validity of and reliability of this instrument has been established (Laessle *et al.* 1989).

2.5.1.2 Dutch Eating Behaviour Questionnaire (DEBQ)

DEBQ (**Appendix C**) was constructed to reflect three psychological dimensions of eating behaviour: restraint theory, externality theory, and emotionality theory (Elfhag & Morey 2008). The instrument consists of 33 items: restrained eating (10 items), external eating (10 items), and emotional eating (13 items). Emotional eating reflects an inclination to eat in response to negative emotions such as depression, disappointments and feelings of loneliness; *e.g.* 'Do you have the desire to eat when you are irritated?'. External eating displays susceptibility to eating more in response to external food cues such as the sight, smell and taste of food; *e.g.* 'If food smells and looks good, do you eat more than usual?'. Scores were rated on a 5-point Likert scale with categories ranging from 'never' (1) to 'very often' (5). Both reliability and validity of this instrument have been proven to be adequate (Williamson *et al.* 2007).

2.5.2 Standardised Diet

In Chapters 3 and 4, subjects were instructed to consume a controlled diet 3 days prior to each experimental trial. The purpose of this diet was to standardise energy and macronutrient intakes. The measured RMR was multiplied by an activity factor of 1.55, which corresponds to the physical activity level of a non-active adult (Shetty 2005; FAO 1985) to estimate total daily energy expenditure for each subject. To ensure that all subjects consumed the right amount of calories, all meals were provided throughout the study. The diet was designed to provide 20% of the daily energy intake at breakfast, 35% at lunch and 45% at dinner and consisted of whole and frozen foods (*e.g.* cereal, bread, fruits, yogurt, pasta, etc.). The macronutrient ratio of the diet was formulated to consist of 48% carbohydrate, 37% fat and 15% protein to match the typical Scottish daily macronutrient intake (DOE 2004). Energy, protein, lipid, and carbohydrate intake were calculated using nutrient information obtained from respective online sources or food labels. Prior to the provision of each three-day diet, subjects were asked to complete a questionnaire giving information on their preferred i) breakfast cereal, ii) type of milk, iii) type of bread, iv) choice of sandwich filling, v) crisp and yogurt flavour, vi) type of pasta meal, vii) tea and coffee consumption and vii) food dislikes and allergies. Foods were

individually weighed and rationed to provide the calculated energy intake and then packaged and labelled according to meal types (breakfast/lunch/dinner) and days (1/2/3) (**Figure 2.4**).

In addition to the main meals for each day, subjects were provided with a pre-weighed bottle of sugar-free fruit squash to be consumed freely and tea or coffee if needed. They were also given two optional snacks, consisted of an apple and a bag of crisps, which they were allowed to consume should they experience extreme hunger. Subjects were instructed to consume all food provided and to return used containers at the end of the third day including any uneaten food. If subjects had not consumed all of the food or squash provided or if they had eaten any of the snacks during the three days leading up to the first experimental trial, this information was recorded and the diet was adjusted accordingly before being provided again for the days preceding subsequent trials. The three-day diet provisions were identical for all trials. They were instructed to refrain from alcohol throughout the intervention and not to consume foods other than what was provided. An example of the three-day diet can be seen in **Table 2.1.**

Figure 2.3. Individually-packed and weighed food provided for standardised diet

Table 2.1. An example of foods provided during the three days preceding each experimental trial.

	Day 1	Day 2	Day 3
Breakfast	Crunchy nut cornflakes	Crunchy nut cornflakes	Weetabix
	Wholemeal roll	Wholemeal roll	Wholemeal roll
	Margarine*	Margarine*	Margarine*
	Strawberry jam	Strawberry jam	Banana
	Semi-skimmed milk	Semi-skimmed milk	Semi-skimmed milk
Lunch	Wholemeal bread	Wholemeal rolls	Wholemeal bread
	Margarine*	Margarine*	Margarine*
	Cheese slices	Cheese slices	Cheese slices
	Chicken slices	Tuna & sweetcorn	Chicken slices
	Clementines	Clementines	Apple
	Flapjack	Mars bar	Twix bar
Dinner	Lasagne	Bolognese bake	Chicken and potatoes
	Wholemeal rolls	Chicken soup	Hazelnut yogurt
	Margarine*	Wholemeal roll	Wholemeal roll
	Jaffa cake bars	Chocolate mousse	Margarine*
	Grapes	Grapes	Grapes
Drink	Orange squash	Orange squash	Orange squash
Snacks	1 x Apple	1 x Apple	1 x Apple
	1 x packet crisps	1 x packet crisps	1 x packet crisps

***** polyunsaturated fat margarine

2.5.3 2-Day Dietary Record

In Chapter 5, a 2-day food intake diary was provided to subjects for the purpose of recording their food intake for two consecutive days prior to commencing each trial. They were given verbal and written instructions on how to keep the diet records and were instructed to record as detailed as possible every item that they either ate or drank, the time they ate it, the amount they ate in grams, brand names, and recipes. Subjects were then reminded to replicate the 2-day dietary intake for subsequent trials. Diets were analysed using a computerized version of food composition table (CompEat Pro; Nutrition Systems, Banbury, UK).

2.6 Metabolic Day Assessment

Subjects underwent metabolic day assessments in Chapters 3, 4, and 5. In the morning after a 12-hour fast, subjects reported to the Metabolic Suite. They rested quietly for 10 minutes and RMR was measured for 25 minutes by the ventilated hood system. Afterwards, a cannula was inserted into an antecubital vein in a forearm for the purpose of blood sampling. Buffet-style breakfast, lunch, and dinner were served at specific times, according to study protocols stated in Chapters 3, 4, and 5. Appetite sensations, postprandial blood samples and substrate utilisation were obtained at specific intervals throughout the entire day. Apart from the test measurements, subjects were free to do as they pleased while being the metabolic suite, *e.g.* watching television, reading, working or relaxing.

2.6.1 *Ad-Libitum* Energy Intake Assessment

Buffet-style breakfast, lunch and dinner meals were provided during metabolic assessment days to determine *ad libitum* food intake. Foods were provided in excess of typical consumption and subjects were instructed to eat according to their appetite until they were comfortably full. All meals were consumed in isolation. An example of types and amounts of food served for breakfast, lunch, and dinner are presented in **Table 2.2**. Food items were cut into identical portions whenever possible to disguise the amount being offered which could potentially affect eating behaviour, *e.g.* bread and fruits were cut into smaller slices to avoid subjects feeling obliged to finish the food if it was served as a whole. Time allocation for meal consumption was 15 minutes for breakfast and 20 minutes for both lunch and dinner. Subjects were blinded to the purpose of the buffet-type meal setting, which was designed to assess *ad-libitum* energy intake, to avoid conscious eating (de Castro 2000). The foods were covertly weighed before being served to the subjects and reweighed after completion of meal to quantify the intake of each type of food. Energy, protein, lipid, and carbohydrate intake were calculated using information on food labels and food composition tables when food labels were not available. Water consumption as well as reading and watching television were not permitted during all meals as these activities have been shown to influence food intake (Stroebele & De Castro 2004). *Ad libitum* access to water was made available throughout the day after the completion of each meal. All food items were identical for all subjects and across all trials. Additional measures were taken to ensure the foods were served in a standardised

setting for all the trials to avoid any bias in eating behaviour such as using neutral-colored tablewares (*e.g.* white), presenting food in the same dishes for the same type of meals as well as scheduling meals at the same time of the day in every trial. Examples of a buffet-type meal presentation are as in **Figure 2.4**.

Figure 2.4. Examples of buffet-type meals

Table 2.2. An example of foods provided during *ad libitum* buffet meals.

Period	Food	Approx. amount
Breakfast	Cornflakes	350 g
	Weetabix	500 g
	Coco Shreddies	450 g
	Strawberry jam	80 g
	Orange marmalade	80 g
	Margarine*	60 g
	Low-fat croissants	3 large pieces
	Wholemeal toast	6 slices
	Semi-skimmed milk	1 pint
Lunch	Spaghetti bolognaise	1000 g
	Oven chips	400 g
	Salad	150 g
	Salad dressing	100 g
	Low-fat yogurt	700 g
	Crisps	150 g
	Clementines	3 whole fruit
	Banana	2 whole fruit
	Chocolates	100 g
Dinner	Chicken arrabiata	900 g
	Baguette	Whole foot long
	Margarine*	60 g
	Crisps	150 g
	Mini flapjacks	120 g
	Grapes	150 g
	Apples	2 whole fruit

***** polyunsaturated fat margarine

2.6.2 Appetite Rating Assessment

Visual analogue scales (VAS) were used to assess subjective appetite sensations in Chapters 4 and 5. It was consisted of a 100-mm line in length with words anchored at each end expressing the most positive and negative ratings, e.g. 'I am not hungry at all' (0 mm) / 'I have never been more hungry' (100 mm). Subjects were instructed to mark a vertical line on the 100 mm scale anywhere between the two extreme ratings, which they considered to indicate the degree of the subjective feeling being rated. The VAS score was quantified by measuring the distance in millimeters from the 0 mm point to the position of mark. The questionnaire that was used consisted of five scales adapted from Flint *et al.* (2000) (**Appendix D**):

1. How hungry do you feel now?
2. How full do you feel now?
3. How satisfied are you now?
4. What is your desire to eat now?
5. How much can you eat now?

Subjects were given a booklet consisting of several sets of VAS questionnaires, each to be completed at 30 or 60 min intervals up until dinner was served during the metabolic assessment day. Each page of the questionnaire was folded out of view after each rating assessment so they could not refer to previous ratings when marking the VAS. All appetite rating assessments were administered prior to each blood venous sample collection. The areas under curve (AUC) were calculated for each time points to measure the response of each appetite rating over time using the trapezoidal method.

2.7 Daily Physical Activity Assessment

In Chapter 4, daily physical activity was objectively measured using a uniaxial accelerometer for three days prior to metabolic assessment day. The Actitrainer model (Actigraph, FL, USA) is a small (8.5 cm x 3.2 cm x 1.5 cm) and lightweight device that is specially designed to detect the range of movement that corresponds to most activities that humans perform (**Figure 2.6**). The Actitrainer has an internal time clock and

extended memory and is able to record and store the magnitude of acceleration and deceleration associated with movement. The actual data collected by the Actitrainer is a series of numbers representing the intensity of activity in each epoch; translated as raw activity counts and steps. Raw physical activity data can be downloaded to a personal computer via a reader interface unit and later summarised into duration (time spent doing physical activity) and intensity (stepcount·min^{-1}). Subjects were instructed to wear the accelerometers during waking hours (to be removed during water-based activities *e.g.* bathing) for three consecutive days, which was to put in on when they get out of bed in the morning and to take it off when to go to bed at night. The Actitrainer was worn at waist level on the right side, clipped to a belt or trousers. Subjects were given detailed instructions including how to care for and wear their Actitrainer as well as a log sheet to provide details of wearing time, and activity during non-wearing time for each day. The device was initialized using 1-min epochs for data collection. A cut-off point of <100 counts·min^{-1} was chosen to categorise sedentary time, which included activities such as sitting, or working quietly (*e.g.* reading, typing). Time spent in different levels of activity in was summarised based on Freedson's cut-offs: light activity (100-1951 counts·min^{-1}), moderate activity (1952-5724 counts·min^{-1}), hard activity (5725-9498 counts·min^{-1}) and very hard (>9498 counts·min^{-1}) (Freedson *et al.* 1998). A criterion of at least 20 minutes of continuous zero counts, as well with diary information, was identified as non-wearing periods.

Figure 2.5. Actitrainer® accelerometer used in the study

2.8 Blood Sampling and Analysis

Venous blood samples were collected during metabolic day assessment in Chapters 3, 4, and 5. Subjects arrived at the metabolic suite in the morning on an overnight fast. After RMR measurement, subjects rested in a semi-supine position while a cannula was inserted into the antecubital vein in a forearm. A baseline or fasting blood sample was drawn after 10 min. Patency of the cannula was maintained by flushing with a small amount of non-heparinized saline 0.9% sodium chloride (B.Braun, Melsungen, Germany) after each blood sample collection. Immediately before each blood samples was drawn, saline waste remaining in the connector tube after flushing was drawn off with a 2 ml syringe. Venous samples of 20 ml each were drawn at 30 or 60 min intervals during the assessment period, as specified in Chapters 3, 4, and 5 protocols. Blood samples were collected into 2 x 10 ml K_2EDTA blood collection tubes (Becton Drive Vacutainer, New Jersey, USA) preserved in ice.

In Chapters 3 and 5, blood samples used for analysis of postprandial metabolites analysis were immediately spun at 3500 rpm for 15 minutes in a refrigerated centrifuge. The plasma supernatant was then aliquoted into Eppendorf tubes and frozen for analysis. In Chapter 4, blood samples used for the analysis of gut peptides were split into two aliquots: (1) 1 ml aliquot of blood into duplicate microtubes treated with bovine aprotinin 500 KIU (Sigma-Aldrich, UK) and 10 µl of dipeptidyl peptidase-IV inhibitor (Calbiochem, Darmstardt, Germany) per ml of blood for determination of peptide YY_{3-36}; and (2) 1.5 ml aliquot of blood into duplicate microtubes treated with 15 µl of phenylmethanesulphonylfluoride (PMSF) (Sigma-Aldrich, UK), for the analysis of acylated ghrelin. After all samples were spun in a microcentrifuge at 3500 rpm for 5 minutes, samples treated with aprotinin were promptly aliquoted into Eppendorf tubes while samples treated with PMSF, were further added with 1 N hydrochloric acid (HCL) per 1 ml of plasma to acidify the samples. Samples were then centrifuged again for 5 minutes and finally aliquoted into separate tubes. All aliquoted samples were immediately frozen in -80ºC until assayed.

2.8.1 Plasma Metabolites Analysis

2.8.1.1 Insulin Analysis

The quantitative determination of insulin was performed using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Mercodia AB, Uppsala, Sweden). The intra- and interassay coefficients of variance for the analysis were 3.4% and 3.6%, respectively. It is based on the sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. Plasma samples (25 µl) were pipetted into the assay wells. A 100 µl of freshly prepared enzyme conjugate solution was then added to each well. Plates were then incubated on a plate shaker for 1 hour at room temperature. During this incubation period, insulin in the samples reacted with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to plate wells. After incubation, the plates were washed and dried 5 times by automatic washer to remove any unbound enzyme labelled antibody using the provided wash buffer solution. Bound conjugates which remained in the wells were detected by adding 200 µl of 3,3',5,5'-tetramethylbenzidine (TMB). The plates were then incubated for 15 minutes at room temperature to allow reaction between substrate TMB and bound conjugates. After incubation, 50 µl of the Stop solution containing 0.5 M sulphuric acid were added to each well to stop the reaction. A yellowish-tint color developed according to the concentration of conjugate-substrate complex. The optical density of each well was read at 450 nm using a spectrophotometer. All samples were run in duplicate together with the standards ranging from 0 to 200 mU/l. A standard curve was obtained by computerised data reduction of the absorbance for the standards against the concentration using cubic spine regression. The concentration of insulin in the samples was then determined by comparing the optical density of the samples to that of the standard curve for each respective plate. All reagents and samples were brought to room temperature before use. Coefficients of variation for the assay were <5%.

2.8.1.2 Glucose Analysis

Determinations for glucose were performed using kit reagents (Glucose HK CP Reagent ABX Pentra, Horiba ABX, France) on an automated Roche Cobas Mira spectrophotometric analyser (Horiba ABX, Montpellier, France). All samples within each subject were performed on a single run and in duplicates with coefficients of variation of <3%. The principle of glucose determination is based on the following colorimetric reactions:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + \text{NADP}^{+} \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-phosphate} + \text{NADPH} + \text{H}^{+}$$

(HK: hexokinase; G-6-PDH: glucose-6-phosphate-dehydrogenase)

2.8.1.3 Triglyceride Analysis

Determinations for TG were performed using kit reagents (Triglycerides CP Reagent ABX Pentra, Horiba ABX, France) on an automated Roche Cobas Mira spectrophotometric analyser (Horiba ABX, Montpellier, France). All samples within each subject were performed on a single run and in duplicates with coefficients of variation of <2%. The principle of TG determination is based on the following colorimetric reactions:

$$\text{Triglycerides} + \text{H}_2\text{O} \xrightarrow{\text{lipoprotein lipase}} \text{glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{glycerokinase}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + \text{O}_2 \xrightarrow{\text{glycerol-3-phosphate oxidase}} \text{DHAP} + \text{H}_2\text{O}_2$$

$$\text{H}_2\text{O}_2 + \text{4-AAP} + \text{p-chlorophenol} \xrightarrow{\text{peroxidase}} \text{Quinoneime} + 4\,\text{H}_2\text{O}$$

(DHAP: dihydroxyacetote phosphate; 4-AAP: 4-aminoantipyrine)

2.8.1.4 NEFA Analysis

Determinations for NEFA were performed using kit reagents (NEFA-HR Reagent, Wako Chemicals USA Inc., USA) on an automated Roche Cobas Mira spectrophotometric analyser (Horiba ABX, Montpellier, France). All samples within each subject were performed on a single run and in duplicates with coefficients of variation of <2%. The principle of NEFA determination is based on the following colorimetric reactions:

$$\text{RCOOH (NEFA)} + \text{ATP} + \text{CoA-SH} \xrightarrow{\text{ACS}} \text{Acyl-CoA} + \text{AMP} + \text{Ppi}$$

$$\text{Acyl-CoA} + O_2 \xrightarrow{\text{ACOD}} \text{2,3-trans-Enoyl-CoA} + H_2O_2$$

$$2\ H_2O_2 + \text{4-Aminoantipyrine} + \text{MEHA} \xrightarrow{\text{peroxidase}} \text{blue purple pigment} + 3\ H_2O$$

(ACS: acyl-CoA synthetase; ACOD: acyl-CoA oxidase; MEHA: 3-methyl-N-ethyl-N-β-hydoxyethyl-aniline)

2.8.1.5 Total Cholesterol Analysis

Determinations for total cholesterol were performed using kit reagents (Cholesterol CP Reagent ABX Pentra, Horiba ABX, France) on an automated Roche Cobas Mira spectrophotometric analyser (Horiba ABX, Montpellier, France). All samples within each subject were performed on a single run with coefficients of variation of <4%. The principle of cholesterol determination is based on the following colorimetric reactions:

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{RCOOH}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholesterol-3-one} + H_2O_2$$

$$2\ H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneime} + 4\ H_2O$$

2.8.1.6 HDL Cholesterol Analysis

Determinations for HDL-C were performed using kit reagents (HDL Direct Reagent ABX Pentra, Horiba ABX, France) on an automated Roche Cobas Mira spectrophotometric analyser (Horiba ABX, Montpellier, France). All samples within each subject were performed on a single run with coefficients of variation of 3%. The principle of cholesterol determination is based on the following colorimetric reactions:

$$\text{HDL-C, LDL-C, VLDL, chylomicron} \xrightarrow[\text{DSBmT + peroxidase}]{\text{cholesterol oxidase}} \text{non-reactive LDL-C, VLDL, chylomicron}$$

$$\text{HDL} \xrightarrow{\text{HDL-specific detergent}} \text{HDL disrupted}$$

$$\text{HDL cholesterol} \xrightarrow[\text{cholesterol oxidase}]{\text{cholesterol esterase}} \Delta^4\text{-cholestenone} + H_2O_2$$

$$2\ H_2O_2 + \text{DSBmT} + \text{4-AAP} \xrightarrow{\text{peroxidase}} \text{color development} + 5\ H_2O$$

(DSBmT: N,N-bis(4sulphobutyl)-m-toluidine-disodium; 4-AAP: 4-aminoantipyrine)

2.8.1.7 Gut Peptides Analysis

Quantitative analysis of acylated ghrelin and PYY_{3-36} from plasma samples was determined by competitive binding radioimmunoassay using a commercial kit (Millipore, MO, USA). Radioimmunoassay is based on the antigen-antibody reaction in which tracer amounts of the radio-labelled antigen competes with endogenous antigen for limited binding sites of the specific antibody against the same antigen. Thereafter, a standard curve was generated using a set of known concentrations of the unlabeled standards and from this curve the amount of antigen in unknown samples can then be calculated. All the procedures were carried out by a colleague at the Medical Genetics Department, Yorkhill Hospital, Glasgow. Samples were run in duplicates and the procedure was carried out according to manufacturer's instructions. The active form of ghrelin molecule is very unstable in the serum or plasma due to the nature of the octanoyl group on serine-3 position. In order to prevent degradation and loss of the octanoyl group, acylated ghrelin samples were processed with 1 N HCL and the addition of phenylmethylsulfonyl fluoride

(PMSF) per 1 ml of plasma. PYY_{3-36} samples were treated with DDP-IV inhibitor per 1 ml of blood to prevent the action of DPP-IV in the blood which could cause abnormal release of this peptide form upon storage and measurement, thus avoiding false interpretation of PYY increase. The addition of aprotinin is to protect against degradation by serine protease enzymes. Radioactivity in the processed samples was counted with a gamma counter (ARC-600, Aloka, Tokyo). The intra- and inter-assay CV, as given by the manufacturer, were 6.5 – 9.5% and 9.6 – 16.2% respectively for acylated ghrelin and 6.4 – 11.0% and 7.0 – 15.0% for PYY_{3-36}. The procedures for the acylated ghrelin and PYY_{3-36} assays were detailed in **Appendix F** and **Appendix G** respectively. The intrassay CV for acylated ghrelin and PYY_{3-36} samples were 8.9% and 7.6% respectively.

CHAPTER 3

Effects of Exercise on Postprandial Responses to *Ad Libitum* Feeding in Overweight Men

3.1 Introduction

A number of lines of evidence indicate that exaggerated metabolic disturbances occurring during the postprandial period contribute to the development of vascular and metabolic diseases. High postprandial concentrations of triglyceride (TG)-rich lipoproteins are the primary driver of the atherogenic lipoprotein phenotype characterised by a preponderance of small dense low-density lipoprotein (LDL) and low concentrations of high-density lipoprotein (HDL) (Cohn 1998), and postprandial lipoproteins and their remnants can contribute to endothelial dysfunction (Vogel *et al.* 1997) and may deposit into the arterial wall (Zilversmit 1979). Furthermore, it has been proposed that exaggerated postprandial insulin excursions may contribute to development of atherosclerosis (Frayn 2002; Boquist *et al.* 2000; Tsuchihashi *et al.* 1999), chronic insulin resistance and type 2 diabetes (Yki-Jarvinen 1990). As humans spend much of their days in a postprandial state, repeated episodes of exaggerated postprandial metabolism represent a daily, recurring atherogenic environment (Karpe & Hamsten 1995). Thus, interventions which reduce postprandial TG and insulin disturbances may play a role in the preventing the development of vascular and metabolic diseases.

Exercise is a potent regulator of postprandial lipid metabolism. There is a large body of evidence showing that postprandial lipemia can be attenuated by a prior session of exercise performed ~12-18 hours before a meal, with the magnitude of TG-lowering being essentially proportional to the exercise energy expenditure (Gill & Hardman 2003; Petitt & Cureton 2003). The TG-attenuation from an exercise-induced energy deficit is greater than that elicited by an equivalent diet-induced energy deficit (Gill & Hardman 2000), however, replacement of the energy expended during exercise by increasing subsequent energy intake markedly attenuates or abolishes the exercise-induced TG reductions (Harrison *et al.* 2009; Burton *et al.* 2008). In addition, replacing the energy expended during exercise has been shown to attenuate exercise-induced reductions in postprandial insulin concentrations and increases in postprandial fat oxidation (Burton *et al.* 2008). This has potential implications for the 'real world' effectiveness of exercise in

the regulation of postprandial metabolism. The available evidence suggests that individuals replace some, but not all, of the energy expended during exercise in subsequent meals when fed *ad libitum* (Whybrow *et al.* 2008; Pomerleau *et al*. 2004). Thus, controlled laboratory experiments in which either all or none of the additional energy expended during exercise (compared to a control trial) is replaced do not provide information about the likely extent of changes to postprandial metabolism which might occur following exercise in a 'real-world' setting with *ad libitum* post-exercise energy intake. It is therefore important to determine the effects of prior exercise on postprandial responses to *ad libitum* feeding. We hypothesised that that performing exercise prior to consumption of *ad libitum* buffet meals would lead to lower postprandial TG and insulin responses, and increased postprandial fat oxidation.

While increasing exercise energy expenditure (and therefore exercise-induced energy deficit) up to ~800 kcal, on the day prior to a postprandial challenge has been shown to increase the postprandial TG-attenuation in a dose-dependent manner (Gill *et al.* 2002; Tsetsonis & Hardman 1996), it is unclear whether inducing larger exercise-induced energy deficits by exercising on consecutive days would augment this effect. This would help understanding about whether the potential for exercise to lower TG is maximised by a single exercise session or whether an augmented acute effect can be seen by exercising on consecutive days, without increasing energy intake, to incur a larger exercise-induced energy deficit. We hypothesised that, by inducing a larger energy deficit, three days of consecutive exercise would have greater effects on postprandial responses to *ad libitum* buffet meals than a single exercise session. Overweight/obese men were chosen for the study, as this group typically has exaggerated postprandial metabolic responses (Gill *et al.* 2004), which would benefit from attenuation via exercise.

3.2 Methods

3.2.1 Participants

Ten overweight men, (mean ± SD) aged 35 ± 6 years, with body mass 90.6 ± 7.2 kg, body mass index (BMI) 28.2 ± 2.4 kg·m^{-2}, waist circumference 94.4 ± 6.4 cm, body fat 24.6 ± 3.1%, systolic blood pressure 126 ± 6 mm Hg, diastolic blood pressure 79 ± 7 mm Hg, and predicted maximal oxygen uptake ($\dot{V}O_2$max) 43.0 ± 6.4 ml·kg^{-1}·min^{-1} volunteered to participate in this study. All volunteers were healthy, normocholesterolemic, non-smokers, were not consuming any type of specialised diet, had a sedentary to moderately active lifestyle (less than two hours of planned exercise per week), and were not highly restrained eaters. Exclusion criteria included BMI < 25 kg·m^{-2}, fasting blood glucose > 7.0 mmol·l^{-1}, total cholesterol levels > 6.0 mmol.l-1, diagnosed heart disease, presence of diseases known to cause metabolic disturbances, current tobacco use, and use of any medications that are known to alter carbohydrate or lipid metabolism or energy intake behaviours. The study was approved by the Faculty of Biomedical and Life Sciences Research Ethics Committee at the University of Glasgow, and all procedures complied with the Declaration of Helsinki. Each participant provided written, informed consent before participation. They were asked to remain in their normal daily activities and to refrain from consuming alcohol during the course of the study.

3.2.2 Experimental design

Each participant undertook three main trials, in counter-balanced order, with an interval of at least seven days with, no exercise (CON), a single exercise session (EX-1) and three exercise sessions (EX-3), as the intervention. Each trial was conducted over four days. In CON, participants performed no exercise on Days 1 to 3; in EX-1, participants performed a single exercise session on Day 3; and in EX-3 participants undertook exercise sessions on Days 1, 2 and 3. On Day 4 of each trial participants attended the metabolic investigation suite for a 7-h metabolic assessment, described in detail below. On Days 1 to 3 in all trials, participants were provided with a controlled diet by the experimenters (see below for description) and, other than the imposed exercise in the EX-1 and EX-3 trials, were asked to refrain from planned exercise and maintain their usual day-to-day activities during this period. An overview of the experimental protocol is shown in **Figure 3.1**.

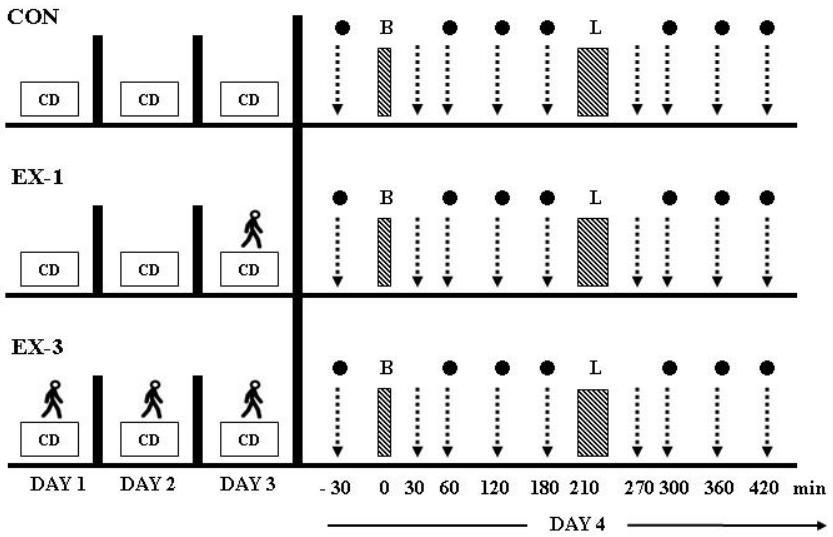


Figure 3.1. **Experimental design**. Subjects completed three trials: Control (CON), single-exercise trial (EX-1), and three-exercise trial (EX-3). Controlled diet foods (CD) were provided on Days 1-3. Exercise sessions (🚶) were performed to expend 8 kcal·kg^{-1}. Expired air (●), blood samples, and appetite questionnaires (↓) were collected at regular intervals on Day 4. *Ad libitum* buffet breakfast (B), and lunch (L) were provided at designated times.

3.2.3 Preliminary tests

Before undertaking the main experimental trials, participants undertook a number of preliminary tests. Resting metabolic rate (RMR) was measured after an overnight fast using a ventilated hood system (Oxycon Pro, Jaeger GmbH, Hoechberg, Germany) as described in section 2.4.1. A four-stage incremental sub-maximal treadmill walk test was performed to estimate $\dot{V}O_2$max and calculate the speed and gradient required to elicit the intensity of 50% $\dot{V}O_2$max for the exercise intervention as described in section 2.3 (ACSM 1995). Blood pressure was measured using an automated blood pressure monitor (Omron HEM705 CP, Omron Healthcare UK Limited, Milton Keynes, UK). Skinfolds were measured at four sites (biceps, triceps, subscapular, suprailiac) to enable estimation of percentage body fat using the equations of Durnin and Womersley (Durnin & Womersley 1974). Height, body mass, waist circumference were measured. Additionally, subjects completed the Three Factor Eating Questionnaire (TFEQ) (Stunkard & Messick 1985) (**Appendix B**) and the Dutch Eating Behaviour Questionnaire (DEBQ) (Van Strien *et al.* 1986) (**Appendix C**). Scores on the TFEQ and DEBQ were (mean ± SD): 7.1 ± 4.1 and 2.3 ± 0.7 respectively; none of the participants was classified as a restrained eater.

3.2.4 Main trials

a) Days 1 to 3: Experimental intervention days

Control trial (CON). Participants refrained from alcohol and all planned exercise over Days 1 to 3 of CON. They were provided with all of their food and drink by the experimenters in diets designed to maintain energy balance over this period. Energy intakes were calculated as RMR multiplied by a physical activity level of 1.55, which corresponds to the energy requirement of a non-active adult (FAO 1985). The macronutrient content of the diet reflected the average Scottish diet (49% carbohydrate, 37% fat and 14% protein) (DOE 2004), with 20% of energy provided at breakfast, 35% at lunch, and 45% at dinner. Participants were allowed *ad libitum* access to water and sugar-free fruit cordial. Average daily energy intake was 2693 ± 66 kcal (mean ± SD). Dietary adherence was monitored and verified by daily email and telephone contact.

Single exercise session trial (EX-1). On Days 1 to 3 of EX-1, participants consumed exactly the same diet as in CON (*i.e.* energy intake 1.55 x RMR), consumed no alcohol and refrained from all planned exercise other than that undertaken as part of the intervention. Participants performed a single exercise session on the afternoon of Day 3, in which they walked on a treadmill at an intensity of 50% $\dot{V}O_2$max to induce a net energy expenditure of 8 kcal·kg^{-1} body mass. Thus, relative to CON, participants were in negative energy deficit by 8 kcal·kg^{-1} body mass at the start of Day 4. The duration of the walk differed between individuals, ranging from 65 to 110 min. Expired air samples were collected in Douglas bags at rest, at 15-min intervals during the walk and for 15 min after the completion of exercise for the determination of oxygen uptake and carbon dioxide production. Exercise energy expenditure was determined using indirect calorimetry (Frayn 1983) described in section 2.4.3. The net energy expenditure of exercise was determined by subtracting resting energy expenditure from the gross energy expenditure of exercise. Heart rates and ratings of perceived exertion were recorded at 15-min intervals during the exercise.

Repeated exercise session trial (EX-3). This trial was identical to EX-1 except participants walked at 50% $\dot{V}O_2$max to induce a net energy expenditure of 8 kcal·kg^{-1} body mass on each of Days 1 to 3. Thus, on the morning of Day 4, participants were in negative energy balance by 24 kcal·kg^{-1} body mass relative to CON.

b) Day 4: Metabolic assessment

Participants reported to the metabolic suite on the morning of Day 4 after a 12-hour overnight fast, approximately 14-16 h after completion of exercise in the EX-1 and EX-3 trials. Following a 10-min supine rest on a couch, a 25-min expired air measurement was taken using the ventilated hood system to determine resting metabolic rate and substrate utilisation. A cannula was inserted into an antecubital vein and, after a 10-min interval, a fasting blood sample was taken. Immediately after fasting measurements were made, an *ad libitum* buffet-style breakfast was provided containing a variety of breakfast cereals, semi-skimmed milk, toast, croissants, margarine, jam and marmalade. A total of ~4600 kcal of energy was available in the buffet and participants were instructed to eat according to their appetite until they felt comfortably full. They were given 20 min to complete this meal. Participants were not informed that consumption was being measured, and consumed breakfast without experimenters present, to minimise potential alterations to usual feeding behaviour (Herman & Polivy 2005). All foods were covertly weighed before they were made available to subjects and re-weighed again after meal ingestion to quantify food intake. An *ad libitum* buffet lunch, containing spaghetti Bolognese, salad, vinaigrette dressing, bread, margarine, potato crisps, fruit, yogurt and chocolate cake (~3700 kcal of energy available) was provided 3.5 h after breakfast in a similar manner, and participants were given 30 min to consume this meal. Participants were not provided with drinks during the meals but *ad libitum* access to water was made available throughout the day after the completion of each meal. During the observation period, blood samples were collected at 30, 60, 120, 180 min after breakfast and the same pattern was repeated after lunch (270, 300, 360 and 420 min after the start of the observation period). Fifteen-min expired air measurements were made using the ventilated hood immediately following the 60, 120, 180, 300, 360 and 420 min blood samples.

3.2.5 Blood analysis

Venous blood samples were collected into potassium EDTA tubes and placed on ice before centrifugation to separate plasma within 15 min of collection. Plasma was stored at -80°C until analysis. Glucose, triglycerides (TG), non-esterified fatty acid (NEFA), total cholesterol and HDL cholesterol concentrations were determined by enzymatic colorimetric methods using commercially available kits (Horiba ABX, Montpellier, France; and Wako Chemicals GmbH, Neuss, Germany). LDL cholesterol was calculated using the Friedewald equation (Friedewald *et al.* 1972). Insulin was determined using a commercially-available enzyme-linked immunoassay (ELISA) with < 0.01% cross-

reactivity with pro-insulin (Mercodia, Uppsala, Sweden). All samples for each subject were analysed in a single analyser run.

3.2.6 Statistical analysis

Statistical analyses were performed using Statistica (version 6.0, StatSoft Inc., Tulsa, USA) and Minitab (version 13.1, Minitab Inc., State College, Pennsylvania). Data were tested for normality using the Ryan-Joiner normality test and transformed as appropriate. Box-Cox plots were used to determine the most appropriate transformation for data which did not follow a normal distribution. Consequently, statistical analyses for insulin and TG were performed on reciprocal-transformed data and are presented as values back-transformed to their original units. The total areas under the 420-min variable vs. time curve (AUC), calculated using the trapezium rule, and the incremental AUC, calculated as the increment in AUC over baseline concentrations, were used as summary measures of the postprandial responses. One-way repeated measures ANOVAs were used to compare fasting values, summary data and energy intakes across the three trials. Two-way repeated measures ANOVAs (trial × time) were used to compare changes over time and across the three trials. *Post-hoc* Tukey tests were used to identify where differences lay. Associations between variables were determined using Pearson product-moment correlations. A priori power calculations, based on our data for intra-subject reproducibility of postprandial TG responses and insulin responses in men (between-day coefficients of variation 10.1% and 22.9%, respectively) (Gill *et al.* 2006) indicated that 10 participants would enable detection of exercise-induced changes of ~10% in the TG response and ~23% in the insulin response with 80% power. Data are presented as means ± SEM, unless otherwise stated. Statistical significance was accepted at $p < 0.05$.

3.3 Results

3.3.1 Responses to the exercise sessions

The duration, and treadmill speed and gradient for each of the four exercise sessions (one session in EX-1, three sessions in EX-3) were identical within each participant. Participants walked on the treadmill at a speed of 5.3 ± 0.1 $km \cdot h^{-1}$at a gradient of 6.7 ± 0.7% for a duration of 93.5 ± 2.2 min. Mean oxygen uptakes and heart rates over the course of the exercise sessions were 21.6 ± 1.1 $mL \cdot kg^{-1} \cdot min^{-1}$ (50.2 ± 0.4% $\dot{V}O_2$ max) and 122 ± 5 $beats \cdot min^{-1}$, respectively in EX-1, and 21.8 ± 1.1 $mL \cdot kg^{-1} \cdot min^{-1}$ (50.6 ± 0.4% $\dot{V}O_2$ max) and 124 ± 3 $beats \cdot min^{-1}$, respectively in EX-3. These values did not differ between EX-1 and EX-3. Net exercise energy expenditure was 715 ± 25 kcal in EX-1 and 2140 ± 74 kcal in EX-3. Net fat oxidation during exercise was 29.6 ± 2.6 g and 96.9 ± 8.2 g for EX-1 and EX-3 respectively. Net carbohydrate oxidation during exercise was 114.8 ± 10.4 g for EX-1 and 322.9 ± 23.3 g for EX-3.

3.3.2 Responses in the fasted state

Due to difficulties with blood sampling in one participant, data for plasma variables are presented for $n = 9$; data for the energy intake and substrate utilisation are presented for $n = 10$. A summary of all fasting values is shown in **Table 3.1**. Compared to CON, fasting TG concentrations were 17% lower in EX-1, and 15% lower in EX-3 ($p < 0.05$ for both). Fasting NEFA concentrations were significantly higher in EX-1 than CON ($p < 0.01$). There were no significant differences between trials in fasting insulin, glucose, or total, HDL or LDL cholesterol. There were no differences between trials in resting metabolic rate, but rate of fat oxidation was 16% higher in EX-1 ($p < 0.05$) and 39% higher in EX-3 ($p < 0.01$), compared to CON. Reciprocally, carbohydrate oxidation was 39% lower in EX-3 condition compared to CON ($p < 0.01$). There were no differences between EX-1 and EX-3 for any other measured variables.

Table 3.1. Summary of fasting plasma and metabolic values in all trials (n = 9). Values are mean ± SEM.

Variables	CON	EX-1	EX-3
Triglyceride (mmol·l^{-1})	1.48 ± 0.23	1.23 ± 0.30*	1.26 ± 0.28*
Insulin (mU·l^{-1})	8.89 ± 1.96	9.06 ± 2.04	8.18 ± 2.31
Glucose (mmol·l^{-1})	5.45 ± 0.14	5.41 ± 0.11	5.23 ± 0.14
NEFA (mmol·l^{-1})	0.50 ± 0.03	0.63 ± 0.03**	0.58 ± 0.05
Total cholesterol (mmol·l^{-1})	5.38 ± 0.44	4.74 ± 0.44	4.63 ± 0.14
HDL-C (mmol·l^{-1})	1.07 ± 0.08	1.05 ± 0.05	1.11 ± 0.04
LDL-C (mmol·l^{-1})	3.63 ± 0.45	3.13 ± 0.33	2.94 ± 0.11
Resting metabolic rate (kcal·day^{-1})	1801 ± 47	1819 ± 49	1819 ± 68
Fat oxidation (g·h^{-1})	4.1 ± 0.4	5.1 ± 0.4*	5.7 ± 0.3**
Carbohydrate oxidation (g·h^{-1})	10.1 ± 1.0	7.7 ± 1.0	6.1 ± 0.7**

CON, control; EX-1, single exercise session; EX-3, three exercise sessions; NEFA, non-esterified fatty acids; HDL, high density lipoprotein; LDL, low density lipoprotein. * significantly different from CON (p < 0.05); ** (p < 0.001)

3.3.3 Ad libitum energy intake

Energy and macronutrient intakes at the buffet breakfast and lunch meals are presented in **Table 3.2**. There were no differences between trials in energy, fat, carbohydrate or protein intakes at breakfast, but energy, carbohydrate and protein intakes at lunch were significantly higher in EX-3 than both CON and EX-1. For breakfast and lunch combined, energy intake was significantly higher in EX-3 than CON and protein intake was significantly higher in EX-3 than CON and EX-1. There were no differences in energy or macronutrient intake between EX-1 and CON, and fat intake did not differ significantly between any of the trials at either breakfast or lunch.

Table 3.2. Buffet meal energy and macronutrient intake (n = 10). Values are mean ± SEM.

	CON	EX-1	EX-3
Breakfast			
Energy intake (kcal)	656 ± 77	731 ± 94	745 ± 93
Fat intake (g)	7.6 ± 1.4	8.5 ± 1.8	8.3 ± 1.7
Carbohydrate intake (g)	124.0 ± 14.0	136.7 ± 16.8	139.3 ± 17.0
Protein intake (g)	19.2 ± 2.3	23.0 ± 2.5	24.0 ± 2.4*
Lunch			
Energy intake (kcal)	1222 ± 65	1261 ± 99	1458 ± 84**,††
Fat intake (g)	32.7 ± 3.4	35.7 ± 3.8	40.5 ± 3.3
Carbohydrate intake (g)	170.0 ± 12.5	169.7 ± 16.6	197.0 ± 15.9*, †
Protein intake (g)	55.1 ± 2.8	58.3 ± 3.4	68.4 ± 3.9**, ††
Breakfast plus lunch			
Energy intake (kcal)	1878 ± 117	1992 ± 163	2202 ± 160*
Fat intake (g)	40.4 ± 3.6	44.2 ± 4.3	48.8 ± 4.4
Carbohydrate intake (g)	294.0 ± 21.4	306.3 ± 27.5	336.3 ± 27.3
Protein intake (g)	74.3 ± 4.7	81.3 ± 5.7	92.5 ± 6.0**,†

CON, control; EX-1, single exercise session; EX-3, three exercise sessions; * significantly different from CON ($p < 0.05$); ** ($p < 0.001$); † significantly different from EX-1 ($p < 0.05$); †† ($p < 0.01$)

3.3.4 Postprandial plasma metabolic responses

Postprandial responses for plasma variables over the 7-h observation period are presented in **Figure 3.2**. Two-way ANOVA revealed a significant trial effect for TG (p = 0.031) and insulin responses (p = 0.006), but not for glucose and NEFA. Summary measures of these responses are shown in **Table 3.3**. The postprandial TG total AUC was 27% lower in EX-1 and 25% lower in EX-3 than CON (both $p < 0.05$). Incremental TG AUC did not differ between trials. Total insulin AUC was 31% lower in EX-3 than CON ($p < 0.05$); the 26% reduction in insulin AUC between CON and EX-1 did not quite achieve statistical significance (p = 0.06). No differences were observed in NEFA AUC between trials, but incremental NEFA AUC was significantly lower in EX-1 than CON ($p < 0.05$), indicating greater postprandial NEFA suppression. Postprandial glucose responses did not differ between trials. None of these values differed between EX-1 and EX-3.

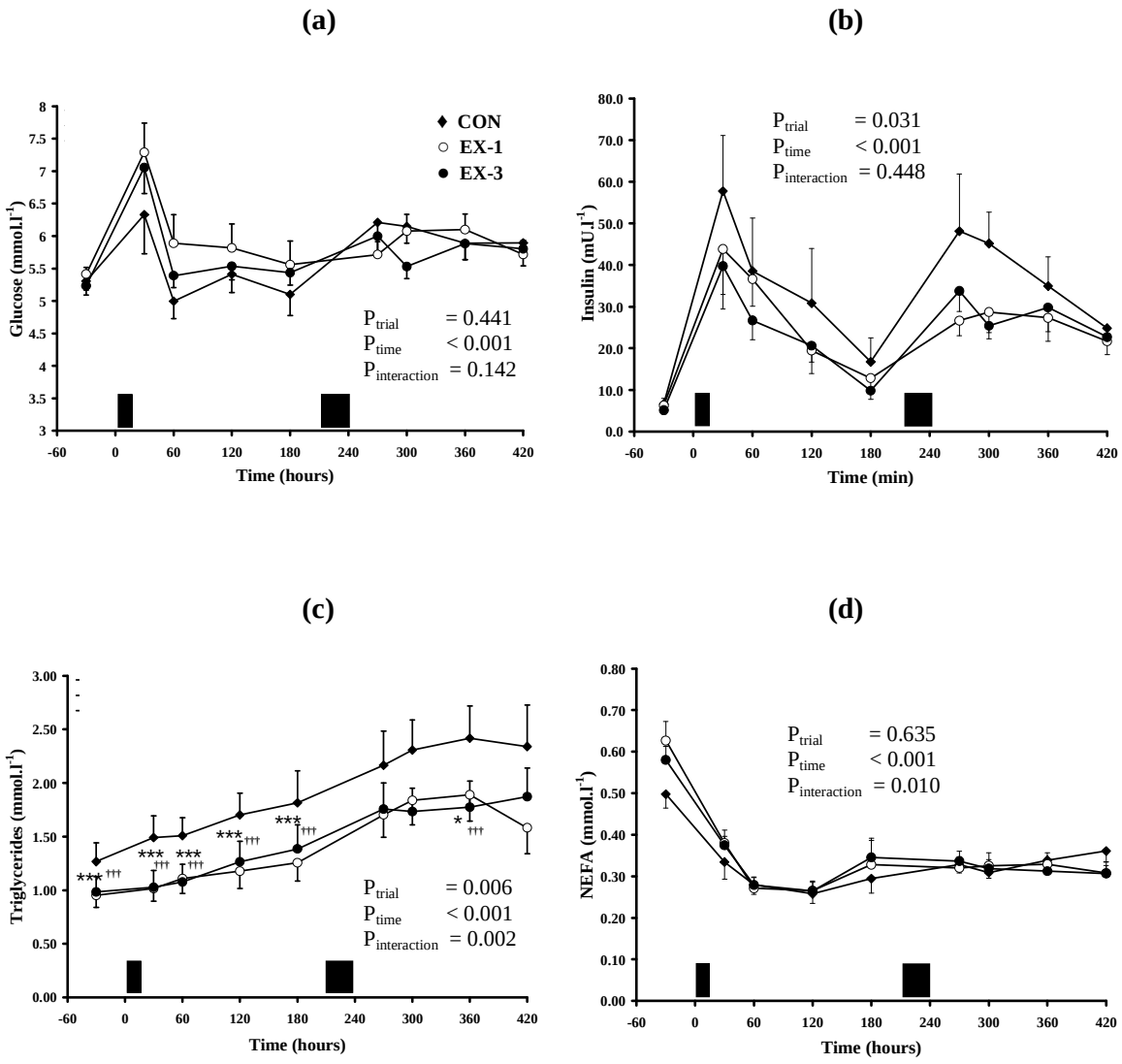


Figure 3.2. Postprandial plasma (a) glucose, (b) insulin, (c) triglyceride and (d) NEFA responses in CON (♦), EX-1 (○), and EX-3 (●) trials. Rectangles indicate the times at which the buffet meals were provided. Values are expressed as means, with standard errors represented by vertical bars (n = 9). (*) significantly different from CON ($p < 0.05$); (***) ($p < 0.001$); (†††) significantly different from EX-1 ($p < 0.001$)

Table 3.3. Postprandial total AUC and incremental AUC for plasma metabolic variables over 7-h observation period (n = 9). Statistical analyses for insulin and TG performed on reciprocal-transformed data and values are presented as means back-transformed to original units with positive and negative SEMs in brackets below. Values are mean ± SEM for glucose and NEFA.

	CON	**EX-1**	**EX-3**
TG (mmol·l^{-1})			
Total AUC	840	617	628
	(-84, +105)	(-77, +102)*	(-67, +85)*
Incremental AUC	204	160	172
	(-45, +80)	(-56, +188)	(-38, +68)
Insulin (mU·l^{-1})			
Total AUC	16783	12367	11649
	(-2250, +3076)	(-1847, +2635)	(-1694, +2390) *
Incremental AUC	13034	8896	9206
	(-2000, +2885)	(-1475, +2207)	(-1299, +1809)
Glucose (mmol·l^{-1})			
Total AUC	2413 ± 58	2484 ± 96	2408 ± 64
Incremental AUC	125 ± 58	211 ± 71	211 ± 30
NEFA (mmol·l^{-1})			
Total AUC	132 ± 7	136 ± 7	137 ± 11
Incremental AUC	-77 ± 12	-127 ± 9*	-107 ± 17

CON, control; EX-1, single exercise session; EX-3, three exercise sessions; * significantly different from CON ($p < 0.05$)

3.3.5 Postprandial energy expenditure and substrate utilisation

Postprandial energy, fat, and carbohydrate utilisation, measured over the 7-h observation period for each trial are shown in **Figure 3.3**. Summary AUC for energy expenditure and substrate utilisation data are presented in **Table 3.4**. There were no differences between trials in energy expenditure over the 7-h postprandial observation period, but the relative contribution of energy from fat and carbohydrate oxidation were different between trials (fat, $p = 0.003$; carbohydrate, $p = 0.001$). Fat oxidation over this period was 20% higher in EX-1 and 27% higher in EX-3 than CON ($p < 0.05$ for both). Reciprocally, carbohydrate oxidation was 18% lower in EX-1 and 26% lower in EX-3 than CON ($p < 0.05$ for both). No trial *x* time interaction effects were observed for any of the measures and none of these values differed between EX-1 and EX-3.

Table 3.4. Postprandial area under curve for energy expenditure and substrate utilisation over the 7-h observation period ($n = 10$). Values are means ± SEM.

	CON	**EX-1**	**EX-3**
AUC energy expenditure (kcal)	607 ± 18	615 ± 24	621 ± 22
AUC fat oxidation (g)	33.9 ± 2.9	40.6 ± 2.1*	42.9 ± 2.3*
AUC carbohydrate oxidation (g)	80.9 ± 5.5	66.5 ± 4.8*	59.7 ± 6.0*

CON, control; EX-1, single exercise session; EX-3, three exercise sessions; * significantly different from CON ($p < 0.05$).

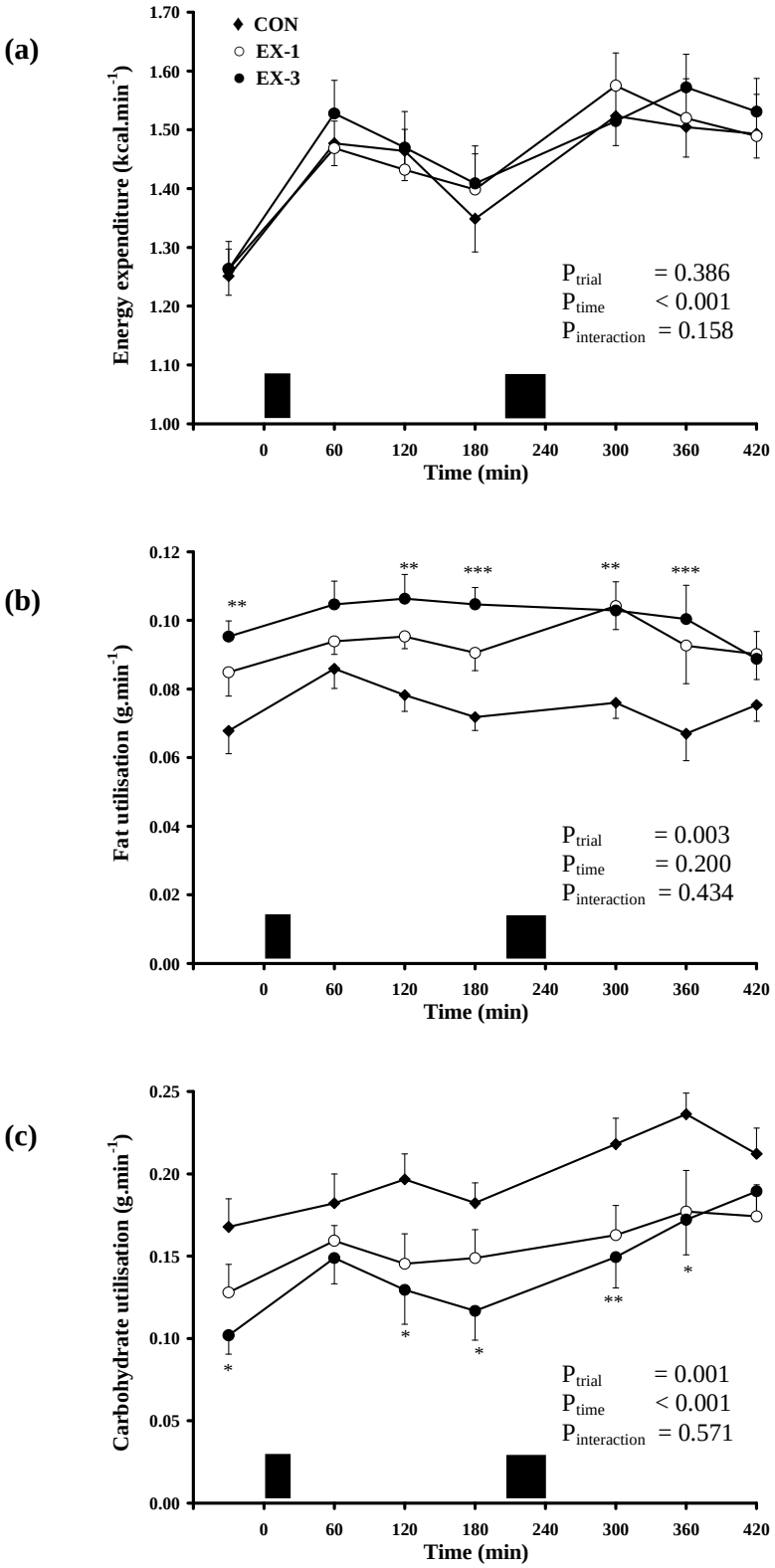


Figure 3.3. Postprandial (a) energy expenditure, (b) fat, and (c) carbohydrate utilisation in CON (♦), EX-1 (○), and EX-3 (●) trials. Rectangles indicate the times at which the buffet meals were provided. Values are expressed as means, with standard errors represented by vertical bars (n = 10). (**) significantly different from CON ($p < 0.01$); (***) ($p < 0.001$).

3.3.6 Correlation between variables

There was a significant positive correlation between the energy deficit-induced change in reciprocal TG AUC (*i.e.* difference between values in EX-1 or EX-3 and CON) and the energy deficit-induced change in postprandial fat oxidation (r = 0.50, p = 0.03) (**Figure 3.4**). As taking the reciprocal of a value reverses the direction of effect, this indicates that participants with the largest increases in postprandial fat oxidation between CON and the exercise trials experienced the largest reductions postprandial TG AUC.

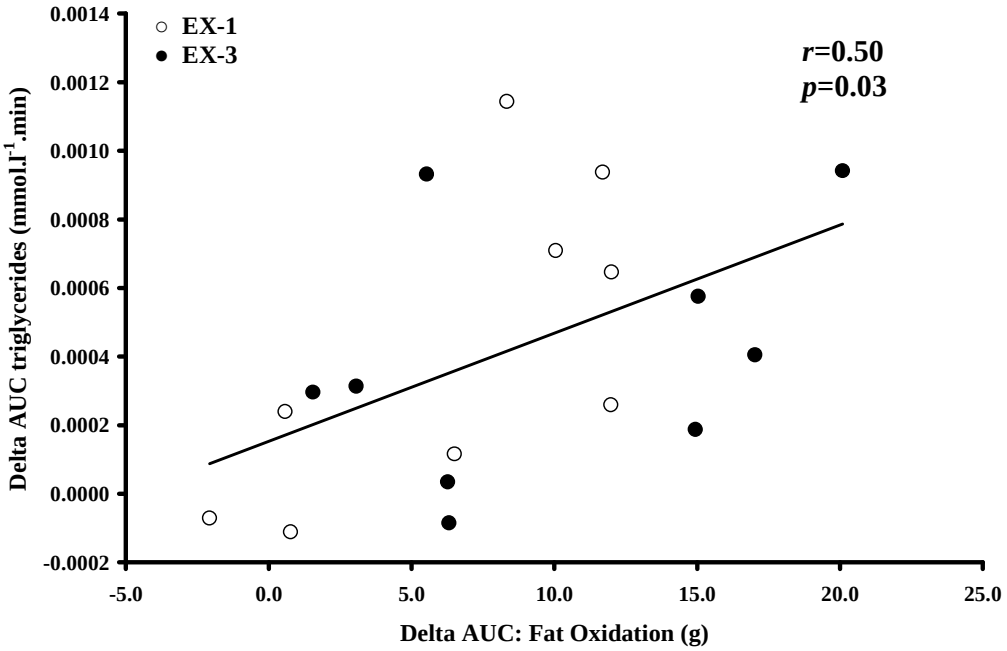


Figure 3.4. Relationship between changes in postprandial fat oxidation and reciprocal postprandial TG total AUC in EX-1 (○), and EX-3 (●) trials.

3.4 Discussion

The aims of this study were: i) to determine the effect of a prior exercise session on postprandial responses to *ad libitum* meal consumption, and ii) to determine whether three days of consecutive exercise altered postprandial responses to *ad libitum* meals to a greater extent than a single exercise session. The present data demonstrate that a single exercise session significantly reduced the postprandial TG response to an *ad libitum* breakfast and lunch by 27%, with no further TG-attenuation seen in response to three consecutive days of exercise (25% lower than control). Postprandial fat oxidation increased significantly and to a similar extent in response to one and three sessions of prior exercise, and while the postprandial insulin response was only significantly attenuated in response to three prior exercise sessions, the reduction in insulin response to the single exercise session approached statistical significance ($p = 0.06$) and was similar in magnitude to the reduction seen in response to three exercise sessions (26% *vs.* 31% reduction). Thus, the findings reveal that the favourable effects of prior moderate exercise on postprandial metabolism remain evident when food is provided *ad libitum* – suggesting that these changes are likely to persist into 'real-world' settings – and that consecutive days of exercise do not markedly augment the effects elicited by a single exercise session.

A key observation in interpreting the present findings is that *ad libitum* energy intake on the day following the single exercise session was not significantly increased compared to the control trial. Thus, in EX-1, the participants were in energy deficit by ~600 kcal compared with CON at the end of the postprandial observation period. This deficit is of similar magnitude to that seen in previously published studies in which prior exercise significantly attenuated postprandial TG responses (Gill & Hardman 2003, Petitt & Cureton 2003). The maintenance of this energy deficit despite *ad libitum* access to food is likely to play an important role in mediating exercise's effects on postprandial responses, in light of recent reports demonstrating that replacement of the exercise-induced energy deficit leads to a marked diminution of prior exercise's TG-lowering effect (Harrison *et al.* 2009; Burton *et al.* 2008).

A number of reports in the literature have shown that the extent of reduction in postprandial TG by of a prior exercise session is broadly proportional to the exercise energy expenditure. Increasing the energy expended in a 90-minute exercise session from ~400 to ~800 kcal by doubling the intensity (from 30% to 60% $\dot{V}O_2$max) increased the

reduction in postprandial lipaemia, compared to a control trial, from 16% to 26% (Tsetsonis & Hardman 1996); and increasing energy expenditure from ~350 to 700 kcal by doubling the duration of exercise at 50% $\dot{V}O_2$ max from 60 to 120 minutes increased postprandial TG-lowering 9% to 23% (Gill *et al.* 2002), in studies on young adults. In addition, a meta-analysis of 13 studies with exercise energy expenditures ranging from ~350 to ~1600 kcal reported a correlation co-efficient of 0.62 between exercise energy expenditure and reduction in postprandial lipaemia (Petitt & Cureton 2003). Thus, the present observation that three exercise sessions performed on consecutive days did not influence postprandial metabolism to a greater extent than a single exercise session is an interesting one. The energy expended in EX-3 was three times as great as in EX-1 (2140 ± 74 *vs.* 715 ± 25 kcal) and although energy intake during the *ad libitum* test meals, particularly at lunch, was higher in the EX-3 than the other two trials, the cumulative energy deficit, compared to CON, was still 3-times as great in EX-3 compared to EX-1 (~1800 kcal *vs.* ~600 kcal).

There are two factors which could potentially explain this observation. Firstly, the first two of the three exercise sessions in the EX-3 trial were performed ~40-64 hours before the postprandial observation period. It is known the effects of exercise on postprandial lipaemia are relatively short-lived, with the maximal effect observed ~8-16 hours post-exercise (Gill & Hardman 2003) and markedly diminishing from ~24 hours onwards (Zhang *et al.* 2004). Thus, despite the energy deficit incurred from the two earlier exercise sessions not being replaced, the TG-lowering effects of these sessions may not have persisted until the postprandial observation day. Secondly, it is likely that the TG-lowering effect of exercise plateaus once a certain threshold energy expenditure is achieved. For example, in a study of young trained men, Ferguson and colleagues found that reductions in fasting TG concentrations 24 hours following an exercise session were similar following exercise sessions expending between 750 kcal and 1500 kcal of energy (Ferguson *et al.* 1998). Thus, it is possible that the potential for exercise to lower postprandial TG concentrations was maximised by the energy expended in the single exercise session undertaken in the EX-1 trial. Irrespective of relative importance of these two effects, in practical terms, the present data imply that the TG-lowering effects of exercise are effectively maximised by a single session of exercise (provided the energy expended in this session is sufficient), and that increasing the total exercise-induced energy deficit via repeated days of exercise do not augment this effect. However, it important to recognise that, because an exercise session was performed on each of Days 1, 2, and 3 in the EX-3 trial, a TG-lowering effect of exercise would likely have been

evident on Days 2 and 3 as well as 4 of this trial (although this was not directly measured), as opposed to only on Day 4 in the EX-1 trial. Thus, although performing exercise on consecutive days does not increase the magnitude of the TG-lowering effect elicited by a single exercise session, repeated daily exercise sessions would act to maintain the TG attenuation incurred in response to the single session.

This study also confirms our previous findings that the extent of attenuation in postprandial TG concentrations in response to exercise is proportional to the exercise-induced increase in postprandial fat oxidation (Burton *et al.* 2008). The exercise-induced increase in whole-body fat oxidation is likely to reflect increased fat oxidation in skeletal muscle and/or the liver. Muscle TG utilisation is elevated for at least 18 hours following prolonged endurance exercise, which is thought to occur to facilitate resynthesis of muscle glycogen depleted during exercise (Kiens & Richter 1998). In addition, post-exercise increases in circulating 3-hydroxybutyrate concentrations, reflecting increased hepatic fatty acid oxidation (Williamson & Whitelaw 1978), are evident for at least 24-hours following exercise (Burton *et al.* 2008, Gill *et al.* 2001), which, analogous to the post-exercise increase in muscle fat oxidation, may occur in response to exercise-induced hepatic glycogen depletion (Casey *et al.* 2000). The responses to glycogen deficits in muscle and/or liver, and the associated increases in fat oxidation, may mediate the TG-lowering effects of exercise by stimulating skeletal muscle LPL activity and increasing TG clearance (Gill & Hardman 2003) and/or by directing the hepatic fatty acid flux towards oxidation and away from re-esterification, thereby reducing VLDL production (Gill *et al.* 2006; Gill & Hardman 2003). Interestingly, the increase in postprandial fat oxidation in EX-3 did not differ significantly from EX-1. Although in energy deficit on the exercise days in EX-3, subjects consumed ~350 g of carbohydrate per day over this period, which should have been sufficient to replace the liver and muscle glycogen used during the exercise sessions (~110 g per session). Thus it is likely that muscle and liver glycogen levels would have been similar at the end of exercise on day 3 in the EX-1 and EX-3 trials, and this might explain the similar increases in postprandial fat oxidation and decreases in postprandial TG concentrations between these two conditions.

It is important to recognise that the energy expended in each exercise session in the present study, at 700 kcal, was relatively large and beyond the level currently recommended in physical activity for health guidelines (Haskell *et al.* 2007), although the moderate nature of the exercise undertaken meant all volunteers completed the sessions without difficulty. Other studies have shown that 30 minutes of brisk walking, performed

in either a single session or multiple smaller sessions spread throughout the day, can effectively lower lipaemic responses to meals of a fixed size (Miyashita *et al.* 2008; Murphy *et al.* 2000). The exercise dose used in the present study, compared to a smaller dose, would be expected to increase the chances of a compensatory increase in energy intake under *ad libitum* feeding conditions. Thus the finding that the exercise induced energy deficit was largely maintained and the effects of exercise on postprandial metabolism were similar to those observed in response to test meals of fixed size, would likely extend to studies utilising smaller exercise doses. However, it is not clear whether the finding that the TG-lowering effect of a single exercise session was not augmented by exercise on consecutive days would still hold if the exercise doses were smaller. If an energy expenditure threshold exists for maximising the TG-lowering effect of exercise, is possible that the TG-lowering effect of a single session eliciting, say, 250-350 kcal exercise-induced energy deficit would be augmented by sessions of repeated exercise on consecutive days. This possibility warrants further investigation. Further study is also needed to determine whether the present findings extend to women, who may alter energy intake in response to exercise in a different manner to men (Stubbs *et al.* 2002a, Stubbs *et al.* 2002b).

3.5 Summary

In conclusion, the results of this study extend the literature on the effects of prior exercise on postprandial metabolism in two important ways. Firstly the data show that the exercise-induced attenuation of postprandial TG concentrations, previously documented in response to meals of a fixed size, persist when meals are consumed *ad libitum*. This suggests that the TG-lowering effect of prior exercise is likely to extend into a 'real-world' setting where food intake is not carefully controlled. Secondly, we have demonstrated that the effects of a single exercise session on postprandial metabolism are not augmented by inducing a larger energy deficit by exercising on consecutive days, which implies that the potential for exercise to attenuate postprandial TG is effectively maximised by a single session of exercise.

CHAPTER 4

Effects of Exercise on *Ad-Libitum* Energy Intake, Appetite, and Gut Peptide Responses in Overweight Men

4.1 Introduction

Exercise can be effective for creating the energy deficit needed for weight loss, maintaining energy balance for the primary prevention of weight gain, as well as for preventing weight re-gain in the formerly obese (Donnelly *et al.* 2009). However, the ability of exercise in the absence of dietary restriction to facilitate weight loss is less certain, as some individuals lose less weight than predicted in response to an exercise intervention (King *et al.* 2008). A possible explanation for this is that exercise may induce a compensatory increase in appetite and hence, energy intake. It is common sense to believe that a regulatory mechanism will trigger an increase in energy intake in order to match the energy expenditure expended at some stage (Schwartz *et al.* 2000). Therefore, the energy deficit created by exercise may not lead to appreciable weight loss if individuals increase their food intake following exercise. However, the extent to which the compensation in energy intake occurs still remains unclear.

Short-term exercise studies have attempted to examine the relationship between energy intake and energy expenditure over relatively short periods ranging from hours to a day. Some have found a strong coupling between energy intake and energy expenditure demonstrated by an increase in energy intake corresponding to an acute increase in exercise-induced energy expenditure (Finlayson *et al.* 2009; Pomerleau *et al.* 2004; George & Morganstein 2003; Verger *et al.* 1992). Conversely, studies that found no compensatory increases in energy intake suggest no direct link between exercise and energy intake (King *et al.* 2010a; King *et al.* 2010b; Harris & George 2008; Imbeault *et al.* 1997; King *et al.* 1997a; King *et al.* 1996; Thompson *et al.* 1988; Durrant *et al.* 1982). Such conflicting results may partly stem from differences in study protocols, exercise intensities, participant characteristics (*i.e.* lean *vs.* overweight/obese, dietary restraint level), and energy expenditures. High intensity exercise appears to suppress appetite to a greater extent than low to moderate intensities (King *et al.* 1997a, Imbeault *et al.* 1997;

Kissileff *et al.* 1990), while one study showed that a mild bout of exercise neither suppressed nor stimulated appetite (King *et al.* 1994). It is possible that exercise-induced energy expenditures is an important factor that influence post-exercise energy intake as studies have shown that expending ~300-500 kcal resulted in the absence of any compensation after exercise (King *et al.* 2010b; Harris & George 2008; Imbeault *et al.* 1997; Thompson *et al.* 1988; Durrant *et al.* 1982). It could be that expending a larger amount of energy expenditures would drive an increase in energy intake due to the state of greater negative energy balance the body is in. Having said that, others who have investigated the effects of higher exercise-induced energy expenditures (*i.e.* ~1200 kcal) on subsequent energy intake have not observed any compensatory responses (King *et al.* 2010a, King *et al.* 1997a). It is worthwhile mentioning however, that lean men were involved in these studies, thus it is possible that responses may be different in overweight/obese subjects. Furthermore, although most short-term studies have shown that exercise may not lead to an automatic increase in energy intake, there are evidence that when exercise is continued over several days, energy intake appears to track energy expenditure (Whybrow *et al.* 2008, Stubbs *et al.* 2002a).

Changes in energy balance can have a marked impact on the hormonal responses that modulate appetite, and energy intake (Suzuki *et al.* 2010). Peripheral gut hormones such as cholecystokinin (CCK), peptide YY (PYY), glucagon-like peptide 1 (GLP-1), and ghrelin are integral to the process in mediating short-term sensations of hunger and satiety (Suzuki *et al.* 2010). Ghrelin is the only known orexigenic (appetite-stimulating) peptide and all others act as satiety signals (appetite-suppressing). In the recent past, investigators have reported that changes to ghrelin levels are evident in individuals who underwent a long-term exercise program (Foster-Schubert *et al.* 2005; Leidy *et al.* 2004), suggesting that it possible that exercise may influence ghrelin through modification of energy balance. However, findings on the effects of exercise on ghrelin are similarly divided, with some investigators reporting no change (Burns *et al.* 2007; Martins *et al.* 2007a; Kyriazis *et al.* 2007; Zoladz *et al.* 2005), while others reporting increased (Mackelvie *at al.* 2007; Erdmann *et al.* 2007), as well as suppressed levels (King *et al.* 2011a; Malkova *et al.* 2008; Broom *et al.* 2007; Olive & Miller 2001) following acute exercise. Again, the discrepancies in these study outcomes could be due to the variability in exercise intensities and that the regulation of ghrelin is likely to differ between energy status (*i.e.* energy deficit *vs.* energy balance). Some studies did not include assessment of appetite (Broom *et al.* 2007; Burns, *et al.* 2007; Kyriazis *et al.* 2007; Mackelvie *at al.* 2007), making the physiological and behavioural relevance of the findings unclear. Others only

measured total ghrelin (Burns *et al.* 2007; Erdmann *et al.* 2007; Kyriazis *et al.* 2007; Zoladz *et al.* 2005) instead of acylated ghrelin; the latter is thought to play a more important role in appetite regulation (Suzuki *et al.* 2010). Less is known regarding the response of PYY to exercise, which appears to function in its truncated form; $PYY_{3–36}$. $PYY_{3–36}$ is released into the circulation following food intake and the level of release is directly proportional to caloric load ingested (Chelikani *et al.* 2004). Current evidence are showing that acute exercise transiently increases plasma total PYY (Broom *et al.* 2009; Ueda *et al.* 2009a; Martins *et al.* 2007a) but only one study has measured $PYY_{3–36}$ responses to acute exercise in overweight subjects (Ueda *et al.* 2009b). The role of $PYY_{3–36}$ in exercise and appetite regulation therefore requires more attention.

Many previous studies in the research literature regarding exercise and appetite regulation have tended to assess appetite and energy intake responses within the same day as an exercise bout, but it may be possible that changes may occur over a longer duration, or after several meals have being taken. Indeed, it has been proposed by Edholm (1977) that '*we eat not for today but for the day before yesterday*'. While a single bout of exercise have been shown not to increase appetite and energy intake, it is unclear whether inducing exercising on consecutive days would augment subsequent appetite and energy intake, and whether these compensatory responses are induced by changes in the gut hormones. In addition, to fully understand the effects of exercise on the hormonal regulation of appetite and food intake, it is therefore important to assess all three variables simultaneously within the same study. The purpose of the present study was therefore to examine the effects of two levels of exercise-induced energy expenditures (single session *vs.* three sessions over consecutive days) on *ad libitum* energy intake, appetite sensations, and gut peptide responses under laboratory conditions. The analysis for gut peptides would be focused on acylated ghrelin, and peptide YY_{3-36}, the two hormones that are receiving increasing interest in the literature for their roles in energy balance and appetite regulation. While lean individuals seem to show that they regulate and maintain body weight well, studies in overweight/obese subjects are inconclusive, therefore this population was chosen for the study.

4.2 Methods

4.2.1 Participants

The participants recruited for this study were the same participants who took part in the study in Chapter 3. Ten overweight men, (mean ± SD) aged 35 ± 6 years, with body mass 90.6 ± 7.2 kg, body mass index (BMI) 28.2 ± 2.4 $kg \cdot m^{-2}$, waist circumference 94.4 ± 6.4 cm and predicted maximal oxygen uptake ($\dot{V}O_2 max$) 40.8 ± 10.4 $ml \cdot kg^{-1} \cdot min^{-1}$ volunteered to participate in this study. All volunteers were healthy, normocholesterolemic, non-smokers, were not consuming any type of specialised diet, non-dieters, had a sedentary to moderately active lifestyle (less than two hours of planned exercise per week), and were not highly restrained eaters. Exclusion criteria included BMI < 25 $kg \cdot m^{-2}$, fasting blood glucose > 7.0 $mmol.l^{-1}$, total cholesterol levels > 6.0 $mmol.l^{-1}$, diagnosed heart disease, presence of diseases known to cause metabolic disturbances, current tobacco use, and use of any medications that are known to alter appetite or feeding behaviours. The study was approved by the Faculty of Biomedical and Life Sciences Research Ethics Committee at the University of Glasgow, and all procedures complied with the Declaration of Helsinki. Each participant provided written, informed consent before participation. They were asked to remain in their normal daily activities and to refrain from consuming alcohol during the course of the study.

4.2.2 Experimental design

This chapter utilised the same experimental protocol as described in Chapter 3. Each participant undertook three main trials, in counter-balanced order, with an interval of at least seven days: no exercise (CON), a single exercise session (EX-1) and three exercise sessions (EX-3). Each trial was conducted over four days. In CON, participants performed no exercise on Days 1 to 3; in EX-1, participants performed a single exercise session on Day 3; and in EX-3 participants undertook exercise sessions on Days 1, 2 and 3. On Day 4 of each trial participants attended the metabolic investigation suite for a 7-h metabolic assessment, described in detail below. On Days 1 to 3 in all trials, participants were provided with a controlled diet by the experimenters and, other than the imposed exercise in the EX-1 and EX-3 trials, were asked to refrain from planned exercise and to maintain their usual day-to-day activities during this period. An overview of the experimental protocol is shown in **Figure 4.1**.

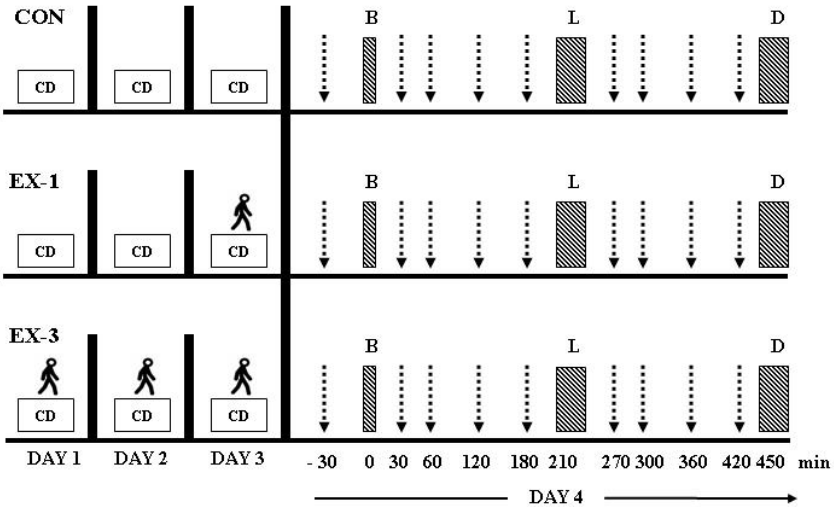


Figure 4.1. **Experimental** design. Subjects completed three trials: Control (CON), single-exercise trial (EX-1), and three-exercise trial (EX-3). Controlled diet foods (CD) were provided on Days 1-3. Exercise sessions (🚶) were performed to expend 8 kcal·kg^{-1}. Blood samples and appetite questionnaires (↓) were collected at regular intervals on Day 4. *Ad libitum* buffet breakfast (B), lunch (L), and dinner (D) were provided at designated times.

4.2.3. Preliminary tests

This chapter utilised the same preliminary tests as described in Chapter 3. Before undertaking the main experimental trials, participants undertook a number of preliminary tests. Resting metabolic rate (RMR) was measured after an overnight fast using a ventilated hood system (Oxycon Pro, Jaeger GmbH, Hoechberg, Germany) as described in section 2.4.1. A four-stage incremental sub-maximal treadmill walk test was performed to estimate $\dot{V}O_2$max and calculate the speed and gradient required to elicit the intensity of 50% $\dot{V}O_2$max for the exercise intervention as described in section 2.3 (ACSM 1995). Height, body mass, waist circumference were measured. Additionally, subjects completed the Three Factor Eating Inventory (TFEI) (Stunkard & Messick 1985) (**Appendix B**) and the Dutch Eating Behaviour Questionnaire (DEBQ) (Van Strien *et al.* 1986) (**Appendix C**). Scores on the TFEI and DEBQ were (mean ± SD): 2.3 ± 0.7 and 7.1 ± 4.1 respectively; none of the participants was classified as a restrained eater.

4.2.4 Main trials

a) Days 1 to 3: Experimental intervention days

Control trial (CON). Participants refrained from alcohol and all planned exercise over Days 1 to 3 of CON. To ensure participants consumed the appropriate amount of energy requirement, all of their food and drink were provided by the experimenters. The energy intakes were calculated as RMR multiplied by a physical activity level of 1.55, which

corresponds to the energy requirement of a non-active adult (FAO 1985). The macronutrient content of the diet reflected the average Scottish diet (49% carbohydrate, 37% fat and 14% protein) (DOE 2004), with 20% of energy provided at breakfast, 35% at lunch, and 45% at dinner. The diet consisted of whole and frozen foods (*e.g.* cereals, bread, fruits, pasta, etc.). Participants were allowed *ad libitum* access to water and sugar-free fruit cordial. They were required to consume all food provided and to return the used containers. Dietary adherence was monitored and verified by daily email and telephone contact. Daily physical activity outside the laboratory during the intervention period was assessed using an Actigraph monitor (Model GT1M, Actigraph, LLC, Florida, USA). Instructions on wearing the Actigraph were provided to all participants, and they were required to wear the activity monitor during waking times for everyday of the 3-d period.

Single-exercise trial (EX-1). On Days 1 to 3, participants consumed exactly the same diet as CON (*i.e.* energy intake 1.55 x RMR), consumed no alcohol and refrained from all planned exercise other than that undertaken as part of the intervention. Participants performed a single exercise session on the afternoon of Day 3, in which they walked on a treadmill at an intensity of 50% $\dot{V}O_2$max to induce a net energy expenditure of 8 kcal·kg^{-1} body mass. Duration of the walk varied for each individual. Expired air samples were collected in Douglas bags at rest, at 15-min intervals during the walk and for 15 min after the completion of exercise for the determination of oxygen uptake and carbon dioxide production. Exercise energy expenditure was calculated using indirect calorimetry, as described in section 2.4.4. The net energy expenditure of exercise was determined by subtracting resting energy expenditure from the gross energy expenditure of exercise. Heart rates and ratings of perceived exertion were recorded at 15-min intervals during the exercise.

Three-exercise trial (EX-3). This trial was identical to EX-1 except participants walked at 50% $\dot{V}O_2$max to induce a net energy expenditure of 8 kcal·kg^{-1} body mass on each of Days 1, 2 and 3.

b) Day 4: Metabolic assessment

Participants reported to the metabolic suite on the morning (~8.00 am) of Day 4 after a 12-hour overnight fast, approximately 14-16 h after completion of exercise in the EX-1 and EX-3 trials. Following a 10-min supine rest on a couch, a 25-min expired air measurement was taken using the ventilated hood system to determine metabolic rate and

substrate utilisation. A cannula was inserted into an antecubital vein and after a 10-min interval, a fasting blood sample was taken. *Ad libitum* breakfast, lunch and dinner were provided during the observation period. Blood samples along with appetite ratings were collected at 30, 60, 120, 180 min after completion of breakfast and the same pattern was repeated after completion of lunch (270, 300, 360 and 420 min).

4.2.5 Ad libitum energy intake

Immediately after fasting measurements were made, an *ad libitum* buffet-style breakfast was provided containing a variety of breakfast cereals, semi-skimmed milk, toast, croissants, margarine, jam and marmalade (~4500 kcal of energy available). They were given 15 min to consume this meal. An *ad libitum* buffet lunch, containing spaghetti Bolognese, salad, vinaigrette dressing, bread, margarine, potato crisps, fruit, yogurt and chocolates (~3700 kcal of energy available) was provided 3.5 h after breakfast in a similar manner. An *ad libitum* buffet dinner, containing penne and mozzarella pasta, salad, vinaigrette dressing, garlic bread, potato crisps, fruit and cakes (~4900 kcal of energy available) was provided at 3.5 h after lunch. Participants were given 30 min to consume lunch and dinner meals. They were instructed to eat according to their appetite until they felt comfortably full. Participants were not informed that consumption was being measured, and consumed breakfast without experimenters present, to minimise potential alterations to usual feeding behaviour (Herman & Polivy 2005). All foods were covertly weighed before they were made available to subjects and re-weighed again after meal ingestion to quantify food intake. Drinks were not provided during the meals but *ad libitum* access to water was made available throughout the day after the completion of each meal.

4.2.6 Visual analogue scales

Subjective assessment of appetite was made using visual analogue scales adapted from Flint *et al.* (2000) (**Appendix D**). Each scale consisted of a 100-mm horizontal line anchored at either end with statements “not at all” and “extremely”. The questionnaire that was used consisted of five visual analogue scales to rate ‘hunger’, ‘fullness’, ‘satisfaction’, ‘desire to eat’ and ‘prospective food consumption’.

4.2.7 Blood analysis for gut hormones

Venous blood samples were collected into potassium EDTA tubes and placed on ice before separation and centrifugation for analysis of gut hormones. Acylated ghrelin and PYY_{3-36} were quantified using commercially-available radioimmunoassay kits (Millipore,

St. Charles, Missouri, USA). Stability of acylated ghrelin in plasma was maintained by treating blood with *p*-hydroxymercuribenzoic acid (PMSF) before centrifugation and 1 N HCL were added to acidify the plasma. Blood samples were treated with aprotinin and dipeptidyl peptidase IV inhibitor (DDP-IV inhibitor) to prevent degradation of PYY_{3-36}. All samples from the same subject were assayed in duplicate and in single assay-run to eliminate the effects of interassay variation.

4.2.8 Statistical analysis

Statistical analyses were performed using Statistica (version 6.0, StatSoft Inc., Tulsa, USA) and SPSS (version 10.0, SPSS Inc., Chicago, US). Data were tested for normality using the Kolmogorov-Smirnov normality test and transformed as appropriate. Box-Cox plots were used to determine the most appropriate transformation for data which did not follow a normal distribution. Consequently, statistical analyses for acylated ghrelin were performed on log-transformed data and are presented as values back-transformed to their original units. The total areas under the 420-min variable *vs.* time curve (AUC), calculated using the trapezium rule were used as summary measures of the postprandial appetite and gut peptide responses. One-way repeated measures ANOVAs were used to compare fasting values across the three trials. Two-way repeated measures ANOVAs (trial × time) were used to examine changes over time and across the three trials for energy and macronutrient intake, appetite, and gut peptides responses. *Post hoc* Tukey tests were used to identify where differences lay. Associations between variables were determined using Pearson product-moment correlations. Data are presented as means ± SEM, unless otherwise stated. Statistical significance was accepted at $p < 0.05$.

4.3 Results

4.3.1 Responses to the exercise sessions

The mean absolute $\dot{V}O_2$ values were similar in all exercise conditions (EX-1: 1.72 ± 0.08 $L \cdot min^{-1}$; EX-3: 1.74 ± 0.07 $L \cdot min^{-1}$). The duration, and treadmill speed and gradient for each of the four exercise sessions (one session in EX-1, three sessions in EX-3) were identical within each participant. The duration of the walk differed between individuals, ranging from 65 to 110 min. Participants walked on the treadmill at a speed of 5.3 ± 0.1 $km \cdot h^{-1}$ at a gradient of 6.7 ± 0.7% for a duration of 93.5 ± 2.2 min. Mean oxygen uptakes and heart rates over the course of the exercise sessions were 21.6 ± 1.1 $ml \cdot kg^{-1} \cdot min^{-1}$ and 122 ± 5 $beats \cdot min^{-1}$, respectively in EX-1, and 21.8 ± 1.1 $ml \cdot kg^{-1} \cdot min^{-1}$ and 124 ± 3 $beats \cdot min^{-1}$, respectively in EX-3. These values did not differ between EX-1 and EX-3. Net exercise energy expenditure (energy expenditure above resting level) was 714 ± 25 kcal in EX-1 and 2140 ± 74 kcal in EX-3.

4.3.2 Metabolic responses in the fasted state

A summary of all fasting values is shown in **Table 4.1**. By design, participants were in negative energy deficit by 8 $kcal \cdot kg^{-1}$ and 24 $kcal \cdot kg^{-1}$ at the start of Day 4 in EX-1 and EX-3 respectively, relative to CON. There were no differences between trials in fasting resting metabolic rate (RMR). Due to difficulties with blood sampling in one participant, data for plasma variables are presented for $n = 9$; data for the energy intake and appetite variables are presented for $n = 10$. No between-trial differences were observed for fasting plasma acylated ghrelin and PYY_{3-36}. Fasting hunger ratings were higher in EX-3 compared CON ($p < 0.001$). There were no differences between EX-1 and EX-3 for any of the measured variables.

Table 4.1. Summary of fasting values in all trials (n = 10). Values are mean ± S.E.M.

	CON	EX-1	EX-3
RMR (kcal·day^{-1})	1802 ± 47	1819 ± 49	1819 ± 68
Acylated ghrelin (pmol·l^{-1})	15.2 ± 1.5	16.0 ± 1.1	17.8 ± 1.8
PYY_{3-36} (pmol·l^{-1})	45.2 ± 5.0	41.5 ± 6.7	43.0 ± 5.9
Hunger ratings (mm)	52 ± 6	61 ± 5	73 ± 4 **
Desire to eat ratings (mm)	51 ± 8	64 ± 6	69 ± 5
PFC ratings (mm)	62 ± 6	66 ± 5	73 ± 5
Satisfaction (mm)	33 ± 5	32 ± 5	25 ± 4
Fullness (mm)	24 ± 4	22 ± 4	20 ± 5

CON, control; EX-1, single exercise session; EX-3, three exercise sessions; * significantly different from CON ($p < 0.05$); ** ($p < 0.001$)

4.3.3 Ad libitum energy intake

Energy intakes at the buffet breakfast, lunch and dinner meals are presented in **Figure 4.2**. Two-way ANOVA showed a main effect of trial ($p = 0.003$) and time ($p < 0.001$), but no trial *x* time interaction. *Post hoc* analysis showed energy intake was 19% greater at lunch in EX-3 (1458 ± 84 kcal) compared to CON (1222 ± 66 kcal; p=0.041), with no difference noted between EX-3 and EX-1 (1261 ± 99 kcal). Energy intakes at breakfast and dinner did not differ between all trials. In total, energy intake was 18% and 13% greater in EX-3 (3364 ± 235 kcal) compared to CON (2844 ± 219 kcal; $p = 0.003$) and EX-1 (2976 ± 201 kcal; $p = 0.022$) respectively. In terms of energy intake compensation [(total energy intake in EX-1 or EX-3 – total energy intake in CON)/net exercise energy expenditure x 100], participants compensated about 18% and 24% of the energy expended during exercise in EX-1 and EX-3 respectively.

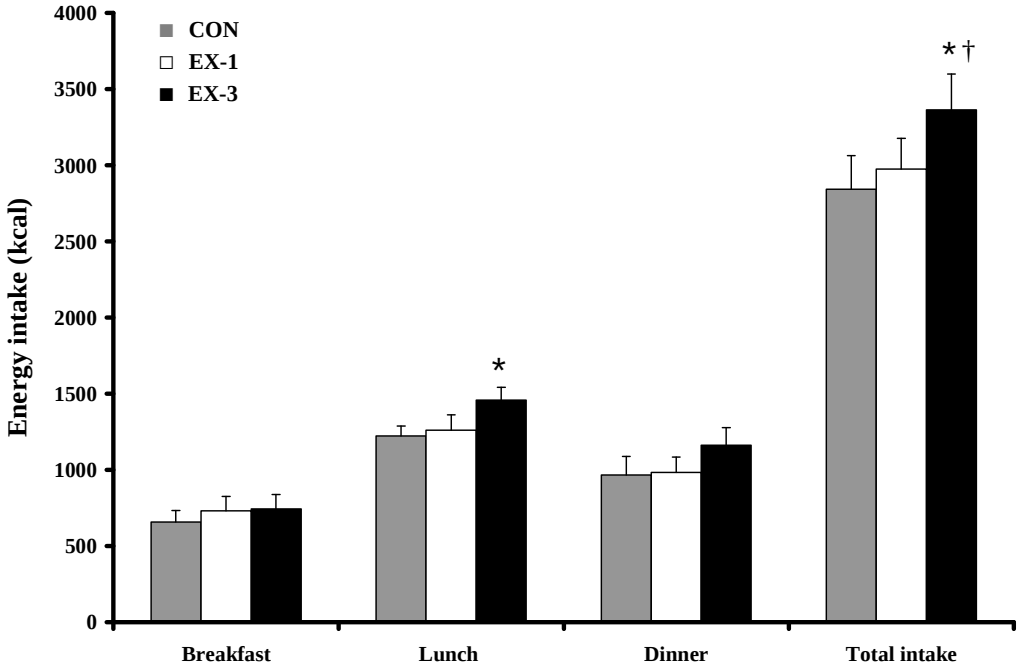


Figure 4.2: *Ad libitum* energy intake at breakfast, lunch, dinner, and total in CON (■), EX-1 (□), and EX-3 (■) trials (n = 10). Values are expressed as means, with standard errors represented by vertical bars. (*) significantly different from CON ($p < 0.05$); (†) significantly different from EX-1 ($p < 0.05$).

4.3.4 Macronutrient intake

Table 4.2 summarised the macronutrient intakes at all meal times in all three trials. For all meals (breakfast, lunch, and dinner) combined, compared to CON, carbohydrate intake was 16.7% higher in EX-3 ($p = 0.011$). The same trend was also observed for fat intake, which was 19.7 % higher in EX-3 ($p = 0.010$) compared to CON. Consumption of protein were 22.6% and 14.5% higher in EX-3 ($p < 0.001$) and EX-1 ($p = 0.001$) respectively, compared to CON. No differences were noted different between EX-1 and EX-3 trials in total carbohydrate and fat intake, and across meals.

Table 4.2. Macronutrient intake during *ad libitum* breakfast, lunch, and dinner for all trials ($n = 10$). Values are expressed as mean ± S.E.M.

	CON	EX-1	EX-3
Carbohydrate			
Breakfast (g)	124.0 ± 14.0	136.7 ± 16.8	139.3 ± 17.0
Lunch (g)	170.0 ± 12.5	169.7 ± 16.6	197.0 ± 15.9
Dinner (g)	130.7 ± 17.5	136.0 ± 13.8	159.1 ± 15.9
Total (g)	424.7 ± 35.5	442.3 ± 31.9	495.4 ± 39.3 *
Fat			
Breakfast (g)	7.6 ± 1.4	8.5 ± 1.8	8.3 ± 1.7
Lunch (g)	32.7 ± 3.4	35.7 ± 3.8	40.5 ± 3.3
Dinner (g)	36.5 ± 4.2	36.6 ± 4.0	43.3 ± 5.1
Total (g)	76.9 ± 7.1	80.7 ± 6.4	92.1 ± 6.8 *
Protein			
Breakfast (g)	19.2 ± 2.3	23.0 ± 2.5	24.0 ± 2.4
Lunch (g)	55.1 ± 2.8	58.3 ± 3.4	68.4 ± 3.9 **†
Dinner (g)	22.6 ± 3.6	21.6 ± 2.6	26.5 ± 3.1
Total (g)	97.0 ± 6.7	103.0 ± 6.0 **	118.9 ± 6.4 **†

* significantly different from CON ($p < 0.05$); ** ($p < 0.001$); † significantly different from EX-1 ($p < 0.05$)

4.3.5 Appetite responses

Time-averaged area under the curve (TAUC) for postprandial appetite scores are summarised in **Table 4.3**. Overall hunger scores were significantly higher in EX-3 compared to CON ($p = 0.011$). No differences were observed for other appetite variables. Appetite responses over the 7-h observation period are illustrated in **Figure 4.3**. Two-way ANOVA revealed a main effect of trial ($p = 0.009$), time ($p < 0.001$), and trial *x* time interaction ($p = 0.027$) for hunger scores. No significant effect of trial was observed for other appetite scores (desire to eat, prospective food consumption, fullness, and satisfaction), although a time effect was evident for these responses ($p < 0.001$), indicating that appetite sensations changed significantly during the observation period but were not influenced by trials.

Table 4.3. Postprandial time-averaged area under curve for subjective ratings of appetite over 7-h observation period ($n = 10$). Values are expressed as mean ± S.E.M.

Appetite variables	CON	EX-1	EX-3
Hunger (mm)	27.2 ± 2.5	30.4 ± 2.4	34.2 ± 2.2 *
Desire to eat (mm)	23.1 ± 3.2	25.0 ± 3.8	28.4 ± 4.0
Prospective food consumption (mm)	27.0 ± 3.0	27.6 ± 3.1	30.8 ± 3.1
Fullness (mm)	67.7 ± 3.9	67.9 ± 4.9	68.8 ± 4.0
Satisfaction (mm)	70.8 ± 4.0	69.4 ± 4.7	68.8 ± 3.9

CON, control; EX-1, single exercise session; EX-3, three exercise sessions; (*) significantly different from CON ($p < 0.05$).

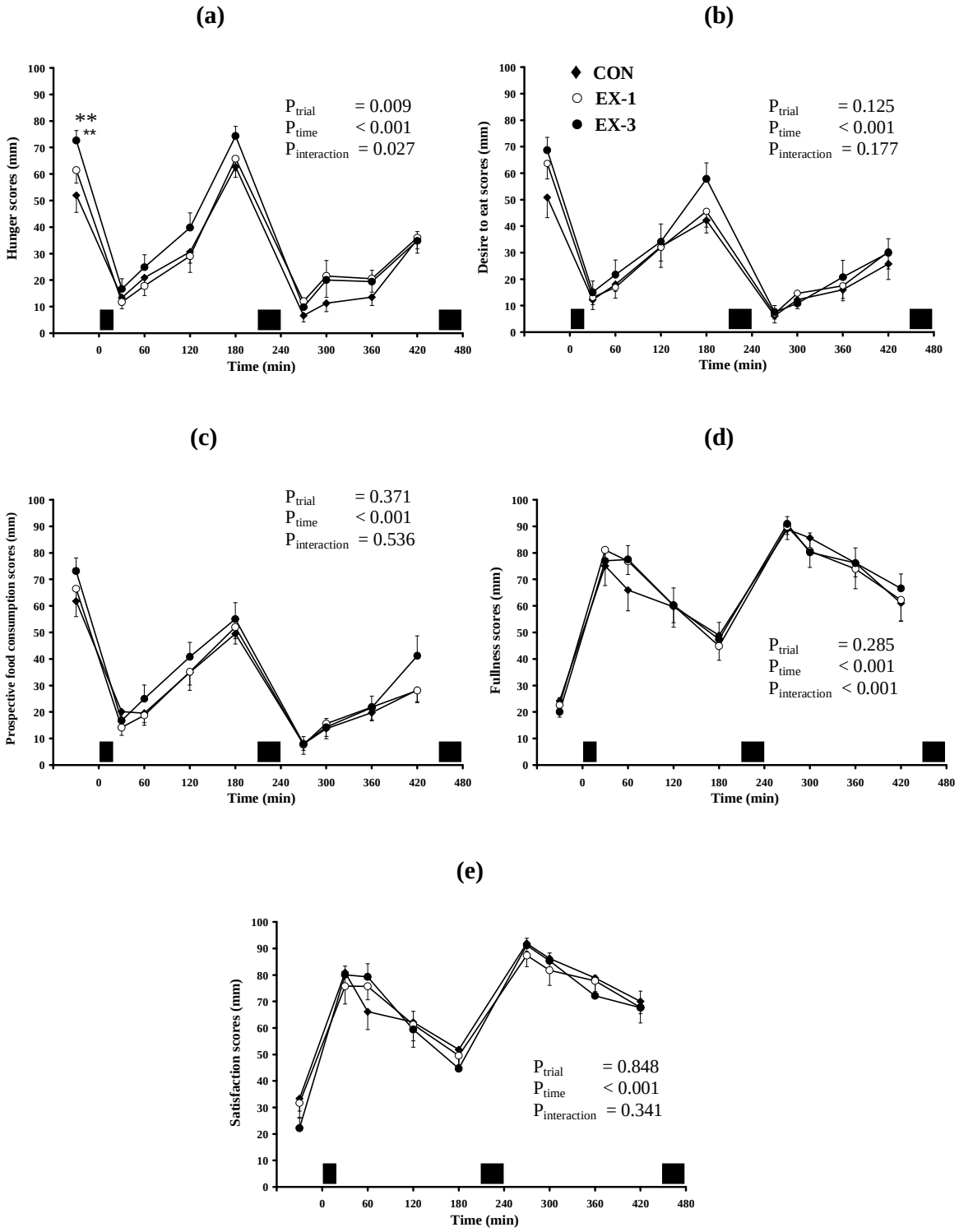


Figure 4.3. Postprandial (a) hunger, (b) desire to eat, (c) prospective food consumption, (d) fullness, and (e) satisfaction in CON (♦), EX-1 (○), and EX-3 (●) trials. Times at which buffet breakfast, lunch, and dinner (■) were provided are shown. Values are expressed as means, with standard errors represented by vertical bars (n = 10). (**) significantly different from CON (p < 0.001).

4.3.6 Gut hormone responses

Statistical analyses for acylated ghrelin were performed on log-transformed data and back-transformed to original units for data reporting. Summaries of area under curve for acylated ghrelin and PYY_{3-36} responses over the 7-h observation period are presented in **Table 4.4.** Total AUC for acylated ghrelin and PYY_{3-36} concentrations *vs*. time curve was not significant between trials. When assessing the AUC in separate intervals, pre-lunch (30-180 min) and post-lunch (270-420 min), no differences were observed for gut peptide responses during these intervals. **Figure 4.4** illustrates the responses of plasma acylated ghrelin and PYY_{3-36} over the observation period. Acylated ghrelin and PYY_{3-36} responses changed significantly over time (p = 0.028 and p = 0.025 respectively), but no trial or interaction effects were found.

Table 4.4. Postprandial time-averaged area under curve (TAUC) for gut peptide responses over 7-h observation period (n = 9). Statistical analyses for acylated ghrelin were performed on log-transformed data and values are presented as means back-transformed to original units with positive and negative S.E.M. Values are expressed as mean ± S.E.M for PYY_{3-36}.

Gut peptides	CON	EX-1	EX-3
Acylated ghrelin (pmol·l^{-1})			
TAUC -30 – 420 min	13.6 ± 1.6, 1.5	14.5 ± 1.6, 1.4	14.5 ± 2.3, 2.0
TAUC 30 – 180 min	13.0 ± 1.5, 1.4	12.9 ± 1.5, 1.4	13.8 ± 2.2, 1.9
TAUC 270– 420 min	13.6 ± 1.8, 1.6	14.9 ± 2.2, 1.9	15.1 ± 2.7, 2.3
PYY_{3-36} (pmol·l^{-1})			
TAUC -30 – 420 min	13.6 ± 0.7	13.3 ± 0.9	13.6 ± 0.9
TAUC 30 – 180 min	12.4 ± 0.9	12.9 ± 1.0	11.8 ± 0.9
TAUC 270– 420 min	14.9 ± 1.0	14.2 ± 1.5	15.3 ± 0.9

CON, control; EX-1, single exercise session; EX-3, three exercise sessions

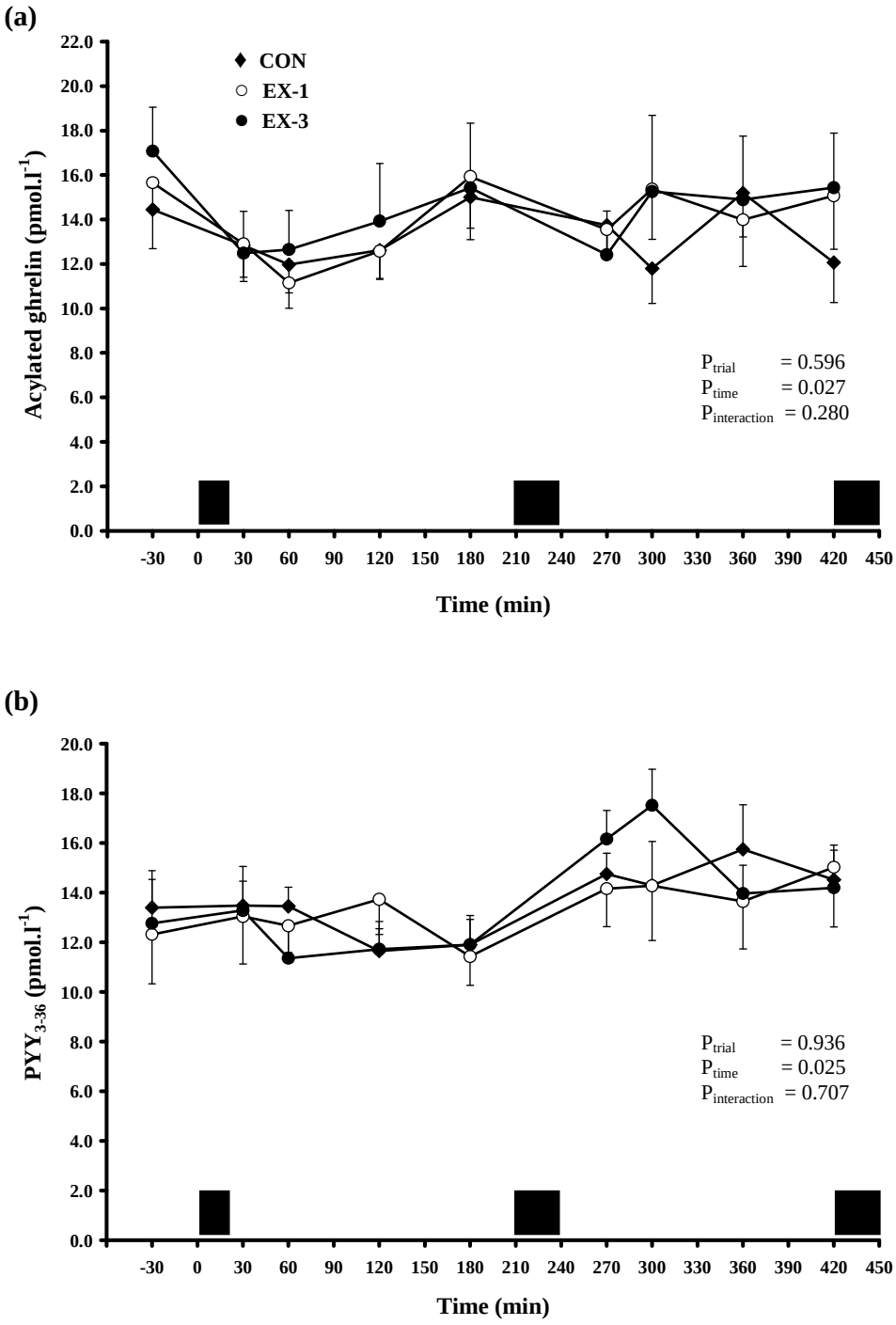


Figure 4.4: Postprandial plasma (a) acylated ghrelin, and (b) PYY_{3-36} during the 7-h observation period in CON (♦), EX-1 (○), and EX-3 (●) trials. Times at which buffet breakfast, lunch, and dinner (■) were provided are shown. Values are expressed as means, with standard errors represented by vertical bars (n = 9).

4.3.7 Physical activity outside laboratory

Physical activity for the preceding 3-d period before observation day (Day 4) is illustrated in **Figure 4.5**. Data is expressed as mean time (minutes) spent in various levels of physical activity as defined by the Freedson's cut-off point for adults (Freedson *et al.* 1998): sedentary (< 100 counts·min^{-1}), light (100-1952 counts·min^{-1}), moderate (1952-5724 counts·min^{-1}), and vigorous activities (> 5724 counts·min^{-1}). Prior to statistical analysis, accelerometry data during the period of exercise bouts performed in EX-1 and EX-3 were substituted with data from the corresponding period in the control trial, so only physical activity outside the laboratory was analysed. About 68-70% of each day was spent in sedentary activities, 23-27% was in the light intensity category, and the remaining time was spent in the at least moderate activity category. Two-way ANOVA revealed a significant level ($p < 0.001$), but no trial and interaction effects, indicating that although the absolute amount of time spent differed between activity levels, daily physical activity outside the structured exercise in the laboratory did not differ across trials.

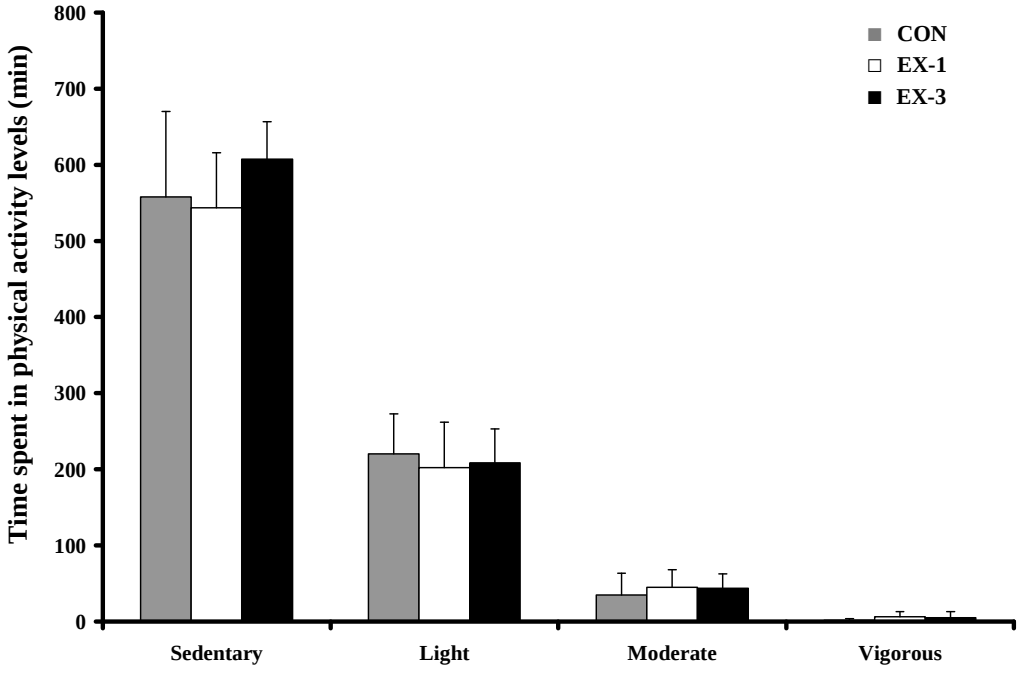


Figure 4.5: Mean time spent in various physical activity levels during the preceding 3-d period in CON (■), EX-1 (□), and EX-3 (■) trials (n = 10). Values are expressed as means, with standard errors represented by vertical bars.

4.4 Discussion

The purpose of this study was to investigate *ad libitum* energy intake, appetite and gut hormone responses to a single session *vs.* consecutive sessions of exercise in overweight men. The findings demonstrated that while a single exercise session did not result in a significant compensation in energy intake, performing exercise for three consecutive days evidently resulted in increased *ad libitum* energy intake and hunger perception. In contrast, the consecutive exercise sessions did not influence plasma acylated ghrelin, PYY_{3-36} and other appetite perceptions. These results support our hypothesis that higher energy deficit stimulated greater energy intake, however, these responses were not related to appetite-related hormones.

The findings of the present study showed that a single exercise session (~700 kcal) led to a slight (~18% of expended energy expenditure) but not significant increase in energy intake. No changes in appetite perceptions were observed. This is somewhat in agreement with previous reports that have shown no differences in energy intake (King *et al*. 2011a; King *et al.* 2010a, King *et al.* 2010b; Unick *et al.* 2010; Erdmann *et al.* 2007; King *et al.* 1997a, Imbeault *et al.* 1997) and appetite responses (Unick *et al.* 2010; King *et al.* 2010a, King *et al.* 2010b; Imbeault *et al.* 1997) in the post-exercise period. However, it is not known whether energy intake would remain unaltered beyond the observation period of these studies. In an elegant study by Edholm *et al.* (1955) five decades ago, a correlation between energy expenditure and energy intake was found only 2 days later, which ignited the possibility of a delayed compensatory augmentation in energy intake. Indeed, Stubbs *et al.* (2002a) found that raising energy expenditure by exercise (~400-800 $kcal \cdot day^{-1}$) for 7 consecutive days in free-living women resulted in a partial compensation (~25-30%) throughout the experimental period, but not in men (Stubbs *et al.* 2002b). In a longer term study lasting 16 days, Whybrow *et al.* (2008) reported ~30% compensation in energy intake due to graded elevations in exercise-induced energy expenditures (~400-900 $kcal \cdot day^{-1}$) in lean men and women feeding *ad libitum*.

In the present study, the participants showed a partial compensation of ~24% (of energy expended in exercise) after expending ~ 2100 kcal through exercise in EX-3. Apart from energy intake, an increase in 'hunger' ratings compared to control was also observed. Given that the accumulated energy expenditure induced by the exercise in EX-3 was

substantially greater than in EX-1, therefore it can be reasonably assumed that the elevation in exercise energy expenditure is likely to induce a compensatory response in hunger and hence, energy intake. In a somewhat similar study, Hagobian *et al.* (2009) reported increased appetite ratings for hunger (*i.e.* hunger, desire to eat, prospective food consumption) in overweight men who underwent 4 days of consecutive exercise (~750 kcal·day^{-1}) with a concurrent energy deficit (daily energy intake not matched to energy expenditure) compared to when studied in energy balance. Collectively, previous findings and the present data indicate that it may be possible that changes in appetite and energy intake may not be evident 1-2 days post-exercise, but instead may take 3-4 days or longer to become apparent. Indeed, Bray *et al.* (2008) recently stated that corrective responses in human food intake to deviations from average energy intake occurs with a lag of 3-4 days, not 1-2 days, thus supporting the findings of the present study. It might also be that overweight/obese individuals are less resistant, compared to lean counterparts, in tolerating substantial negative energy balance that compensation in energy intake will eventually sets in, in the direction to restore energy balance. Furthermore, overweight individuals have been shown to experience greater sensitivity to food-cue reward (Franken & Muris 2005), and this reward-driven behaviour might be implicated in the compensatory behaviour in response to exercise. In the present study, we observed greater macronutrient intakes for carbohydrate, protein, and fat in the EX-3 trial. However, we attributed this finding to the resultant increase in energy intake, and not likely due to changes in macronutrient preferences, as the distributions of energy in meals in this study were typical of a Western diet. A majority of previous work have shown that macronutrient preferences were unaltered in response to exercise (Elder & Roberts 2007).

Changes in energy balance (*i.e.* deficit, surplus) can have a marked impact on appetite-related hormones that modulate energy intake. Many studies in the past have typically measured total ghrelin, and a majority of these studies have consistently shown no effects of exercise on total ghrelin responses (Ueda *et al.* 2009b; Burns *et al.* 2007; Jürimäe *et al.* 2007; Martins *et al.* 2007a; Kraemer *et al.* 2004; Schmidt *et al.* 2004; Dall *et al.* 2002). Although there is a close relationship between total and the biologically-active appetite-stimulating acylated ghrelin (Lucidi *et al.* 2004), it cannot be excluded that this relationship may be somewhat different in response to exercise. It was hypothesised that any increases in appetite perceptions and energy intake in this study would be attributed to increased circulating levels of acylated ghrelin. However the findings demonstrated that this was not the case, as plasma acylated grelin was unaltered in all trials, therefore the increase drive to eat observed in EX-3 could not have been due to effect of this gut

peptide. This apparent uncoupling between feelings of hunger and ghrelin levels has been previously reported in other studies (Hagobian *et al.* 2009; Malkova *et al.* 2008). It is unclear why elevated ghrelin levels were not observed in this investigation. Plasma acylated ghrelin have been measured in a few studies recently but with slightly mixed results. A single bout of running at 70% $\dot{V}O_2$max for 60 min was reported to reduce 9-h AUC acylated ghrelin (Broom *et al.* 2007). In a more recent investigation by Broom *et al.* (2009), it was demonstrated that while acylated ghrelin was suppressed during a 60-min run, the 8-h AUC remained unchanged compared to control. Both studies recruited lean male subjects. A similar trend was observed in a recent study by King *et al.* (2010a), that despite the brief suppression of acylated ghrelin during and immediately after 90-min of treadmill running at 69% $\dot{V}O_2$max, ghrelin levels did not differ from control during the postprandial period, and the following morning in lean, male subjects. Unick *et al.* (2010) reported acylated ghrelin concentrations appeared to be unaffected for two hours following a 40-min brisk-walking exercise in overweight women. In longer term studies, 5 days of consecutive exercise is associated with an increase in 4-h AUC acylated ghrelin in lean male adolescents, but not in the overweight group (Mackelvie *et al.* 2007), while Hagobian *et al.* (2009) observed no change in 2-h AUC acylated ghrelin on the following morning after 4 days of consecutive exercise in overweight male subjects.

Taken together, most of the evidence above seem to suggest that ghrelin may be influenced acutely only during exercise, mostly in the manner of suppression (King *et al.* 2010b; Broom *et al.* 2009; Marzullo *et al.* 2008; Broom *et al.* 2007); and that the effect is only transient and disappears after the cessation of exercise. Only a minority reported sustained suppression (Malkova *et al.* 2008; Broom *et al.* 2007) or increase (Erdmann *et al.* 2007) in the post-exercise period. Furthermore, ghrelin concentrations have been shown to elevate preprandially, and decline postprandially, suggesting that the hormone act episodically to influence acute eating behaviour rather than influence feeding in the long term (Cummings *et al.* 2001; Tschöp *et al.* 2001). Thus, this may conceivably explain the lack of changes in plasma acylated ghrelin observed on the day following exercise in the present study. Furthermore, evidence has also suggested that the relationship between ghrelin and energy balance becomes less significant in obesity, where ghrelin levels seem to be unresponsive to feeding (Maier *et al.* 2010; Maier *et al.* 2008; le Roux *et al.* 2005; Salbe *et al.* 2004), which may partly explain for the lack of plasma ghrelin changes during the postprandial period in our overweight subjects. Alternatively, it could also be possible that responses in ghrelin may be more pronounced

in response to energy deficit caused by reduced energy availability in meals, rather than that induced by exercise (Borer *et al.* 2009). Indeed, increase in fasting ghrelin levels has been observed in individuals following an energy restriction diet to achieve weight loss (Kotidis *et al.* 2006; Hansen *et al.* 2002; Cummings *et al.* 2002). Other authors have observed an increase in total ghrelin and/or acylated ghrelin concentrations with long-term exercise intervention up to one year, but only when it is associated with weight loss (Martins *et al.* 2010; Foster-Schubert *et al.* 2005; Leidy *et al.* 2004). The duration of our study was too short to produce weight loss therefore the lack of exercise-induced effects on acylated ghrelin is somewhat expected.

Very few studies have examined the effects of exercise-induced energy deficit on PYY responses, particularly the more potent form – PYY_{3-36}. In the postprandial state, PYY_{3-36} contributes to ~60% of circulating total PYY and is more potent as a satiety signal than its precursor PYY_{1-36} (Chelikani *et al.* 2004). Similar to acylated ghrelin responses, we demonstrated that there were no changes observed in postprandial PYY_{3-36} levels in response to both exercise trials. Very few studies in the current literature address the effects of short-term exercise-induced energy deficits on PYY_{3-36} responses. So far, evidence are consistently indicating that circulating total PYY and PYY_{3-36} increased during a single bout of exercise (Broom *et al.* 2009; Ueda *et al.* 2009a; Ueda *et al.* 2009b; Martins *et al.* 2007a). All of these studies, except by Martins *et al.* 2007a, reported that plasma PYY remained elevated in the post-exercise period and were different to that of control (no exercise). Perhaps one of the candidate factors contributing to the lack of change observed in plasma PYY in our study is similar to that explaining ghrelin, which is timing of observation, therefore the acute transient changes that may have occurred during the exercise sessions could not be captured on the day following exercise. Similar to ghrelin, some studies have confirmed that fasting and postprandial plasma PYY levels are also attenuated in obesity (le Roux *et al.* 2006; Stock *et al.* 2005; Batterham *et al.* 2003). We also failed to observe any association between PYY_{3-36} and energy intake as well as satiety sensations (*i.e.* fullness and satisfaction). However, this study finding does not rule out the potential role of PYY_{3-36} in appetite regulation in the longer term as Jones *et al.* (2009) recently reported increased fasting total PYY concentrations in overweight adolescents after an 8-month exercise intervention.

By virtue of our study design, we are unable to determine macronutrient balance in this study. Pannacciulli *et al.* (2007) demonstrated that energy intakes were negatively correlated with carbohydrate deficit, independent of energy balance, which indicates that

carbohydrate balance is a contributing metabolic factor affecting food intake. Thus macronutrient imbalances could also be a contributing factor to the feeding responses observed in the present study. Alternatively, we cannot dismiss the contributions of cognitive (*e.g.* palatability, motivation to eat) and environmental (*e.g.* laboratory setting) factors that may have affected the feeding behaviours in our subjects (Berthoud 2006). Furthermore, when allowed to eat *ad libitum*, obese subjects have been showed to consume more than do normal weight subjects (Wing *et al.* 1978).

4.5 Summary

In summary, our findings showed that consecutive days of exercise produced a partial compensation in energy intake (~24%) and increase in hunger perceptions in overweight men, an effect that was not observed with a single exercise. The present study also highlights the findings that the gut hormones are not influenced by the exercise-induced negative energy balance. It would be of interest to examine the hormonal responses during the exercise period and observe how consecutive days of exercise can affect this. Although we cannot clearly distinguish the roles of acylated ghrelin and PYY_{3-36} in this experiment alone, it is not unlikely that these gut peptides can be influenced by exercise in the longer term. Future studies to confirm or refute these initial results are eagerly anticipated.

CHAPTER 5

Effects of Exercise Before or After a Meal on Postprandial Metabolism, Energy Intake and Appetite Responses in Overweight Men

5.1 Introduction

Loss of body fat requires the imposition of a negative fat balance. Fat balance is determined by fat oxidation and fat intake, and negative fat balance is achieved under conditions when more fat is oxidised than is ingested (Schutz 2004). Exercise is effective at increasing fat oxidation, both during and in hours following exercise (Hansen *et al.* 2005). However, the ability of exercise to induce negative energy balance or fat balance within a given period of time depends on its energy expenditure, and its effects on nutritional status across the exercise and the post-exercise periods (Tremblay & Therrien 2006). Beyond the energy cost of exercise, studies have shown that consuming a meal prior to exercise increased the contribution of carbohydrate oxidation to energy expenditure, relative to fat, during the exercise period (Derave *et al.* 2007; Wu *et al.* 2003; Coyle *et al.* 1997; Horowitz *et al.* 1997), while a post-exercise meal can attenuate the shift from carbohydrate to fat oxidation that normally follows exercise (Long *et al.* 2008; Dionne *et al.* 1999; Montain *et al.* 1991). While these research efforts have examined the effects of pre- and post-exercise meals on fat oxidation in controlled laboratory conditions, not much is known regarding which exercise timing around meal ingestion induces greater overall fat oxidation in 'real-life' condition. Schneiter *et al.* (1995) compared the effects of preprandial and postprandial exercise on fat oxidation and suggested that preprandial exercise stimulates greater fat oxidation over an 8-h period. This inference is weak, however, because the experimental condition did not include subsequent food intake and therefore, was not representative of free-living situations. In order to achieve overall negative fat balance, the increased energy expenditure induced by exercise must not be compensated by subsequent food intake. This can prove to be difficult in 'real-life' situations where food intake is often uncontrolled. Furthermore, it is unclear how exercise timing around meal ingestion could conceivably affect appetitive behaviour and *ad libitum* food intake, and how these subsequent meals can influence

variations in substrate oxidation and overall fat balance throughout the post-exercise period.

An additional consideration in the temporal association of exercise and meal ingestion is its effects on postprandial lipid metabolism. Much evidence has shown that exercise is efficient in ameliorating the unfavourable exaggerations in postprandial lipaemia and insulinaemia, adding to the health benefits of physical activity in potentially curbing the progression of atherogenesis (Katsanos 2006). In most of these studies, exercise performed 4-18 hours prior to a high-fat meal effectively reduced postprandial TG and insulin responses (Mestek *et al.* 2008; Miyashita *et al.* 2008; Pfeiffer *et al.* 2005; Kolifa *et al.* 2004; Petridou *et al.* 2004; Gill *et al.* 2002; Gill & Hardman 2000; Murphy *et al.* 2000; Tsetsonis *et al.* 1997). However, in studies which evaluated the effects of post-meal exercise on postprandial lipaemia, findings have been mixed. Some studies reported a decrease in postprandial TG when exercise was performed after a meal (Hardman & Aldred 1995; Klein *et al.* 1992; Schlierf *et al.* 1987) while a study by Zhang *et al.* (1998) reported no change from a no-exercise control. Katsanos & Moffatt (2004) compared the effects of pre-meal and post-meal exercise on postprandial lipaemia and found that the exercise-induced reduction in TG is irrespective of timing of exercise around meal ingestion. The finding of this study however, is limited by the absence of subsequent meals in the post-exercise period. Subsequent meal ingestion in the post-exercise period can have an impact on the magnitude and duration of the exercise-induced metabolic responses, *e.g.* dietary carbohydrate consumed after exercise has been shown to reduce the exercise-induced improvement in insulin sensitivity up until the next day (Newsom *et al.* 2010). Therefore, it is not known how exercise timing relative to meal ingestion and subsequent food ingestion may impact postprandial lipid metabolism.

To improve the effectiveness of exercise and its role in increasing fat oxidation and improving postprandial metabolism, further investigation is required to clarify which conditions of exercise timing relative to meal ingestion permits the greatest overall negative fat balance and magnitude of hypotriglyceridaemic effect across daily meals and the post-exercise period. The purpose of this study is therefore, to investigate the effects of an acute bout of moderate intensity exercise, preceding or after a standardised breakfast meal, on macronutrient and postprandial metabolism, as well as appetite responses and subsequent energy intake in overweight subjects.

5.2 Methods

5.2.1 Participants

Ten healthy, overweight sedentary (engagement in exercise activity <1 $h \cdot wk^{-1}$) men were recruited to participate in this study. Their age, BMI, waist circumference, and predicted maximum oxygen uptake were (mean ± SD) 28.1 ± 10.7 years, 29.0 ± 2.8 $kg \cdot m^{-1}$, 93.2 ± 8.6 cm, and 39.1 ± 5.4 $kg \cdot ml \cdot min^{-1}$ respectively. Participants were healthy, normocholesterolemic, non-smokers, with no known history of CVD or diabetes, and were not consuming any type of specialised diet or taking a medication thought to interfere with energy substrate metabolism and appetite. Dietary restraint was measured by the restraint scale on the Three Factor Eating Questionnaire (TFEQ) (Stunkard & Messick 1985) (**Appendix B**) and Dutch Eating Behaviour Questionnaire (DEBQ) (Van Strien *et al.* 1986) (**Appendix C**). The dietary restraint scores were 6.1 ± 3.0 and 2.4 ± 0.4 respectively. None of the participants was classified as a restrained eater. The present study was conducted according to the guidelines stated in the Declaration of Helsinki and all procedures involving human subjects were approved by the Faculty of Biomedical and Life Sciences Ethics Committee at the University of Glasgow, UK. Each participant gave written informed consent prior to participation.

5.2.2 Experimental design

After preliminary testing, each participant completed three, 8.5 h experimental trials in counter-balanced order with an interval of 1-2 weeks: exercise before breakfast-meal (EXM), exercise after breakfast-meal (MEX), and control (CON). An overview of the study protocol is shown in **Figure 5.1**. For 2 days prior to their first trial, participants recorded all of their food and drink intake and were instructed to replicate this diet for the two days preceding their subsequent experimental trials. They were also asked to refrain from alcohol and planned exercise and maintain their usual day-to-day activities during this recording period.

5.2.3 Preliminary tests

Before undertaking the main experimental trials, participants undertook a four-stage incremental sub-maximal treadmill walk test, described in section 2.3, to estimate $\dot{V}O_2$max and calculate the speed and gradient required to elicit the intensity of 50% $\dot{V}O_2$max for the exercise intervention. Height, body mass, waist circumference were measured. Each participant was also asked about food they disliked and whether they

have any food allergies. This information was used to ensure that foods provided in the *ad libitum* buffet were suitable and acceptable for their consumption.

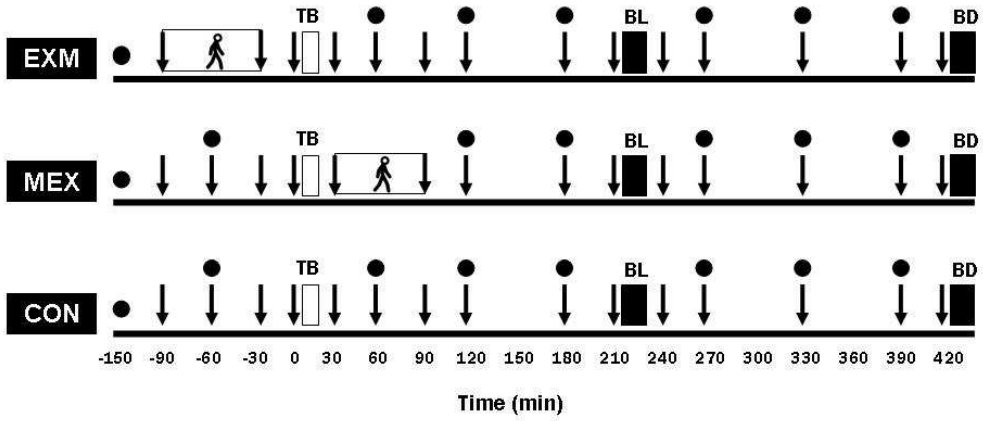


Figure 5.1. **Experimental design**. Subjects completed three trials: exercise before breakfast-meal (EXM), exercise after breakfast-meal (MEX), and control (CON). Expired air measurements (●), as well as blood samples and appetite questionnaires (↓) were collected at regular intervals. Test breakfast (TB), buffet lunch (BL), and buffet dinner (BD) were provided at designated times.

5.2.4 Main trials

Exercise before breakfast-meal (EXM). Participants arrived at the metabolic suite at 8.00 am after a 12-hour overnight fast. Following 10 min supine rest on a couch, a 25 min fasting expired sample was collected using a ventilated hood system (Oxycon Pro, Jaeger GmbH, Hoechberg, Germany) to determine resting metabolic rate and substrate utilisation, described in section 2.4.1. A cannula was then introduced into an antecubital vein for repeated blood sampling. Participants were asked to rate their fasting appetite sensations using 100-mm visual analogue scales and a fasting blood sample was taken immediately before the commencement of exercise session. The exercise session began at 9.00 am (-90 min in **Figure 5.1**) and all participants completed a 60-min treadmill walk at 50% of $\dot{V}O_2$ max. Expired air samples were collected at 15-min intervals during the walk, and for 15 min in the recovery period using Douglas bags for the determination of oxygen uptake and carbon dioxide production. Calculation of fat and carbohydrate oxidation during exercise were determined using indirect calorimetry (Frayn 1983) described in section 2.4.3. Heart rate and ratings of perceived exertion were recorded every 15 min during the walk. Blood samples were collected at 30 min during the walk and immediately after the walk ended. Participants also recorded their appetite sensation ratings at the end of walk. At 10.30 am (0 min in **Figure 5.1**), 30 minutes after completion of the exercise session, participants were provided with a test breakfast as described below. On completion of the meal, participants underwent a 7-hour postprandial observation period, during which blood samples and VAS ratings were

collected at 30, 60, 90, 120, 180, 210, 240, 270, 330, 390, and 420 min. Subsequent expired air measurements were made using the ventilated hood system for 15 min at 60, 120, 180, 270, 330, and 390 min. *Ad libitum* lunch and dinner, as described below, were served at 210 (2.00 pm) and 420 min (5.30 pm).

Exercise after breakfast-meal trial (MEX). This trial was identical to the EXM trial, except that participants rested for 1 h from 9.00 – 10.00 am, and performed the 1-h exercise session at 11.00 am (30 min in **Figure 5.1**), 30 minutes after test breakfast was provided.

Control trial (CON). This trial was identical to both exercise trial, except that participant remained rested during the periods (*i.e.* 9.00 – 10.00 am and 11.00 am – 12.00 pm) which corresponded to the exercise session in EXM and MEX trials respectively.

5.2.5 Test breakfast

Participants were provided with a standardised breakfast made up to provide 5 $kcal \cdot kg^{-1}$ of body mass. The meal comprised a bagel, polyunsaturated fat margarine, and a meal-replacement drink (Complan Foods Ltd, UK) made with whole milk, and provided 49% of energy from carbohydrate, 37% from fat, and 14% from protein. Participants were asked to consume the meal within 10 min.

5.2.6 Ad libitum energy intake

An *ad libitum* buffet lunch, containing spaghetti Bolognese, salad, vinaigrette dressing, microwaved chips, potato crisps, fruit, yogurt and chocolate (~3500 kcal of energy available) was provided 3.5 h after breakfast. Participants were given 20 min to consume this meal. *Ad libitum* dinner was provided 3.5 h after lunch, consisting of chicken arrabiata pasta, bread, margarine, potato crisps, fruit, and cakes (~3500 kcal of energy available). Food was presented in excess of expected consumption and participants were told to eat until they felt comfortably full. Participants were given 20 min to consume both lunch and dinner. They were not informed that consumption was being measured, and consumed all meals without experimenters present, to minimise potential alterations to usual feeding behaviour (Herman & Polivy 2005). All foods were covertly weighed before they were made available to subjects and re-weighed again after meal ingestion to quantify food intake. Participants were not provided with water during the meals but *ad libitum* access to water was made available throughout the day after the completion of each meal.

5.2.7 Visual analogue scales

Subjective assessment of appetite and mood was made using subjective visual analogue scales adapted from Flint *et al.* (2000) described in section 2.6.2 (**Appendix D**).

5.2.8 Blood analysis

Venous blood samples were collected into pre-cooled potassium EDTA tubes and placed on ice before centrifugation to separate plasma within 15 min of collection. Plasma was stored at -80°C until analysis. Glucose, TG, total cholesterol and HDL cholesterol concentrations were determined by enzymatic colorimetric methods using commercially available kits (Horiba ABX, Montpellier, France; and Wako Chemicals GmbH, Neuss, Germany). Insulin was determined using a commercially-available enzyme-linked immunoassay (ELISA) with <0.01% cross-reactivity with pro-insulin (Mercodia, Uppsala, Sweden). All samples for each subject were analysed in a single analyser run.

5.2.9 Statistical analysis

Statistical analyses were performed using Statistica (version 6.0, StatSoft Inc., Tulsa, USA) and SPSS (version 10.0, SPSS Inc., Chicago, US). Data were tested for normality prior to analysis. The total areas under the variable *vs.* time curve (AUC), calculated using the trapezium rule, and the incremental AUC, calculated as the increment in AUC over baseline concentrations, were used as summary measures of the postprandial responses. One-way repeated measures ANOVAs were used to compare fasting values, summary data and energy intakes across the three trials. Two-way repeated measures ANOVAs (trial x time) were used to compare changes over time and across the three trials. *Post hoc* Tukey tests were used to identify where differences lay. Data are presented as means ± SEM, unless otherwise stated. Statistical significance was accepted at $p < 0.05$.

5.3 Results

5.3.1 Responses during the treadmill walk

The treadmill speed and gradient for both exercise sessions (EXM and MEX) were identical within each participant. Participants walked for 60 min at an average speed of 5.5 ± 0.1 km·h^{-1} on a gradient of 4.3 ± 0.8%. All exercise sessions were completed without difficulty, participants rated the exercise as 'light' on the Borg scale of 6-20 in both EXM (10.4 ± 0.6) and MEX (10.5 ± 0.6) trials. Mean oxygen uptakes and heart rates over the course of the exercise sessions were 20.1 ± 0.8 ml·kg^{-1}·min^{-1} and 127 ± 4 beats·min^{-1}, respectively in EXM, and 19.5 ± 0.8 ml·kg^{-1}·min^{-1} and 129 ± 4 beats·min^{-1}, respectively in MEX. Values were not different between trials.

5.3.2 Metabolic responses in the fasted state

Resting metabolic rate, measured first thing in the morning at 8.00 am in all trials, was not different between trials, nor were resting fat oxidation rate and resting carbohydrate oxidation rate between trials. There were no significant differences between trials in fasting triglyceride, insulin, glucose, or total-, HDL- or LDL- cholesterol (**Table 5.1**).

Table 5.1. Summary of fasting metabolic and plasma values (n = 10). Values are mean ± S.E.M.

	EXM	**MEX**	**CON**
RMR (kcal·day^{-1})	1810 ± 65	1842 ± 73	1831 ± 48
Fat oxidation (g·day^{-1})	108.4 ± 12.3	129.5 ± 11.7	121.1 ± 7.0
CHO oxidation (g·day^{-1})	216.7 ± 24.5	172.3 ± 24.3	190.3 ± 20.9
TG (mmol·l^{-1})	0.97 ± 0.08	0.99 ± 0.10	1.01 ± 0.99
Insulin (mU·l^{-1})	6.16 ± 0.95	6.97 ± 1.07	7.05 ± 0.82
Glucose (mmol·l^{-1})	5.11 ±0.16	5.32 ± 0.14	5.17 ± 0.11
Total cholesterol (mmol·l^{-1})	4.61 ±0.30	4.27 ± 0.31	4.39 ± 0.36
HDL-C (mmol·l^{-1})	1.22 ±0.10	1.20 ± 0.10	1.20 ± 0.07
LDL-C (mmol·l^{-1})	2.95 ± 0.34	2.62 ± 0.35	2.73 ±0.34

EXM, exercise before meal; MEX, exercise after meal; CHO, carbohydrate; TG, triglycerides.

5.3.3 Metabolic responses during exercise

Summary data for energy expenditure and substrate utilisation during exercise are presented in **Table 5.2**. The total net exercise energy expenditure was similar during both exercise trials (p = 0.657). The net amount of fat oxidised during exercise was 33% greater in the EXM compared to MEX trial (p = 0.005). Conversely, the net amount of carbohydrate oxidised was 18% greater in the MEX trial than in EXM (p = 0.003). The relative contributions of fat and carbohydrate oxidation to energy expenditure during the 15-min post exercise recovery were however, not different between EXM (fat: 1.2 ± 0.2 g; carbohydrate: 4.4 ± 0.4 g) and MEX (fat: 1.1 ± 0.1 g; carbohydrate: 5.1± 0.1 g).

5.3.4 Postprandial energy and macronutrient utilisation

The summary AUC for postprandial energy expenditure and substrate utilisation are presented in **Table 5.2**. As expected, total energy expenditure over the 8.5 h observation period was greater in both EXM and MEX trials, compared to CON (p < 0.001 for both). Total fat oxidation over this period was 53% and 43% greater in EXM and MEX respectively compared to CON (p < 0.001 for both). Similarly, total carbohydrate oxidation was 55% greater in EXM and 65% greater in MEX than CON (p < 0.001 for both). None of these values differed between EXM and MEX. However, when the net energy expenditure and substrate utilisation during exercise are subtracted from the total AUC, there were no differences observed in energy expenditure and substrate utilisation over the observation period between trials.

Table 5.2. Total net energy expenditure and substrate utilisation during exercise, and postprandial area under curve for energy expenditure and substrate utilisation over 8.5 h observation period (*n* = 10). Values are expressed as mean ± S.E.M.

	EXM	MEX	CON
During exercise:			
Energy expenditure (kcal)	433 ± 24	429 ± 24	
Fat oxidation (g)	23.0 ± 1.3 †	17.3 ± 1.3	
CHO oxidation (g)	59.2 ± 6.7 †	72.4 ± 6.5	
Total AUC (including exercise):			
Energy expenditure (kcal)	1207 ± 40 **	1192 ± 43 **	783 ± 26
Fat oxidation (g)	76.1 ± 4.6 **	71.1 ± 2.8 **	49.6 ± 2.2
CHO oxidation (g)	134.9 ± 12.7 **	143.9 ± 8.0 **	87.0 ± 5.3
Total AUC (excluding exercise):			
Energy expenditure (kcal)	773± 24	763 ± 23	783 ± 26
Fat oxidation (g)	53.0 ± 3.8	53.6 ± 2.5	49.6 ± 2.2
CHO oxidation (g)	75.8 ± 8.4	72.4 ± 5.5	87.0 ± 5.3

**significantly different from CON ($p < 0.001$); † significantly different from MEX ($p < 0.01$); CHO, carbohydrate; EXM, exercise before meal; MEX, exercise after meal

5.3.5 Ad libitum energy intake and relative energy intake

Table 5.3 shows the energy intake and macronutrient intake for *ad libitum* lunch and dinner. Two-factor ANOVA showed no trial or interaction (trial × meal) main effects for energy intake ($p > 0.05$). Thus, *ad libitum* energy intake for lunch, dinner, and total were not significantly different between trials. Calculation of the relative energy intake [total energy intake - (exercise energy expenditure - resting energy expenditure)] however, showed that this was significantly lower in the EXM (1470 ± 121 kcal) and MEX (1525 ± 156 kcal) trials, compared to the control trial (1929 ± 158 kcal, $p < 0.001$ for both). After adjusting for the net energy expenditure of exercise, there was an energy deficit of 433 ± 24 kcal and 429 ± 24 kcal in EXM and MEX trials respectively, compared to CON. There was no significant difference between trials in the quantity of energy consumed derived from fat, carbohydrate and protein.

Table 5.3. *Ad libitum* energy intake and macronutrient intake for lunch and dinner (n = 10). Values are expressed as mean ± S.E.M.

	EXM	MEX	CON
Lunch intake (kcal)	1206 ± 90	1239 ± 106	1247 ± 94
Carbohydrate (kcal)	645 ± 47	652 ± 54	665 ± 53
Fat (kcal)	311 ± 35	338 ± 48	334 ± 33
Protein (kcal)	249 ± 16	248 ± 15	247 ± 18
Dinner intake (kcal)	767 ± 76	715 ± 75	682 ± 106
Carbohydrate (kcal)	393 ± 35	408 ± 34	389 ± 51
Fat (kcal)	174 ± 25	167 ± 29	166 ± 37
Protein (kcal)	130 ± 16	140 ± 19	127 ± 24
Total energy intake (kcal)	1882 ± 130	1955 ± 162	1929 ± 158
Carbohydrate (kcal)	1038 ± 64	1061 ± 78	1055 ± 79
Fat (kcal)	486 ± 53	506 ± 74	501 ± 62
Protein (kcal)	379 ± 13	388 ± 21	373 ± 27

5.3.6 Postprandial appetite responses

Time-averaged AUC values for postprandial appetite ratings are shown in **Table 5.4**. Postprandial responses for appetite sensations (hunger, prospective food consumption, desire to eat, satisfaction, and fullness) over the 8.5 h observation period are illustrated in **Figure 5.2**. Appetite ratings changed significantly over time ($p < 0.001$) but no trial or interaction main effects were found for any of the appetite ratings assessed (hunger, prospective food consumption, desire to eat, satisfaction, and fullness), indicating that appetite ratings changed significantly during the trials but were not influenced by exercise.

Table 5.4. Postprandial time-averaged area under curve (TAUC) for subjective ratings of appetite over 8.5 h observation period (n = 10). Values are expressed as mean ± S.E.M.

Appetite variables (mm)	EXM	MEX	CON
TAUC Hunger	44.6 ± 2.8	40.4 ± 3.5	43.7 ± 4.2
TAUC Desire to eat	44.7 ± 3.2	40.0 ± 4.1	43.1 ± 3.8
TAUC Prospective food consumption	50.2 ± 2.0	45.9 ± 3.1	48.1 ± 3.2
TAUC Fullness	49.1 ± 3.1	51.8 ± 2.7	51.7 ± 3.1
TAUC Satisfaction	49.6 ± 2.4	40.4 ± 3.5	51.5 ± 3.0

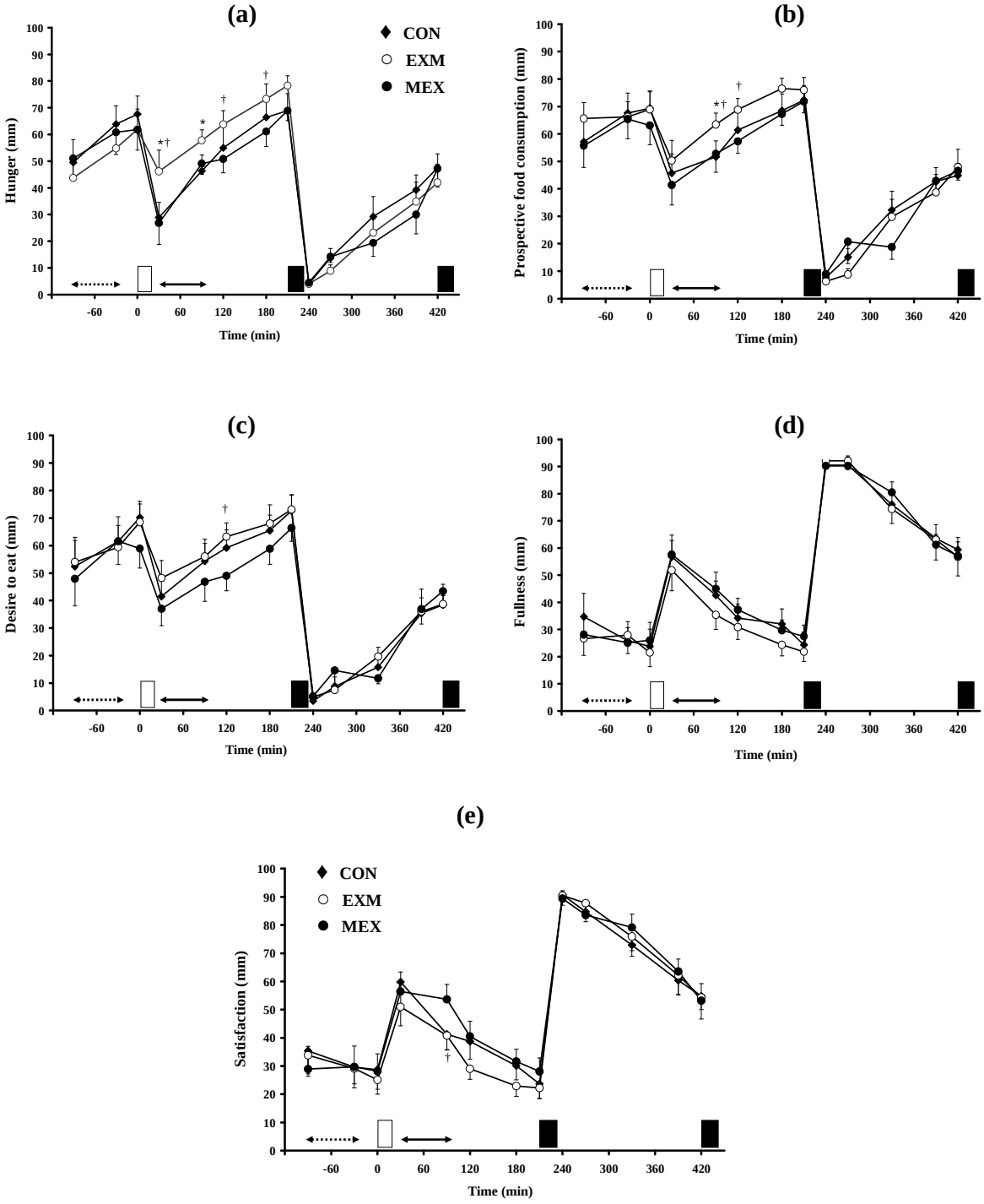


Figure 5.2: Postprandial (a) hunger, (b) prospective food consumption, (c) desire to eat, (d) fullness, and (e) satisfaction in CON (♦), EXM (○), and MEX (●) trials. Dotted arrow (◄┈┈►) represents exercise session in EXM, black arrow (◄──►) represents exercise session in MEX. Times at which test breakfast (□) and buffet meals (■) were provided are shown. Values are expressed as means, with standard errors represented by vertical bars. (*) significantly different from CON ($p < 0.05$), (†) significantly different between exercise trials ($p < 0.05$).

5.3.7 Cumulative energy balances

Cumulative energy, fat, and carbohydrate balances, measured over an 8.5 h observation period during each trial are shown in **Figure 5.3**. Two-factor ANOVA revealed a significant main effect of trial ($p < 0.001$), time ($p < 0.001$), and interaction (trial × time) ($p < 0.001$) for cumulative energy, fat, and carbohydrate balances as well as substrate utilization over time. Energy balance at the end of the 8.5 h observation period (after consumption of *ad libitum* dinner) did not differ between EXM (+1045 ± 122 kcal) and MEX (+1128 ± 145 kcal) trials but were significantly lower than CON (+1549 ± 146 kcal; $p < 0.001$ and $p < 0.01$ respectively). Cumulative carbohydrate balance remained positive in all three trials but were significantly lower in EXM (+708 ± 54 kcal) and MEX (+695 ± 69 kcal) compared to CON (+916 ± 63 kcal, $p < 0.001$ for both). Compared to CON (+190 ± 70 kcal), participants remained in negative fat balance at the end of the observation period in both EXM (-106 ± 86 kcal, $p < 0.001$) and MEX (-22 ± 72 kcal; $p < 0.001$), but these did not differ between exercise trials.

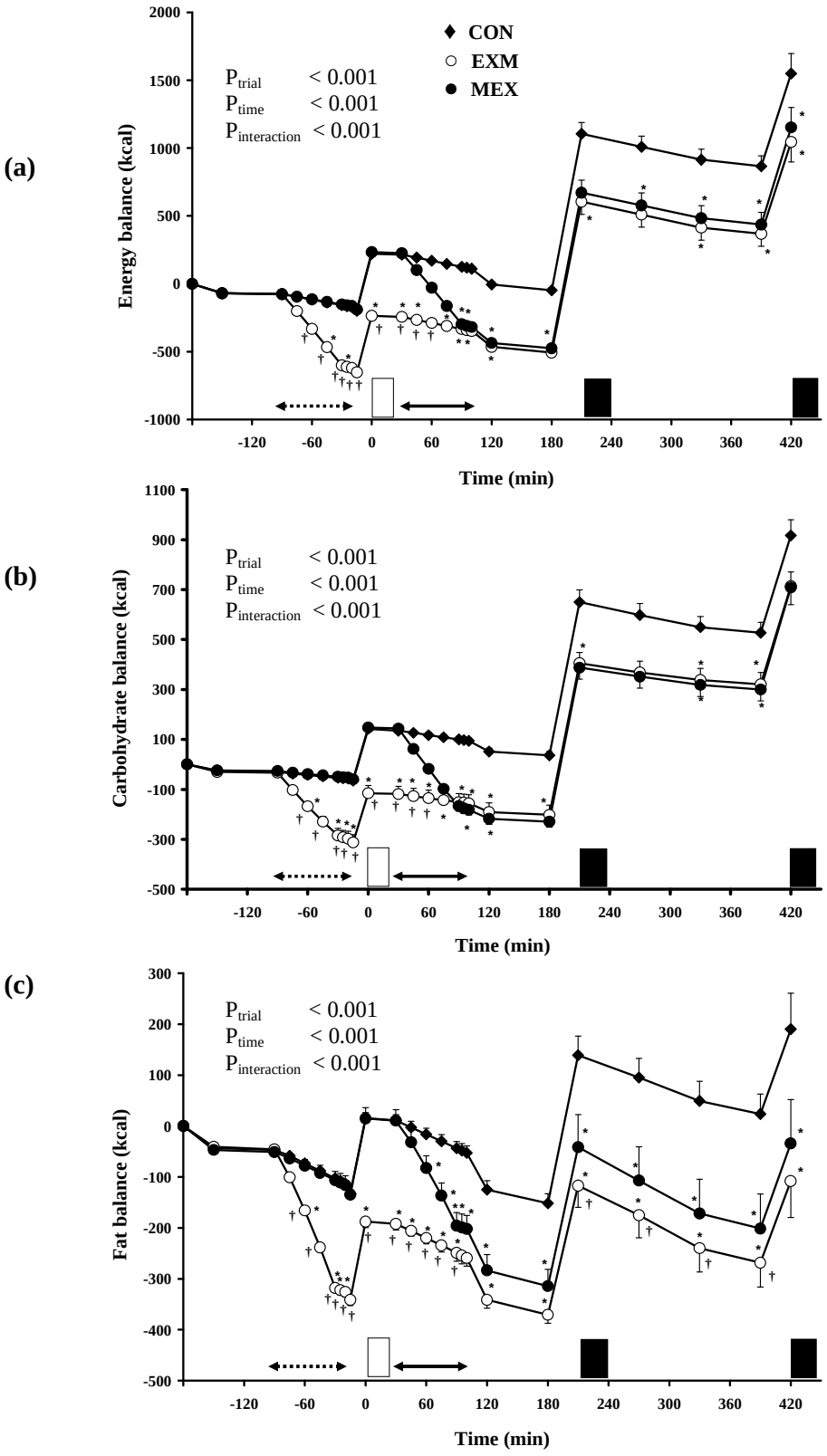


Figure 5.3: Cumulative (a) energy balance, (b) carbohydrate balance, and (c) fat balance in CON (♦), EXM (○), and MEX (●) trials. Dotted arrow (◄┈┈►) represents exercise session in EXM, black arrow (◄━━►) represents exercise session in MEX. Times at which test breakfast (□) and buffet meals (■) were provided are shown. Values are expressed as means, with standard errors represented by vertical bars. (*) significantly different from CON ($p < 0.001$), (†) significantly different between exercise trials ($p < 0.001$).

5.3.8 Plasma metabolic responses during exercise

Figure 5.4 summarises the plasma metabolic variables during 0, 30, and 60 min of exercise period in EXM and MEX trials. TG concentrations were significantly higher in the MEX trial at 30 and 60 min of exercise ($p < 0.05$). Similarly, insulin concentrations were significantly higher at all time points in MEX compared to EXM. There were no differences in glucose concentrations across both exercise conditions.

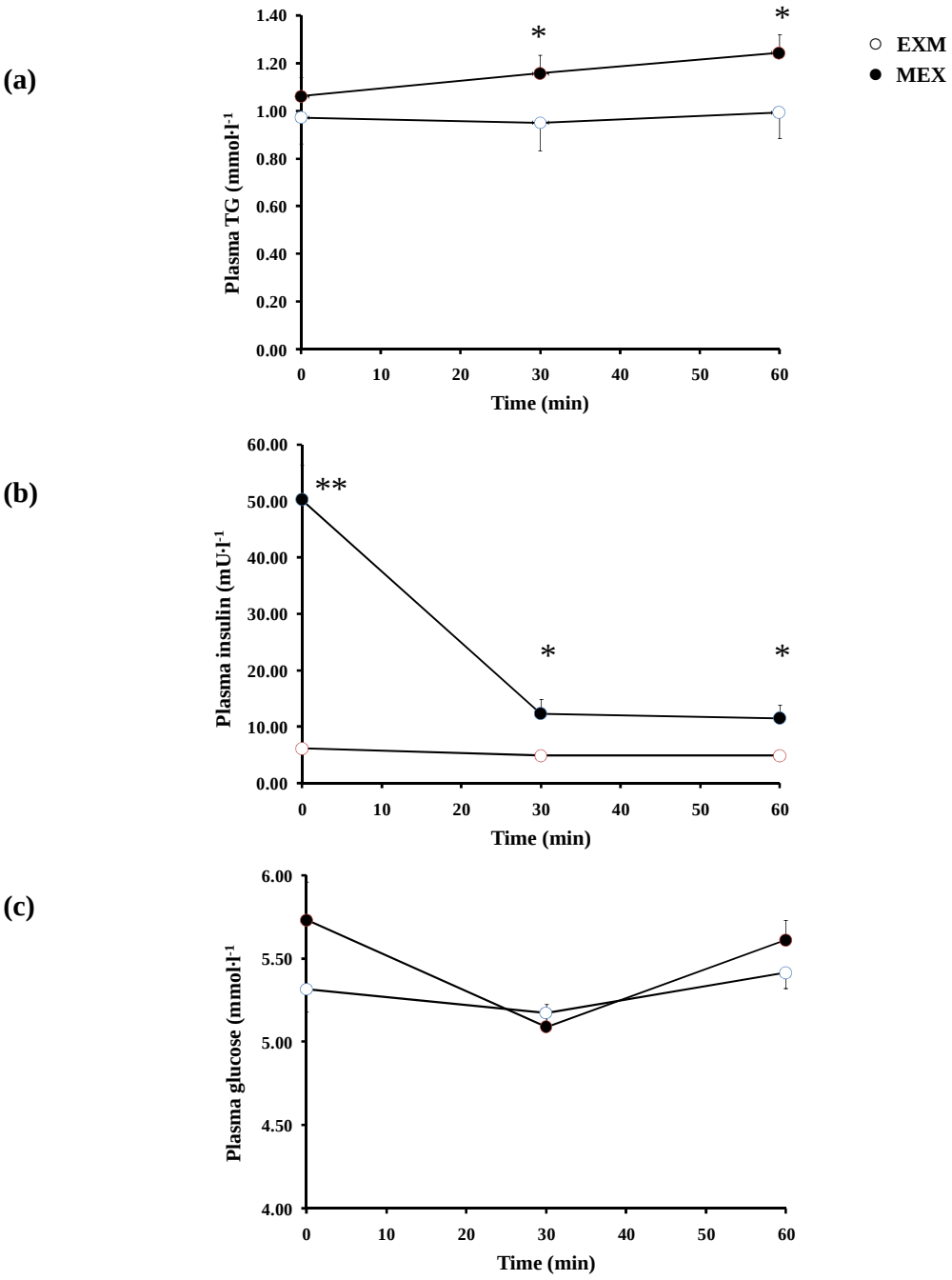


Figure 5.4: Plasma (a) triglycerides, (b) insulin, and (c) glucose during 0, 30, and 60 min of exercise in EXM (○), and MEX (●) trials. Values are expressed as means, with standard errors represented by vertical bars. (*) significantly different between trials ($p < 0.05$), (**) ($p < 0.001$).

5.3.9 Postprandial plasma metabolic responses across time

Table 5.5 summarises the overall postprandial responses for TG, insulin, and glucose. **Figure 5.5** illustrates the postprandial responses for TG, insulin, and glucose over the 8.5 h observation period. Two-factor ANOVA revealed a significant main effect of trial (p = 0.043), time ($p < 0.001$), and interaction (trial × time) ($p < 0.001$) for postprandial TG response. Plasma TG concentrations increased progressively over time and peaked after the consumption of lunch. The overall postprandial TG response was 17% lower in EXM compared to CON (p = 0.02) and no significant changes were observed between MEX and EXM or between MEX and CON. Similar to TG responses, insulin responses differed over time ($p < 0.001$), and across trials (p = 0.009), with a significant trial × time interaction observed ($p < 0.001$). Plasma insulin concentrations peaked from fasting values following both breakfast and lunch in all trials, followed by a gradual decline throughout the rest of the 4-h postprandial period. Compared to CON, the overall postprandial insulin response was 19% (p = 0.008) and 23% ($p < 0.001$) lower in EXM and MEX respectively. The postprandial glucose response did not differ across between all trials.

Table 5.5. Postprandial time-averaged and incremental area under curve (AUC) for plasma metabolic variables over 8.5 h observation period (n = 10). Values are mean ± S.E.M.

	EXM	**MEX**	**CON**
TG (mmol·l^{-1})			
Total TAUC	1.24 ± 0.10 *	1.34 ± 0.13	1.50 ± 0.15
Incremental TAUC	0.27 ± 0.08 *	0.35 ± 0.09	0.48 ± 0.10
Insulin (mU·l^{-1})			
Total TAUC	25.05 ± 4.09 **	23.61 ± 3.49 **	30.85 ± 4.76
Incremental TAUC	18.89 ± 3.27 *	16.64 ± 2.53 **	23.80 ± 4.15
Glucose (mmol·l^{-1})			
Total TAUC	5.50 ± 0.09	5.43 ± 0.13	5.32 ± 0.14
Incremental TAUC	0.32 ± 0.11	0.18 ± 0.16	0.15 ± 0.12

* significantly different from CON ($p < 0.05$); ** ($p < 0.001$)

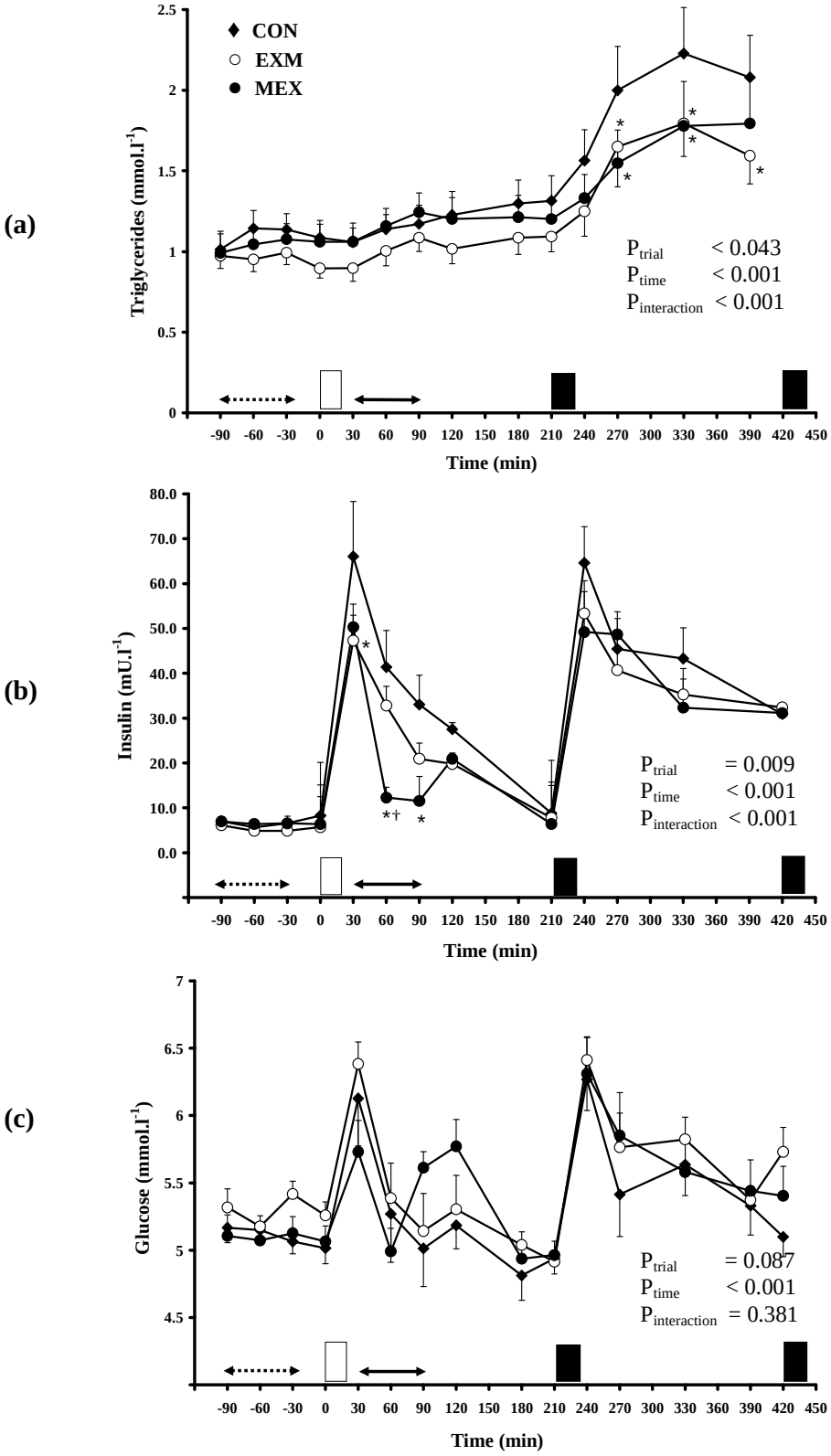


Figure 5.5: Postprandial plasma (a) triglycerides, (b) insulin, and (c) glucose in CON (♦), EXM (○), and MEX (●) trials. Dotted arrow (◄┈┈►) represents exercise session in EXM, black arrow (◄──►) represents exercise session in MEX. Times at which test breakfast (□) and buffet meals (■) were provided are shown. Values are expressed as means, with standard errors represented by vertical bars. (*) significantly different from CON ($p < 0.001$), (†) significantly different between exercise trials ($p < 0.001$).

5.3.10 Postprandial plasma metabolic responses across intervals

AUC for TG, insulin, and glucose in three separate intervals: pre-breakfast (-90 – 0 min), post-breakfast (0 – 210 min) and post-lunch (210 – 420 min) are shown in **Figure 5.6**. When analysed in separate intervals, postprandial TG responses following lunch (210 – 240 min) were 20% (p = 0.02) and 17% (p = 0.03) lower in EXM and MEX respectively, than CON, with no difference between all trials for pre-breakfast or post-breakfast intervals. In the pre-breakfast interval, the insulin responses were 21% lower in EXM compared to CON (p = 0.007), and 20% lower compared to MEX (p = 0.010). Following post-breakfast interval, insulin responses were 27% (p = 0.033) and 39% (p = 0.002) lower in EXM and MEX respectively, compared to CON, with no differences between EXM and MEX. However, insulin responses did not differ between trials in the post-lunch interval. There were no changes in glucose responses across all intervals.

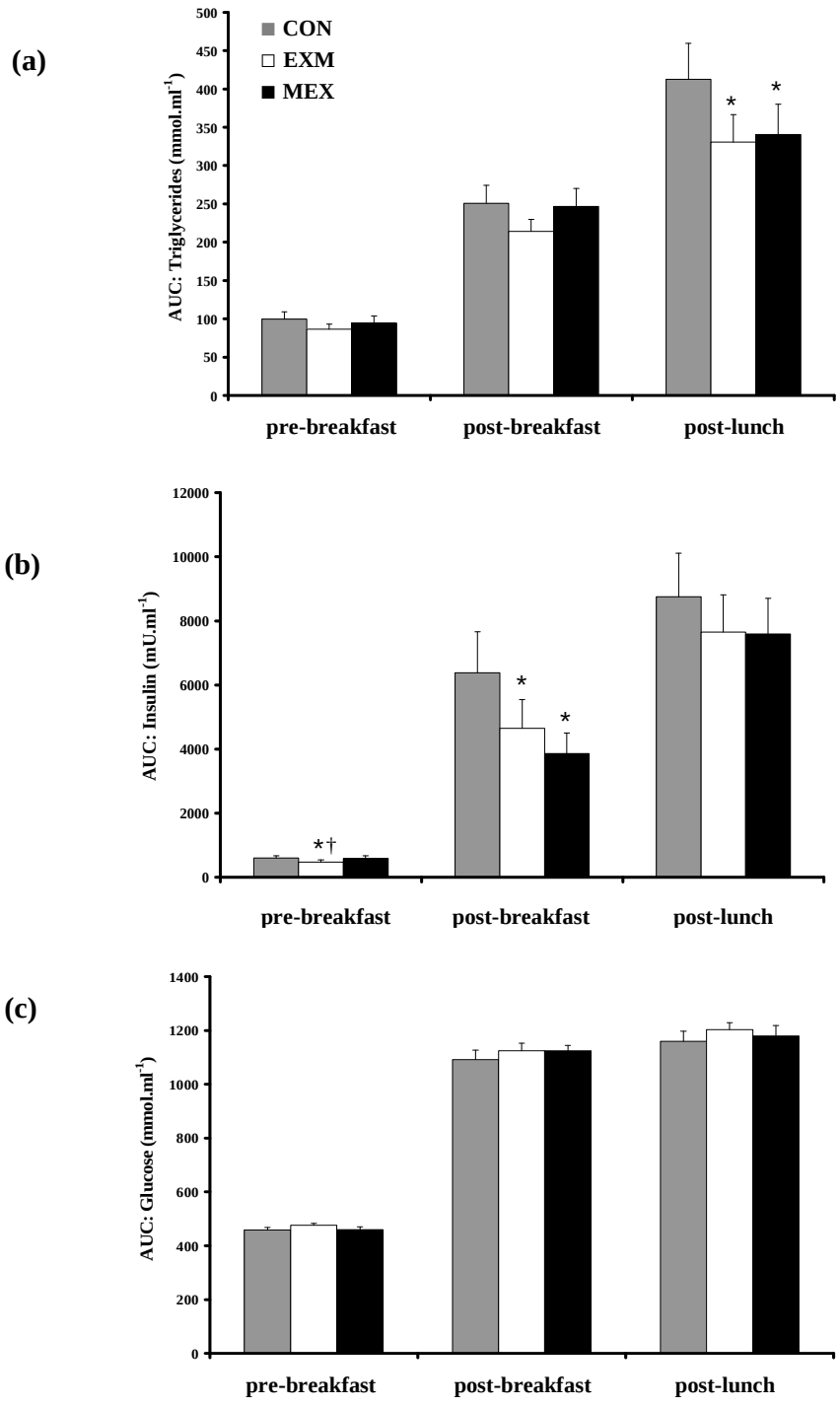


Figure 5.6. Area under curve for plasma (a) TG, (b) insulin, and (c) glucose, in separate intervals: pre-breakfast (-90 – 0 min), post-breakfast (0 – 210 min) and post-lunch (210 – 420 min) in CON (■), EXM (□), and MEX (■) trials. Values are expressed as means, with standard errors represented by vertical bars. (*) significantly different from CON ($p < 0.05$), (†) significantly different between exercise trials ($p < 0.05$).

5.4 Discussion

The aim of the present study was to determine the effects of exercise undertaken before or after a meal on macronutrient and postprandial metabolism, appetite responses and *ad libitum* feeding. The main finding of the study was that an acute bout of moderate intensity exercise elicited negative fat balance and greater total fat oxidation at the end of the day, irrespective of timing of exercise relative to consumption of a meal. Despite inducing a moderate energy deficit, exercise did not seem to modify appetite, or increase *ad libitum* energy intake. In addition, overall postprandial insulin responses were lower, in both exercise conditions compared to no exercise, and postprandial TG responses were decreased after the consumption of *ad libitum* lunch. These findings lend support for the role of exercise in weight control as well as improving postprandial lipid metabolism.

There have been a large number of studies examining the effects of pre-exercise meal ingestion on macronutrient metabolism during exercise. The finding of the present investigation is in agreement with previous work that showed pre-exercise feeding altered substrate utilisation during the exercise period in favour for carbohydrate oxidation, compared to exercise in the fasted state (Derave *et al.* 2007; Wu *et al.* 2003; Coyle *et al.* 1997; Horowitz *et al.* 1997; Schneiter *et al.* 1995; Montain *et al*. 1991). Reciprocally, the rate of fat oxidation during exercise was greater when exercise was performed in the fasted state prior to a meal. Consumption of carbohydrate prior to exercise has been shown to suppress fat oxidation and this is largely due to the concomitant rise in plasma insulin and insulin-induced suppression of lipolysis (Hansen *et al.* 2005; Horowitz *et al.* 1997). Therefore, the present findings support the prevailing notion that, with regards to macronutrient partitioning, exercise performed before a meal (*e.g.* fasted state) maximises fat oxidation during the exercise itself whilst ingesting a meal prior to exercise results in greater carbohydrate disposal by the working muscles.

Although a considerable body of evidence has shown that fat oxidation during exercise is enhanced in the fasted state compared to after meal ingestion, relatively few studies have directly compared the effects of exercise timing around meal ingestion on macronutrient oxidation in the post-exercise period. Data from these studies (Bennard & Doucet 2006; Schneiter *et al.* 1995) reported that exercise performed before a morning meal resulted in a greater total amount of fat oxidised in the postprandial period of 2 – 8 hours compared

to exercise after a meal. However, it is important to note that meals were not provided in the post-exercise period in these studies, which does not reflect 'real-life' setting. To mimic a more realistic, typical daily life scenario, we extended the protocol by including *ad libitum* lunch and dinner meals in the present study. The finding of this study demonstrates that, overall fat oxidation was greater in both exercise conditions, compared to control, and that exercise before a meal is no more efficient in stimulating greater overall fat oxidation than exercise after a meal, even with *ad libitum* lunch in the post-exercise period. Upon closer inspection however, when the energy costs of exercise were adjusted for, it appears that fat oxidation rates were highly congruent in all trials. Thus, the greater amount of fat oxidised at the end of the day in both exercise conditions was mainly contributed by the exercise sessions, and not during the post-exercise postprandial period. Some studies have shown that whole-body fat oxidation rate induced by acute exercise still remained elevated until the following day, despite food consumption, compared to no exercise (Burton *et al.* 2008; Folch *et al.* 2001; Gill *et al.* 2001a; Bielinski *et al.* 1985). Therefore, this present finding seems to contradict the notion that exercise substantially increases whole-body fat oxidation in the post-exercise period. Perhaps the discrepancy between our findings and previous studies can be explained by the greater caloric expenditure (~700-2100 kcal) others had imposed on their subjects, compared to ~400 kcal session in this study. Additionally, it could also be that any increases in fat oxidation during the post-exercise period in this study are offset by subsequent meal/carbohydrate ingestion, which induced hyperinsulinaemia and attenuated the exercise-induced fat oxidation in the postprandial period (Achten & Jeukendrup 2004).

Although many studies have examined the effects of short-term exercise on meal consumption and appetite, research examining the potential interactive effects of exercise timing and appetitive behaviour is lacking. To the author's knowledge, only one published study has compared the effects of pre-meal and post-meal exercise on appetite responses. Cheng *et al.* (2009) reported that 50-min of exercise at 60% $\dot{V}O_2$max after consumption of a test meal appears to suppress overall appetite and prolong depressed hunger scores for a longer time (~5 h) compared to exercise before a meal. It is postulated that exercise helped to extend the postprandial satiating effect caused by meal ingestion, compared to meal alone, in the postprandial period. However, Cheng's study did not assess *ad libitum* food intake, therefore it is unknown if the observed appetite suppression would translate into lower food intake during the postprandial period. In the present

study, exercise, irrespective of its timing relative to meal ingestion, did not seem to influence appetite and food intake in the post-exercise period, as energy intake was similar between trials. This observation also confirms previous findings that have typically shown that short term energy intake (1-2 d) is unaltered in response to an acute exercise bout (Hopkins *et al.* 2010).

More importantly, when addressing the effects of exercise in maximising fat oxidation, substrate balances should be looked into. In the present study, energy balance was positive, but lower than control, in both exercise conditions at the end of the 8.5 h postprandial observation period. The individual substrate balances however, differed markedly. Fat balance was negative at the end, whereas carbohydrate balance was positive, in both exercise conditions respectively, and this is irrespective of exercise timing relative to meal ingestion. Also key to the present findings is that participants did not compensate in energy intake, as demonstrated by the lower relative energy intake, therefore they were in energy deficit relative to control in both exercise conditions. However, the present study was only short term (~9 h), and energy intakes and physical activity outside the laboratory were not recorded, therefore 24-h fat balance could not be determined. In 24-h room calorimeter studies comparing the effects of exercise on 24-h fat oxidation, findings have consistently shown that fat oxidation on the days with exercise did not differ from a sedentary control day in lean adults (Melanson *et al.* 2002), obese individuals (Melanson *et al.* 2009a; Saris & Schrauwen 2004) and endurance-trained individuals (Melanson *et al.* 2008) when subjects were fed to maintain energy balance. Nonetheless, it is not impossible to believe that in the absence of deliberate attempts to restore energy balance in the post-exercise period, transient exercise-induced energy deficit and negative fat balance may still persist through one or more postprandial periods under real-life conditions.

This study also highlights the effect of timing of exercise relative to meal ingestion on TG and insulin responses. It is well established that pre-meal exercise performed 4-18 h before a meal is effective in attenuating the lipaemic response to a fatty meal (Mestek *et al.* 2008; Miyashita *et al.* 2008; Pfeiffer *et al.* 2005; Kolifa *et al.* 2004; Petridou *et al.* 2004; Gill *et al.* 2002; Gill & Hardman 2000; Murphy *et al.* 2000; Tsetsonis *et al.* 1997). Reports have suggested that decreased hepatic VLDL secretion (Gill & Hardman 2003; Malkova *et al.* 2000), diminished entry of intestinally-derived TG into the circulation (Kolifa *et al.* 2004; Hardman & Aldred 1995) or increased LPL-mediated TG clearance

(Petit & Cureton 2003; Zhang *et al.* 1998; Tsetsonis & Hardman 1997) may have contributed to the hypotriglyceridaemic effect observed following exercise. However, the effect of exercise timing on postprandial TG is less clear. Specifically, only Katsanos & Moffatt (2004) and Zhang *et al.* (1998) directly compared the effects of exercise timing relative to meal ingestion on postprandial lipemia (without *ad libitum* feeding), but produced contradicting results. Both studies reported attenuation in postprandial TG when exercise was performed before a meal, and while Katsanos & Moffatt (2004) found a similar attenuation with post-meal exercise, the earlier study reported that post-meal exercise did not have any effect on postprandial TG (Zhang *et al.* 1998). But because these two studies did not include *ad libitum* food consumption, it remains a topic of debate whether exercise performed during postprandial period is as effective in everyday living. In agreement with previous findings in the literature, our findings showed that exercise prior to meal ingestion caused an overall reduction in postprandial TG responses. However, contrary to previous reports that found lower lipaemic response to exercise in the postprandial state (Katsanos & Moffatt 2004; Hardman & Aldred 1995; Klein *et al.* 1992; Schlierf *et al.* 1987), the overall attenuation in TG response was not evident when exercise was performed after a meal in this study, implying that the timing of exercise relative to meal ingestion appears to be important. Support for this finding is found in the work of others who showed that plasma TG was unaltered following post-meal exercise bouts on the same day (Henderson *et al.* 2010; Zhang *et al.* 1998; Chinnici & Zaugner 1971).

However, when examining the data for postprandial TG in separate intervals, we observed lower postprandial TG in the post-lunch interval in both exercise conditions compared to control. This data suggests that the TG-reduction effect may be delayed when exercise was performed after meal ingestion, as the exercise session began 2 h later than when exercise was performed prior to a meal. This delayed response in postprandial TG following exercise after meal ingestion may be due to a delayed increase in exercise-induced LPL activity, as LPL has been reported to increase, not immediately, but ~3 – 4 h after exercise (Perreault *et al.* 2004; Kiens *et al.* 1989). This would tie in with the present observation in the exercise after meal condition, as the reduction in postprandial TG is only evident after consuming *ad libitum* lunch, and not earlier. However this is only speculative. Reductions in postprandial TG have been documented without a concomitant increase in muscle LPL activity (Miyashita & Tokuyama 2008; Gill *et al.* 2003; Herd *et al.* 2001), thereby suggesting that the reduction in TG may be accounted for by mechanisms other than increased LPL. It could also be that exercise after a meal acutely

decreased the rate of VLDL-TG secretion into the circulation (Magkos *et al.* 2006) or increased clearance rate of chylomicron/VLDL-TG (Gill *et al.* 2001). Despite the delayed response in the attenuation of plasma TG when exercise was performed after consumption of a meal, the beneficial effects of exercise in ameliorating postprandial TG can still be achieved and maintained beyond the observation period, as reductions in plasma TG following exercise has been shown to last for at least 12 – 24 hours post exercise (Burton *et al.* 2008; Silvestre *et al.* 2007; Gill & Hardman 2000).

A single session of exercise can enhance insulin sensitivity for up to 48 h post-exercise (Cartee *et al.* 1989; King *et al* 1988). Although exercise typically reduces postprandial TG, the effect on postprandial insulin however, is less consistent, as some studies reported reduction (Burton *et al.* 2008; Gill et al. 2007; Hagobian & Braun 2006; Hardman & Aldred 1995), while others did not (Harrison *et al.* 2009; Tsetsonis & Hardman 1996). In addition, others have reported that changes in postprandial TG and insulin are not correlated (Gill *et al.* 2002). The present study demonstrated lower overall insulin responses in both exercise conditions compared to control, with no effect of exercise timing. Exercise-induced suppressions of insulin levels were particularly evident during exercise in both exercise conditions, as has been previously noted (Hardman & Aldred 1995; Welle 1984), and this effect was also independent of timing of meal ingestion. When analysing the data in separate intervals, neither exercise conditions had an effect on insulin responses post *ad libitum* lunch as plasma insulin were restored to levels similar to that of control. Together, these findings suggest that the exercise-induced reduction effect on insulin is only transitory and that an acute bout of exercise in the morning, either before or after a breakfast meal, had no effect on postprandial insulin responses to a meal ingested 2-4 hours later. It is conceivable that the consumption of lunch had diminished the insulin-reducing effect of exercise. This observation confirms recent findings (Harrison *et al.* 2009; Holtz *et al.* 2008), that providing replacement energy after exercise, especially in the form of carbohydrate, will therefore reverse the attenuation effect of exercise on postprandial insulin concentrations. This finding also confirms the dissociation between insulin and triglyceride responses, as reported in Chapter 3.

5.5 Summary

It is well-recognised that exercise is a key component of a healthy lifestyle, and abundant evidence clearly dictates that significant metabolic health improvements occur even after a single exercise session. Our findings demonstrated that exercise performed prior to a breakfast meal is no more beneficial than exercise after a meal in reducing fat balance and promoting greater overall fat oxidation at the end of the day, despite consuming *ad libitum*. Secondly, an acute bout of moderate intensity exercise did not lead to a subsequent increase in appetite (*i.e.* hunger, prospective food consumption, and desire to eat) and energy intake. Additionally, we demonstrated that postprandial TG responses to *ad libitum* lunch were lower with exercise, irrespective of exercise timing. From a practical standpoint, it shows that timing of exercise relative to meal ingestion is not a major factor influencing the beneficial effects of exercise observed in the present study. Furthermore, it is also the case that exercise after one meal is equivalent to exercise before another meal, when this is extended into a 'real-world' setting where exercise and food ingestion are often interspersed with each other.

CHAPTER 6

An Assessment of the Effects of a Single Bout of Exercise on Appetite-Related Measures Using a Computer-Based Approach: a Pilot Study

6.1 Introduction

Individuals who undertake high levels of physical activity maintain their energy balance and achieve a stable body weight more effectively than their sedentary peers (Wareham *et al.* 2005; Jeffery *et al.* 2003) and long-term maintenance of weight loss in the formerly obese is facilitated by high physical activity (Schoeller 1998). In support of this, studies measuring food intake have fairly consistently shown that energy intake remains unchanged immediately following an exercise session (Hopkins *et al.* 2010). However, over more prolonged periods of time, evidence suggests that some degree of dietary compensation for the exercise-induced energy expenditure may occur, which perhaps explain why some overweight/obese individuals in particular, are resistant to the theoretical weight loss benefits of exercise (King *et al.* 2008; Franz et al. 2007). Thus, there appears to be a discrepancy between the short and long term effects of exercise on energy intake. This may be a true effect, but may also reflect difficulties in obtaining accurate functional measures of appetite and 'usual' food intake in response to acute exercise in a laboratory setting as the setting is not completely natural and this may lead to under or overconsumption relative to 'usual' feeding behaviour (Blundell *et al.* 2010). This is because the initiation and maintenance of feeding behavior is co-determined by metabolic and non-metabolic factors. Among the latter, environmental cues, as well as reward, cognitive, and emotional factors, play an important role, particularly in human food intake in the modern world (Berthoud 2006).

In appetite research, the optimal experimental protocol to determine short-term energy intake still remain elusive because of the complex and multifaceted nature of eating behaviour (Blundell *et al.* 2010). The standard laboratory practice of offering different foods in a buffet meal scenario will not necessarily guarantee a sensitive experimental protocol as food consumption are likely to be influenced by a range of external factors

such as amount/volume of food presented (Rolls *et al.* 2006), food variety (Norton *et al.* 2006), and palatability (Yeomans *et al.* 2001), all of which can override internal appetite cues and delay satiation, as well as promote increased food intake (Hetherington *et al.* 2006). These issues make it difficult to determine whether exercise acutely influences the regulation of appetite. Thus, measuring food intake *per se* using the commonly used method (*i.e. ad libitum* buffet meals) may not be the best way of detecting changes in eating behavior and appetite as it may not be sensitive enough to detect more subtle alterations in the non-metabolic processes that can influence energy intake. How much and what we eat might be influenced by a brief period of cognitive activity during which we select a particular food and portion-size shortly before the onset of a meal (Brunstrom *et al.* 2007), therefore it is possible that exercise may affect how much people perceive they should eat and some aspects of food preference or liking. Differences in the impact of exercise on these perceptual factors may explain some inter-individual variability in compensatory eating after exercise. If exercise can influence the decisions associated with meal planning, it could then help understanding of if and how exercise influences subsequent energy intake.

Therefore the objective of the present pilot study is to determine the effects of acute, moderate-intensity exercise on non-metabolic measures of food intake (*i.e.* ideal portion size, liking, food utility) that may potentially affect decisions associated with energy intake, using a novel, computer-based procedure, across an array of food items. This may provide a more sensitive tool to detect, perhaps fairly subtle changes in appetite variables compared to buffet meals, as it can provide aggregate measures of 'ideal portion sizes' (in kcal units) for a number of different foods at the same time (analogous to performing a number of buffet meal trials) and enables us to explore temporal changes of such effects, a measurement that a test meal will not be able to do. In addition, further information about other appetite-related measures can be obtained in concert, providing further information about how exercise can influence energy intake over time. This pilot study aims to answer several research questions: (1) Does exercise influence how individuals perceive their ideal food portion size, food liking and food utility? (2) If exercise does influence the above measures, do these responses differ across sex, lean/overweight, and dietary restraint? In addition, measurements of disinhibition, loss aversion, as well as adult attachment anxiety will be obtained from all subjects, to determine whether these behavioural factors may also explain some of the variance in the effects of exercise.

6.2 Methods

6.2.1 Participants

Twenty-seven (men = 14, women = 13) healthy participants were recruited from the staff and student population of University of Glasgow. Their age, body mass, and BMI were (mean ± SD) 31.2 ± 8.9 years, 72.7 ± 14.2 kg, and 24.6 ± 3.6 $kg \cdot m^{-1}$. Participants had no known history of CVD or diabetes, were non-vegetarians, and were not consuming any type of specialised diet or taking a medication thought to interfere with appetite. The present study was conducted according to the guidelines stated in the Declaration of Helsinki and all procedures involving human subjects were approved by the Faculty of Biomedical and Life Sciences Ethics Committee at the University of Glasgow, UK. Each participant gave written informed consent prior to participation.

6.2.2 Questionnaires

Dietary restraint and disinhibition were measured by the Three Factor Eating Questionnaire (TFEQ) (Stunkard & Messink 1985) (**Appendix B**) with mean scores of 8.1 ± 5.5 and 5.7 ± 2.9 respectively. Restrained eaters were classified by a score of above 10 (Stubbs *et al.* 2002b). Attachment anxiety style was evaluated using Experiences in Close Relationships questionnaire (ECR) (**Appendix E**) with a mean score of 3.1 ± 0.7. A high score is taken as evidence of an insecure attachment orientation (Brennan 1998).

6.2.3 Experimental design

Each participant completed 2 experimental trials in counter-balanced conditions separated by approximately one week: exercise (EX), control (CON). An overview of the study protocol is shown in **Figure 6.1**. Participants were asked to refrain from alcohol and planned exercise on the day before each trial.

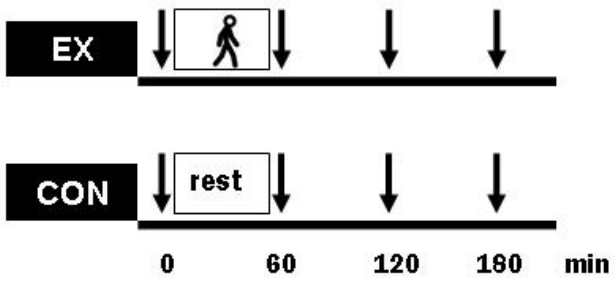


Figure 6.1. **Experimental design**. Subjects completed two trials: exercise (EX), and control (CON). Computer tasks (↓) were completed at 0, 60, 120 and 180 min.

a) Exercise trial (EX)

Participants arrived at the metabolic suite at 9.00 am after a 12-hour overnight fast. They were asked to complete a set of computer tasks (described below) before performing the exercise session (0 min). The exercise bout began at ~9.30 am, which involved a 60-min treadmill walk at a speed of 5.5 $km \cdot h^{-1}$ and 4.5% gradient for all participants (intensity ~6 METs). Expired air samples were collected at 15-min intervals during the walk, for the determination of oxygen uptake and carbon dioxide production. Heart rate and rate of perceived exertion were recorded every 15 min during the walk. Participants completed their responses on the computer immediately, 60, 120, and 180 min after the completion of the exercise. They were free to sit, read, use a computer, relax, or work at a desk between the measurements. Participants are allowed to consume only water *ad-libitum* throughout the measurement period. The trial ended after the last computer task measurement was taken.

b) Control trial (CON)

This trial was identical to the exercise trial, except that participants remained rested for from 9.30 – 10.30 am, the period during which corresponded to the exercise session in EX trial.

6.2.4 Computer task procedure

A set of computer tasks designed to provide stimulus to explore the several behavioural responses towards certain foods was used in this study. The software was developed by Dr. J.M. Brunstrom and his colleagues from the University of Bristol, as described in Brunstrom & Rogers (2009). The procedure is comprised of 5 tasks designed to assess: 1) appetite sensations, 2) mood, 3) food utility, 4) food liking, and 5) ideal portion sizes. Measures of ideal portion size, utility, and liking involved showing subjects pictures of 16 different test foods that are commonly consumed in the UK (fish fingers, pasta and tomato sauce, raw banana, pizza, crackers, chicken tikka masala, Jaffa cakes, oven chips, Pringles potato crisps, peanut M&Ms, garlic bread, KitKat, potato salad, chicken chow mein, cheese baguette and cornflakes). The composition of macronutrient for each test food is listed in **Table 6.1**. Responses were recorded for each food item before the next item was shown. Each food was photographed using a high-resolution digital camera on the same white, 255-mm diameter plate. Constant lighting and camera angle were maintained for each picture. For all food except cornflakes, picture number 1 showed a 20 kcal portion and for the subsequent pictures, the portion is increased by 20-kcal (*i.e.* picture 2 = 40 kcal, picture 3 = 60 kcal, etc). For the cornflakes, each portion picture is

spaced in logarithmic steps, *i.e*. the absolute difference between the amounts of calories shown increases with picture number. In total, each food was photographed between 40 and 70 times, depending on the total amount of food that could be positioned on the plate. Participants were navigated through the procedures at all times by instructions presented on-screen. The code for the computer task procedure was written in Visual Basic and presented on a 17" monitor.

Table 6.1. Macronutrient composition of each test food (values given per 100 g).

Food	Calories (kcal)	Carbohydrate (g)	Fat (g)	Protein (g)
Fish fingers	185.2	15.7	7.4	13.0
Pasta and sauce	152.7	21.4	5.3	5.3
Cornflakes	370.0	84.0	0.8	7.0
Banana	95.0	23.2	0.3	1.2
Pizza	408.2	42.9	18.4	18.4
Crackers	488.0	65.5	20.7	6.9
Tikka masala	168.1	18.5	6.7	9.2
Jaffa cakes	384.0	73.3	8.1	4.4
Oven chips	180.0	31.4	3.2	4.6
Potato crisps	558.0	50.0	37.5	4.6
M&M®	515.0	60.6	26.2	9.6
Garlic bread	370.0	35.9	20.5	7.7
KitKat®	512.8	61.5	25.6	5.1
Potato salad	141.0	10.4	10.6	1.1
Chow mein	82.0	7.1	3.4	5.7
Cheese baguette	318.5	28.0	16.2	15.6

1) Appetite sensations

Subjective appetite sensations for 'hunger' and 'fullness' were recorded using the 100 mm visual analogue scales (VAS), anchored on each end with "not at all" and "extremely".

2) Food utility

Participants were shown a standard-sized portion (200 kcal) of each test food and were instructed to "*Imagine you are having this food RIGHT NOW. What is the maximum you would pay for this food*?" (**Figure 6.2**). Using a computer mouse, participants selected their price on a vertical scale that was displayed to the left of the food image. By moving the scale up increased the value, and moving the scale down decreased the value. Values can be selected in increments of one penny. All test foods were presented in random order each time.

Figure 6.2. Screenshot of food utility task

3) Liking

Participants were shown a standard-sized portion of each test food and were instructed to rate their liking for each test food using a 100-mm visual analogue rating scale with end

anchor points “not at all” and “extremely” (**Figure 6.3**). All test foods were presented in random order each time.

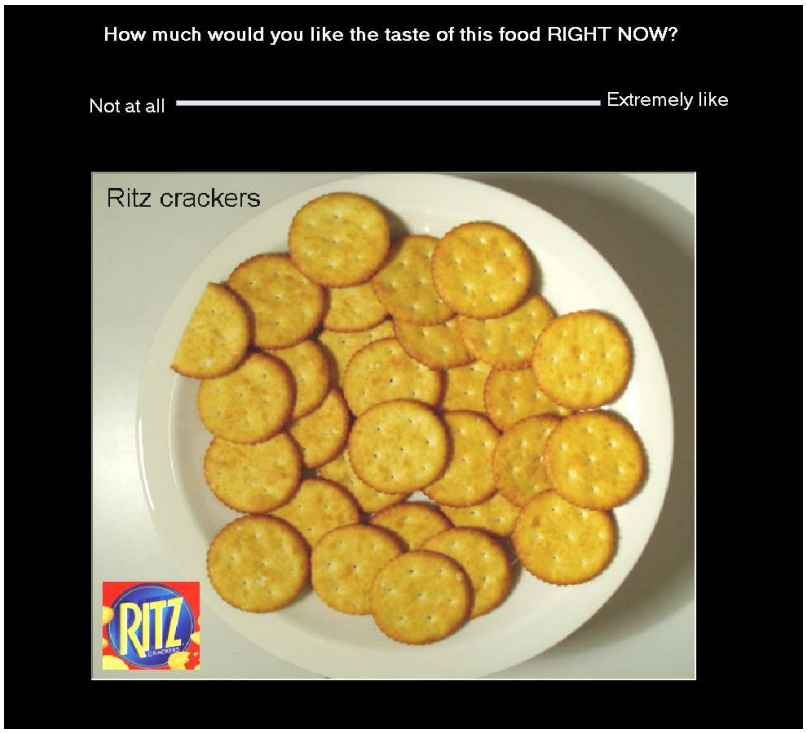


Figure 6.3. Screenshot of food liking task

4) Ideal Portion Size

A randomly-selected portion size of each test food was displayed on the screen. Participants were instructed to “Imagine you are having this food for lunch RIGHT NOW. Select your IDEAL portion size” (**Figure 6.4**). Pressing the left arrow-key on the keyboard caused the portion size to decrease and the right arrow-key to increase portion size. The pictures were loaded with sufficient speed that gave the appearance that the change in portion size was ‘animated’. Once participants selected their ideal portion size, hitting the ‘continue’ button will take them to a different test food. All test foods were presented in random order each time.

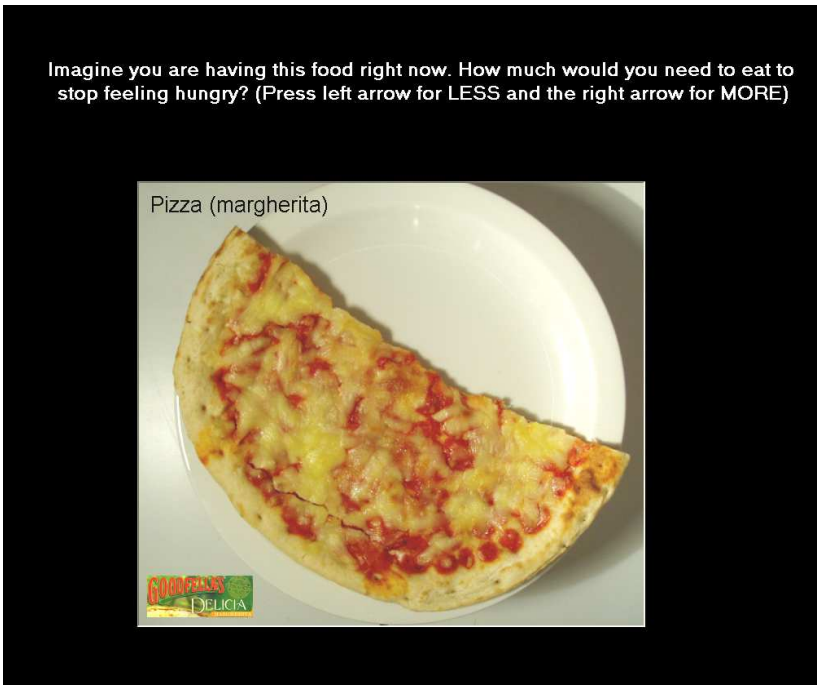


Figure 6.4. Screenshot of ideal portion size task

5) Mood states

Subjective mood states were recorded using a 20-item mood scales to measure positive affect (*attentive, interested, alert, excited, enthusiastic, inspired, proud, determined, strong and active*) and negative affects (*distressed, upset, hostile, irritable, scared, afraid, ashamed, guilty, nervous, and jittery*). Participants were asked to rate the extent to which they were experiencing each particular emotion with reference to a 5-point scale: 'very slightly or not at all', 'a little', 'moderately', 'quite a bit' and 'very much'. The mood scales were based on the Positive and Negative Affect Schedule (PANAS) (Watson *et al.* 1988).

6) Loss aversion cash task

At the end of participation, each participant was asked to accept or reject a series of outcomes of winning or losing a variable amount of money using the classic coin toss procedure. These were presented on a computer screen as the prospective outcomes of a coin flip (**Figure 6.5**). Participants indicated their willingness to take the gamble by

pressing YES or NO. A total of 49 trials were presented. The gamble had potential gains ranging from +£0 to +£50, and potential losses ranging from −£0 to −£50.

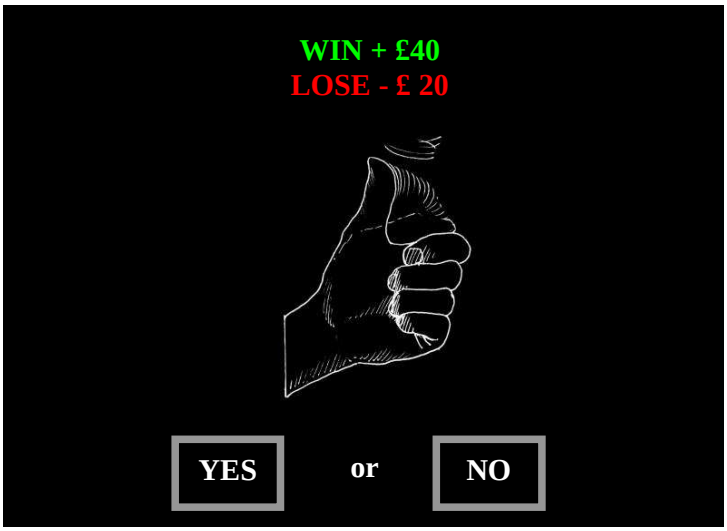


Figure 6.5. Screenshot of loss aversion cash task

6.2.5 Statistical analysis

Statistical analyses were performed using Statistica (version 6.0, StatSoft Inc., Tulsa, USA) and SPSS (version 10.0, SPSS Inc., Chicago, US). The main analyses for this study are focused on the differences in the appetite-related measures across experimental trials (exercise *vs.* control); subjective hunger and fullness, ideal portion size, food liking, and food utility. For liking, food utility, and ideal portion size measures, participants' responses for each test food were compiled and a mean response was calculated. The total areas under the 180-min variable vs. time curve (AUC) were used as summary measures of hunger, fullness, and food liking. Summary measures for ideal portion size and food utility were reported as the sum of the values of each time point. Paired-samples t tests were used to compare summary measures between trials. Two-way repeated measures ANOVAs (trial × time) were used to compare changes over time and across the two trials. *Post hoc* Tukey tests were used to identify where differences lay. Pearson correlations were used to assess the relationship between variables. Data are presented as means ± SEM, unless otherwise stated. Statistical significance was accepted at $p < 0.05$.

6.3 Results

6.3.1 Responses during the treadmill walk

The treadmill speed and gradient in the exercise trial (EX) were identical for all participants. The exercise session was completed without difficulty, participants rated the exercise as 'light' (10.8 ± 1.8) on the Borg scale of 6-20. Mean gross exercise energy expenditure was 444 ± 80 kcal. Mean oxygen uptakes and heart rates over the course of the exercise sessions were 21.4 ± 3.4 $ml \cdot kg^{-1} \cdot min^{-1}$ and 124 ± 16 $beats \cdot min^{-1}$.

6.3.2 Summary responses for appetite-related measures

The summary for appetite-related measures is presented in **Table 6.2**. Participants experienced 17.4% less hunger in the EX trial compared to control (p = 0.004). Participants also chose to eat significantly smaller portions (by -7.7%) in the EX trial compared to control (p = 0.003). Correspondingly, exercise also resulted in lower macronutrient portion size for carbohydrate (p = 0.003), fat (p = 0.004) and protein (p = 0.004). There were no differences observed in AUC liking and fullness, and total food utility between trials.

Table 6.2. Time-averaged area under curve (TAUC) for appetite sensations and liking; total food utility and ideal portion size over 3-h observation period (n = 27). Values are mean ± S.E.M.

Appetite-related measures	CON	EX	p value
TAUC hunger (mm)	56 ± 4	46 ± 4	*0.004*
TAUC fullness (mm)	25± 3	30 ± 3	0.065
Total food utility (£)	6.54 ± 0.33	6.45 ± 0.30	0.528
TAUC food liking (mm)	46 ± 3	44 ±3	0.153
Total ideal portion size (kcal)	1620 ± 111	1495 ± 105	*0.003*
- carbohydrate (kcal)	764 ± 52	705 ± 49	*0.003*
- fat (kcal)	630 ± 44	581 ± 42	*0.004*
- protein (kcal)	189 ± 13	176 ± 12	*0.004*

6.3.3 Appetite sensations: hunger & fullness

Figure 6.6a and **6.6b** summarise the appetite responses over the 180-min observation period in both CON and EX trials. Hunger increased over time in both trials ($p < 0.001$). *Post hoc* analysis revealed that hunger was significantly lower (-31%) immediately post exercise (60 min) compared to the same time point in the control trial ($p = 0.001$) (**Figure 6.6a**). Fullness did not change between trials but changed over time ($p = 0.001$) with progressively lower fullness ratings compared to baseline in both trials (**Figure 6.6b**). No interaction effect was found for hunger and fullness.

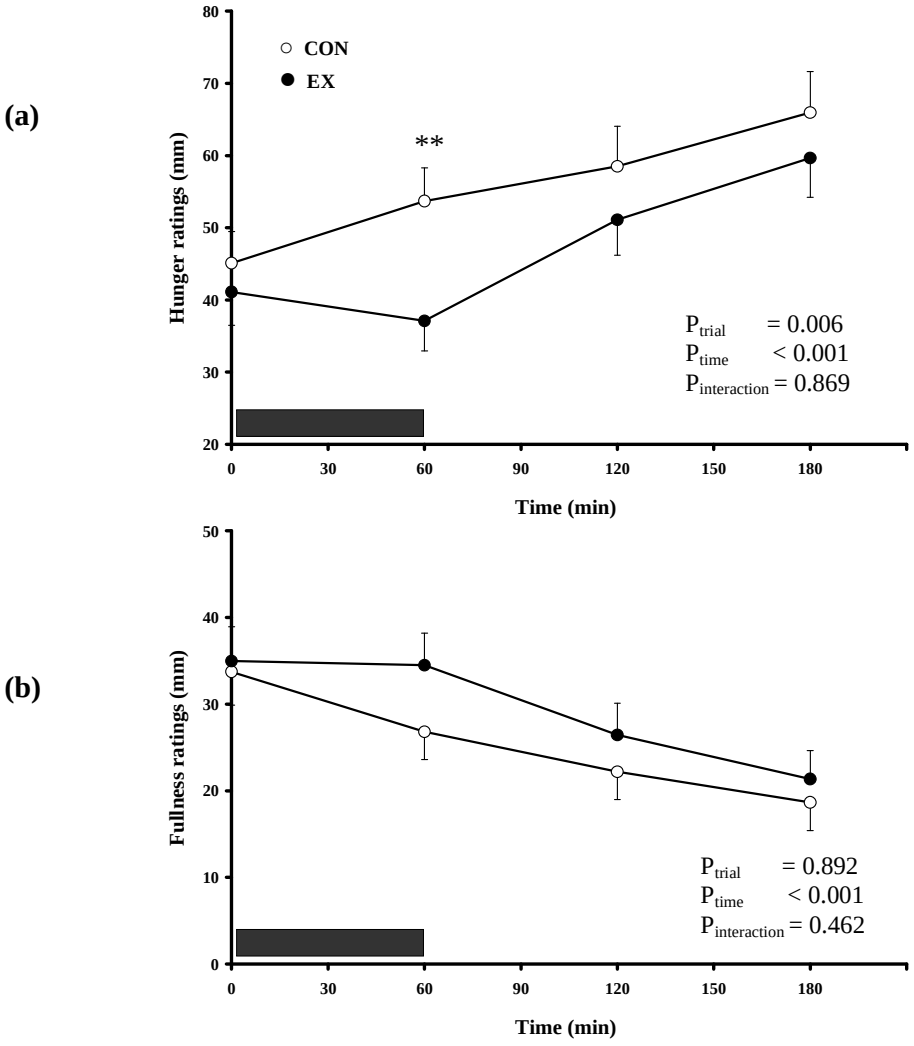


Figure 6.6. Subjective (a) hunger and (b) fullness in control (CON, ○) and exercise (EX, ●) trials ($n = 27$). The 1-h exercise bout is represented by a black rectangle (▬).Values are expressed as means, with standard errors represented by vertical bars. (**) significantly different between trials ($p < 0.001$).

6.3.4 Food utility

Figure 6.7a summarises the food utility responses over the 180-min observation period in both CON and EX trials. Exercise did not affect how much participants were willing to pay for food but a main effect of time ($p < 0.001$) indicated that the values for food utility increased over time in both trials.

6.3.5 Food liking

Figure 6.7b summarises the liking responses over the 180-min observation period in both CON and EX trials. Participants did not experience a change in liking between trials but there was a significant increase in liking scores over time ($p < 0.001$) compared to baseline in both CON and EX trials.

6.3.6 Ideal portion size

Figure 6.7c summarises the ideal portion size responses over the 180-min observation period in both CON and EX trials. Two-factor ANOVA revealed significant main effects of trial ($p < 0.001$) and time ($p < 0.001$) with significantly lower portions (-14.1%) observed immediately post exercise (60 min) ($p < 0.001$) compared to corresponding control. To explore this effect further, the effect of exercise on each food was analysed separately. Overall, participants reported significantly lower portion sizes for pasta ($p = 0.004$), crackers ($p = 0.014$), garlic bread ($p = 0.041$), KitKat ($p = 0.016$), and cheese baguette ($p = 0.014$) in the exercise trial relative to control. There were no significant differences between trials in the ideal portion size for other test foods.

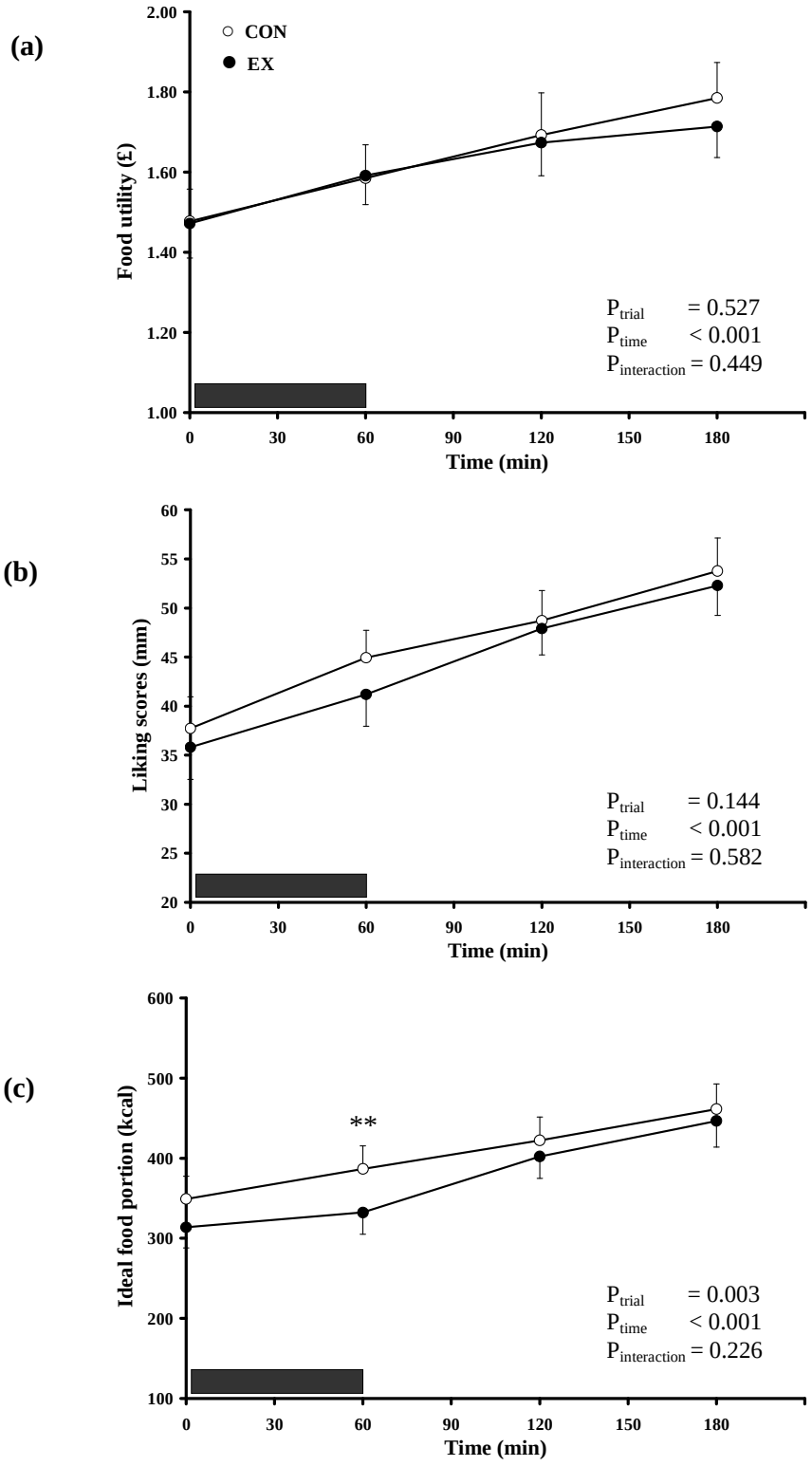


Figure 6.7. (a) Food utility, (b) food liking, and (c) ideal portion size in (CON, ○) and exercise (EX, ●) trials (n = 27). The 1-hr exercise bout is represented by a black rectangle (▬).Values are expressed as means, with standard errors represented by vertical bars. (**) significantly different between trials ($p < 0.001$).

6.3.7 Mood responses: positive & negative affects

Figure 6.8 summarises the positive and negative affect responses respectively over the 180-min observation period in both CON and EX trials. Positive affects did not change between trials but decreased significantly ($p < 0.001$) over time and there was a significant trial × time interaction ($p = 0.049$). Similarly, negative affects were not different between trials but decreased significantly over time ($p < 0.001$) with the interaction effect approaching significance ($p = 0.055$).

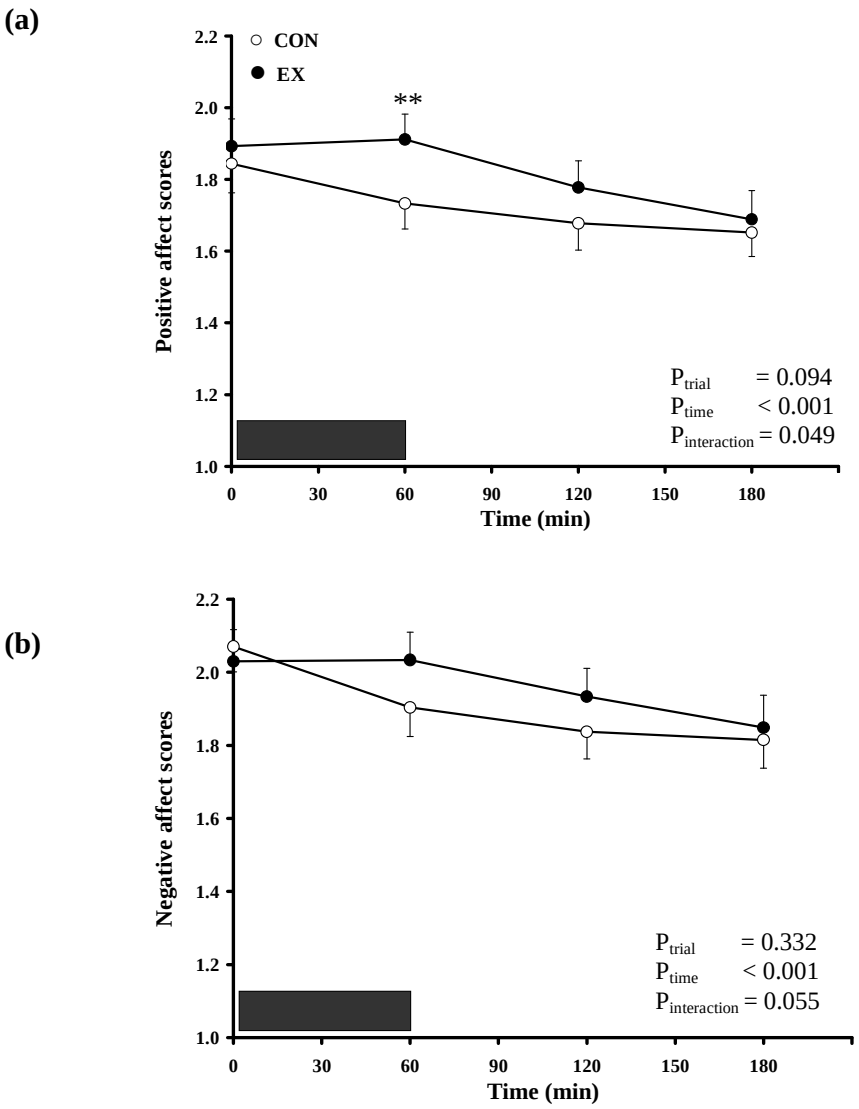


Figure 6.8. (a) Positive affect, and (b) negative affect (CON, ○) and exercise (EX, ●) trials (n = 27). The 1-h exercise bout is represented by a black rectangle (▬).Values are expressed as means, with standard errors represented by vertical bars. (**) significantly different between trials ($p <$ 0.001).

6.3.8 Correlations between appetite-related measures

The associations between summary of ideal portion sizes and hunger, fullness, food utility, and food liking in each experimental condition are illustrated in **Figure 6.9**. Larger ideal portion size was strongly correlated with greater hunger and food liking in control and exercise trial respectively. No correlations were found between ideal portion size with fullness and food utility in both trials.

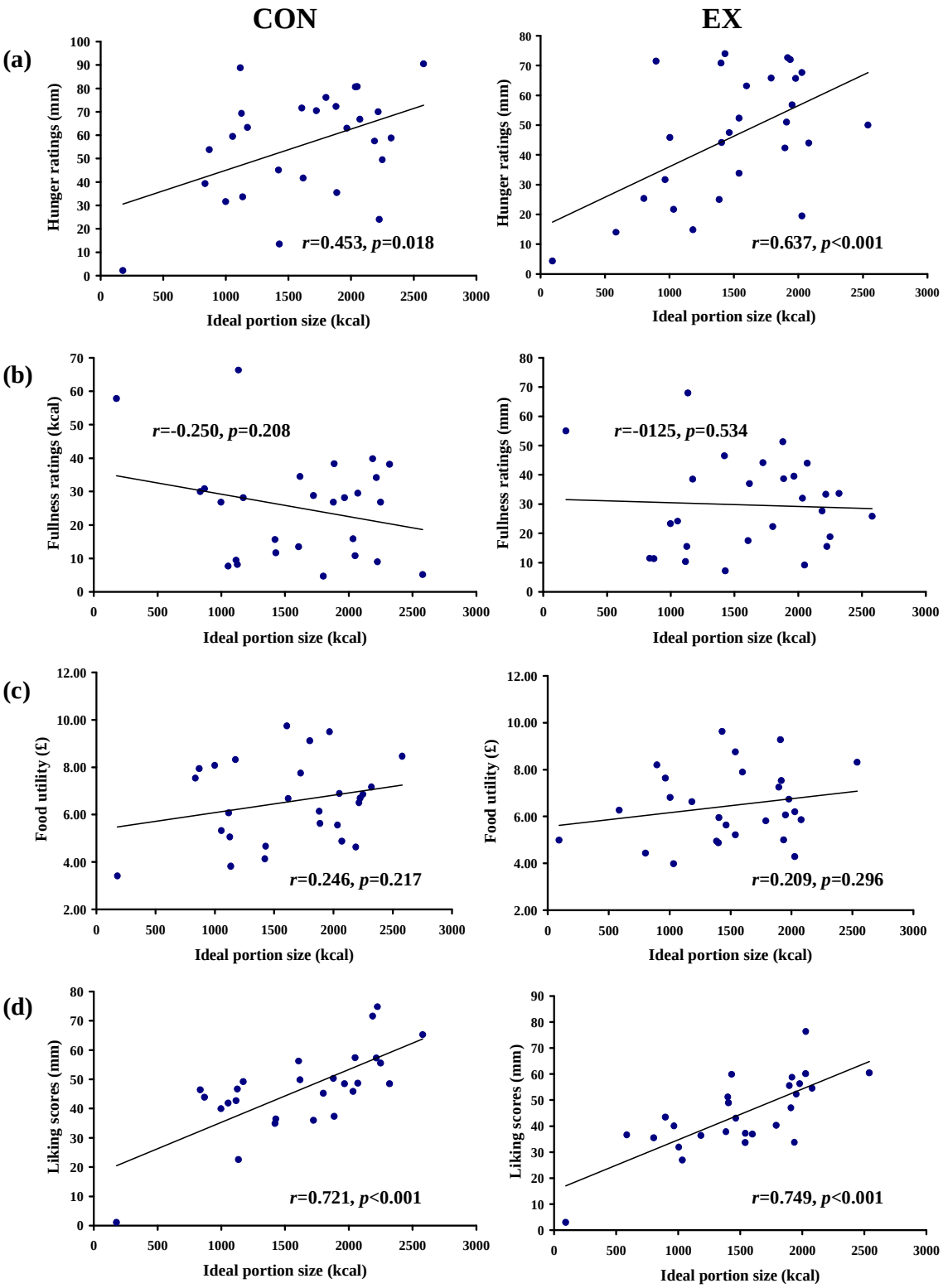


Figure 6.9. Relationship between ideal portion size and (a) hunger, (b) fullness, (c) food utility, and (d) food liking in CON (right panel) and EX (left panel) trials separately. Each panel includes associated *r* and *p* values.

6.3.9 Correlates of the exercise-induced change in ideal portion size

Changes in total ideal portion size between control and exercise trials were related to change in AUC liking scores (r = 0.600, p = 0.001), as illustrated in **Figure 6.10**. The correlations were also significant at 0 min (r = 0.383, $p < 0.05$), 60 min (r = 0.482, $p < 0.05$), and 120 min (r = 0.413, $p < 0.05$). No significant associations were found between change in ideal portion size and change in hunger, fullness, and food utility.

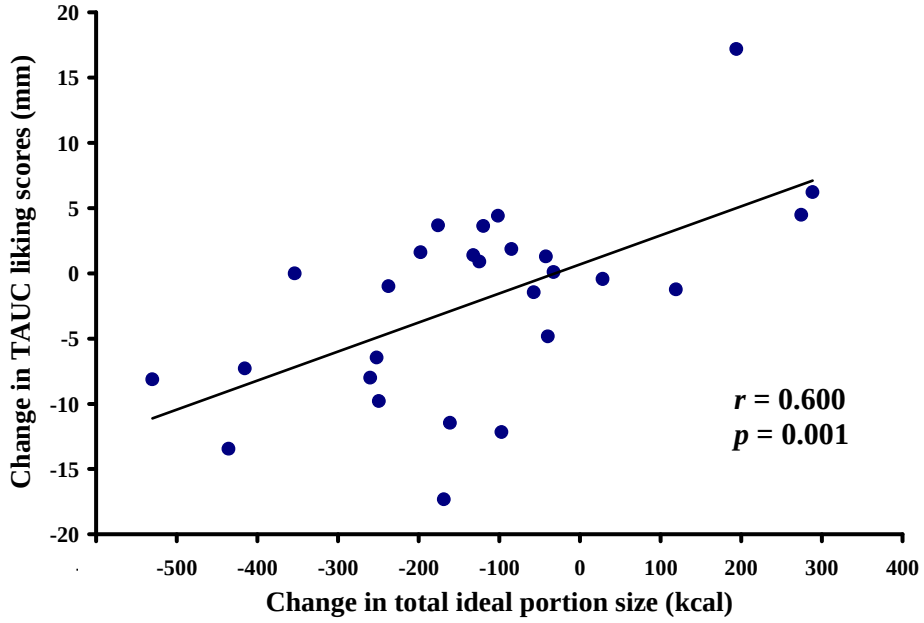


Figure 6.10: Relationship between change in total ideal portion size and change in TAUC food liking (change = exercise trial – control trial).

6.3.10 Effects of sex, BMI, and dietary restraint

The summary of appetite-related measures across sex, BMI, and dietary restraint categories in separate trials is presented in **Table 6.3.** The statistical analyses were repeated using sex (men = 14, women = 13), BMI (lean = 15, overweight/obese = 12), and dietary restraint (non-restrained = 19, restrained = 8) as categorical variables. There was a sex effect for food utility [$F(1,25) = 12.80$, $p = 0.001$] with women willing to pay more for food compared to men. Women, lean, and non-restrained individuals, tended to feel less hungry in the exercise trial compared to control. In contrast, this effect was not observed in men, overweight/obese, and restrained individuals. Despite this, there were no significant main effect of sex, BMI, and dietary restraint or by trial interaction on appetite sensation, food liking, food utility, and ideal portion size.

Table 6.3. Time-averaged area under curve (TAUC) for appetite sensations and liking; total food utility and ideal portion size over 3-h observation period across sex, BMI, and dietary restraint categories. Values are expressed as mean ± S.E.M.

	TAUC hunger (mm)	TAUC fullness (mm)	Food utility (£)	TAUC liking (mm)	Ideal portion size (kcal)
CON trial					
Sex:					
Male (*n*=14)	48 ± 7	28 ± 5	5.55 ± 0.39	46 ± 5	1747 ± 172
Female (*n*=13)	65 ± 5	33 ± 4	7.61 ± 0.38*	47 2	1483 ± 136
BMI:					
Lean (*n*=15)	58 ± 5	24 ± 4	6.98 ± 0.47	49 ± 3	1538 ± 129
Overweight (*n*=12)	53 ± 8	27 ± 4	5.99 ± 0.45	44 ± 5	1722 ± 194
Dietary restraint:					
Non-restraint (*n*=19)	59 ± 5	24 ± 3	6.42 ± 0.40	47 ± 3	1659 ± 121
Restraint (*n*=8)	49 ± 9	29 ± 5	6.82 ± 0.66	45 ± 7	1528 ± 253
EX trial					
Sex:					
Male (*n*=14)	42 ± 6	22 ± 3	5.67 ± 0.31	44 ± 5	1634 ± 160
Female (*n*=13)	51 ± 5 [a]	26 ± 4	7.29 ± 0.42	44 ± 3	1345 ± 127
BMI:					
Lean (*n*=15)	45 ± 5 [a]	29 ± 4	6.59 ± 0.47	46 ± 3	1405 ± 117
Overweight (*n*=12)	48 ± 7	31 ± 4	6.27 ± 0.35	43 ± 5	1607 ± 187
Dietary restraint:					
Non-restraint (*n*=19)	47 ± 5 [a]	29 ± 3	6.30 ± 0.37	44 ± 2	1525 ± 111 [a]
Restraint (*n*=8)	45 ± 8	32 ± 6	6.81 ± 0.51	45 ± 8	1423 ± 250

[a] significantly different from control ($p < 0.05$), * significantly different from men ($p < 0.05$)

6.3.11 Correlations between appetite-related measures across categories

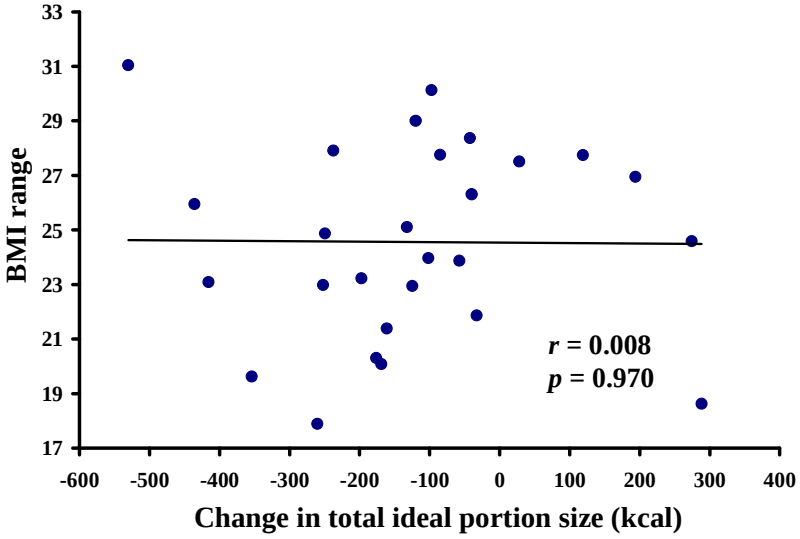
Table 6.4 summarises the relationship between ideal portion size and appetite-related measures across sex, BMI, and dietary restraint categories in separate trials. In both exercise and control trials, ideal portion size was positively correlated with hunger in men and women, and overweight/obese individuals, but not in lean, and restrained eaters. Hunger correlated with ideal portion size in non-restrained individuals in the exercise trial but not in control. Increased in liking is strongly associated in increased ideal portion size across all categories in both trials. There is no relationship between change in total ideal portion size across BMI range and dietary restraint scores as illustrated in **Figure 6.11**.

Table 6.4. Correlation coefficients between appetite-related measures and **ideal portion size** across gender, BMI, and dietary restraint categories

Correlation coefficient	Sex		BMI		Dietary Restraint	
	Male (*n* =14)	**Female (*n* =13)**	**Lean (*n* =15)**	**OW (*n* =12)**	**NR (*n* =19)**	**Restraint (*n* = 8)**
CON trial						
AUC hunger	0.617*	0.580*	0.016	0.779*	0.422	0.476
AUC fullness	-0.439	-0.025	-0.35	-0.516	0.045	-0.792*
Food utility	0.166	0.101	0.130	0.522	0.179	0.406
AUC liking	0.852**	0.554*	0.654**	0.857**	0.644**	0.817*
EX trial						
AUC hunger	0.609*	0.658*	0.466	0.579*	0.657**	0.350
AUC fullness	-0.468	0.298	-0.042	-0.270	0.105	-0.392
Food utility	0.495	0.389	0.233	0.285	0.193	0.305
AUC liking	0.843**	0.635*	0.718**	0.848**	0.604**	0.902*

(*) significant at $p < 0.05$; (**) $p < 0.01$; AUC, area under curve; OW, overweight; NR, non-restraint

(a)



(b)

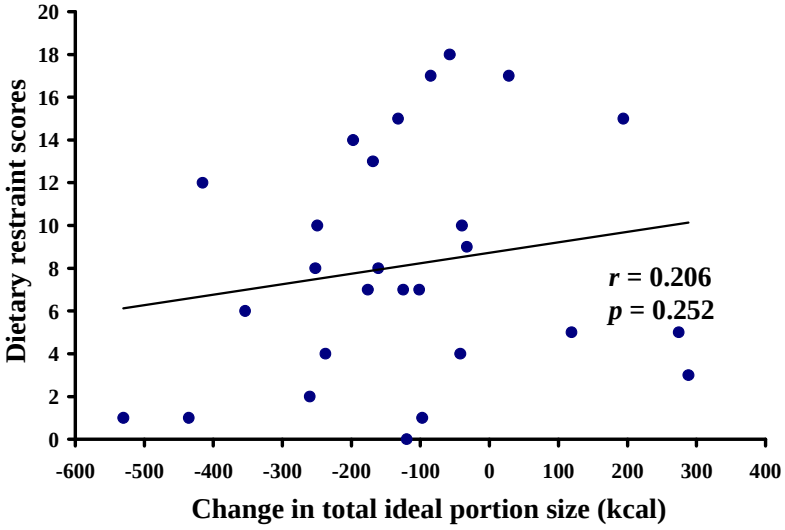


Figure 6.11. Relationship between change in total ideal portion size and (a) BMI range, and (b) dietary restraint scores (as indicated by restraint measures in Three Factor Eating Questionnaire). Each panel includes associated *r* and *p* values. (change = exercise trial – control trial)

6.3.12 Correlations between appetite-related measures and other variables

Table 6.5 summarises the relationship between appetite-related measures and other behavioural variables (*i.e.* disinhibition, attachment anxiety, and loss aversion) in separate experimental trials. Higher disinhibition was associated with increase in food liking in the exercise trial, but not in control. Interestingly, increases in loss aversion scores were significantly correlated with increases in food liking (r = 0.683, p < 0.001) and ideal portion size (r = 0.514, p < 0.05) in both trials. None of the appetite-related measures relate with attachment anxiety scores. There was no relationship between change in ideal portion size across disinhibition, attachment anxiety and loss aversion scores as illustrated in **Figure 6.12**.

Table 6.5. Correlation coefficients between appetite-related measures and disinhibition, attachment anxiety and loss aversion scores in control and exercise trials separately

Correlation coefficients	Disinhibition	Attachment anxiety	Loss aversion
CON trial			
AUC hunger (mm)	-0.063	-0.144	0.337
AUC fullness (mm)	-0.017	-0.039	-0.242
Total food utility (£)	-0.108	0.030	0.184
AUC food liking (mm)	0.268	0.136	0.683**
Total ideal portion size (kcal)	0.306	0.104	0.514**
EX trial			
AUC hunger (mm)	0.101	-0.161	0.422*
AUC fullness (mm)	-0.133	0.056	-0.260
Total food utility (£)	-0.029	-0.162	-0.032
AUC food liking (mm)	0.424*	0.178	0.546**
Total ideal portion size (kcal)	0.338	0.140	0.488*

(*) significant at p < 0.05; (**) p < 0.01; AUC, area under curve; CON, control; EX, exercise

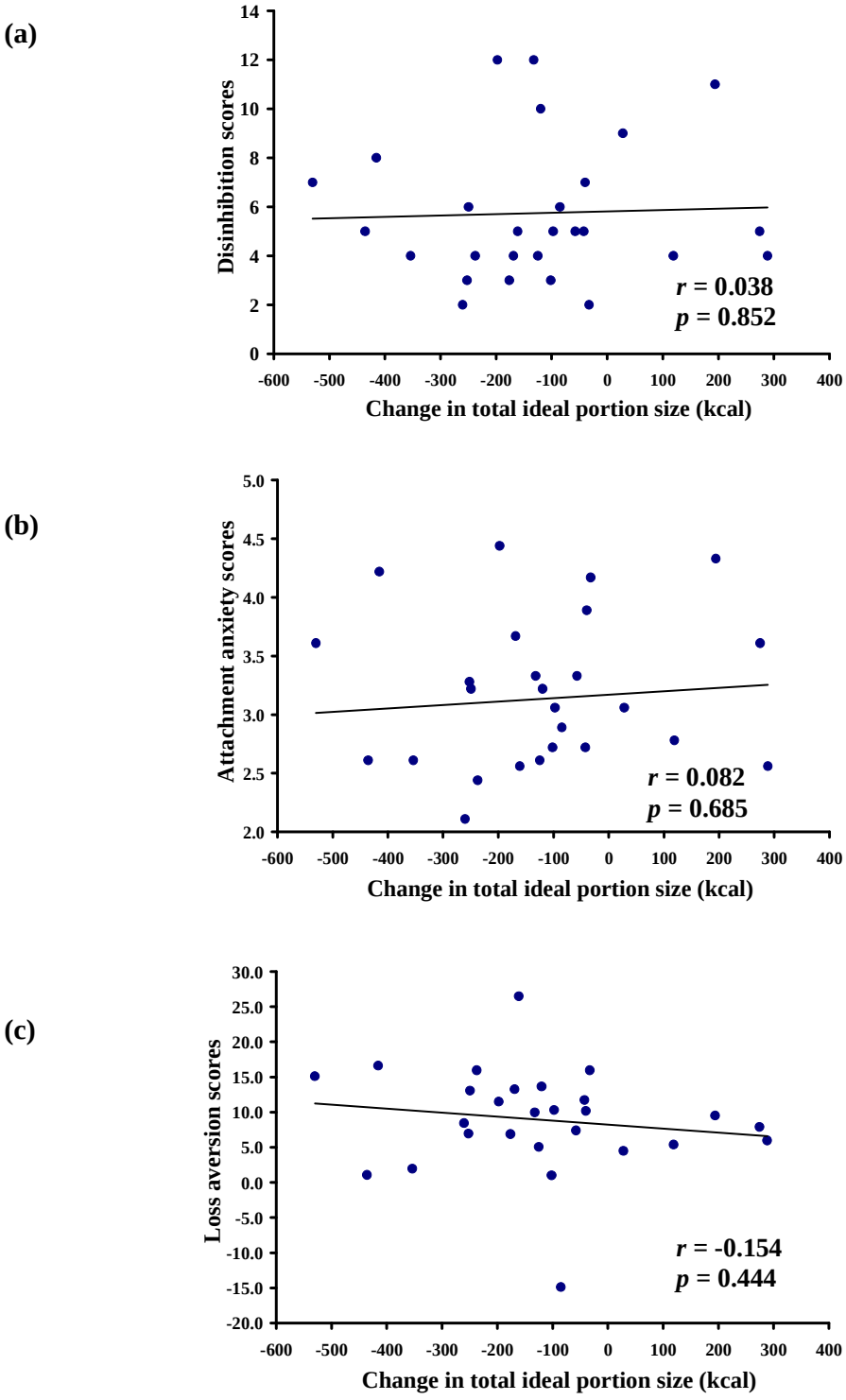


Figure 6.12. Relationship between change in total ideal portion size and (a) disinhibition scores (as indicated by disinhibition measures in Three Factor Eating Questionnaire), (b) attachment anxiety scores and (c) loss aversion scores. Each panel includes associated *r* and *p* values. (change = exercise trial – control trial)

6.4 Discussion

The aim of the present study was to assess the role of exercise in influencing appetite-related outcomes measured using a novel, computer-based procedure. Three hypotheses were proposed: (1) acute exercise influences hunger and fullness, prospective ideal portion size of food, food liking, and how much they want to spend on food; (2) these responses vary across gender, BMI, and dietary restraint; (3) behavioural factors such as disinhibition, attachment anxiety, and loss aversion may correlate with the responses above. The primary finding of the investigation was that a single bout of moderate intensity exercise attenuated hunger and thereby led to lower prospective ideal portion size compared to control. The observed exercise-induced suppression in hunger and perceived food intake are of importance in the perspective of exercise and appetite regulation, and these findings confirm exercise induces an anorexigenic effect in the short term, contrary to the widespread belief that exercise stimulates hunger.

The most prominent decrease in hunger was observed after the cessation of exercise, which is consistent with many studies that reported suppression of hunger following acute exercise (Unick *et al.* 2010; King *et al.* 2010a, King *et al.* 2010b; Westerterp-Plantenga *et al.* 1997; King *et al.* 1994; Thompson *et al.* 1988). Although this suppression effect was short-lived, feelings of hunger for subsequent hours were persistently lower than control. Attenuation in hunger was also accompanied by lower prospective ideal portion size in the exercise trial relative to control. In contrast to hunger, the decrease in ideal portion size was not observed after exercise compared to baseline. This however, was due to participants choosing slightly but not significantly smaller portion sizes at the start of the exercise trial, in addition to feeling less hungry compared to control. These responses could conceivably be explained by the anticipation of exercise which may have caused hunger to be diminished and hence reduced prospective energy intake, due to an increased sympathetic nervous system activity before the commencement of an exercise activity (Frayn 2003). With regards to macronutrient preference, prospective portion size of carbohydrates, fat and protein were reduced simultaneously, indicating that exercise-induced macronutrient preference was not evident in this study.

Using the computer-based approach, we were able to explore additional non-metabolic factors, namely food utility (indicated by the amount of money they would be willing to

spend on food) and food liking in response to exercise. Exercise is also known to activate the dopaminergic neurons in the mesolimbic structures of the brain that is associated with the reward pathway (Chaouloff 1989), therefore it is possible that exercise has a sensitising effect that enhances reward sensitivity to food. Currently, available research on exercise and the components of reward has been limited to liking, wanting, and palatability of food (Finlayson *et al.* 2011; Finlayson *et al.* 2009; Schneider *et al.* 2009) and there are no data examining the effect of exercise on the monetary value of food. Our initial findings showed that how much people were willing to pay for food was not affected by exercise. There also seemed to be a positive association between food utility and ideal portion size, this relationship was however non-significant. Liking is also associated with the food reward component and increased liking may promote an increase in food intake (Finlayson *et al.* 2007). Recently, an enhanced liking for food after acute exercise has been observed in the 'compensators' group (*i.e.* who lost less weight than expected) after a 12-week of exercise program (Finlayson *et al.* 2011), which may help to explain why some people lose less weight than expected during an exercise intervention. The present study demonstrates that exercise did not alter liking scores, suggesting that liking may not be easily affected by acute exercise. Support for this finding was found in the recent study of Finlayson *et al.* (2009), who utilised a computer-based approach in assessing explicit liking and implicit wanting in response to an acute bout of exercise, and found no change in liking, although the 'compensators' group increased their energy intake post-exercise. Instead, they proposed that an enhanced 'implicit wanting' for food after exercise is associated with the compensatory feeding behaviour.

Do these responses vary across groups? Results showed that appetite, food liking, and prospective ideal portion size responses to exercise were not affected by sex, BMI categories or dietary restraint. Women, however, placed more value on food compared to men, independent of exercise. This finding suggests that women (limited to this study sample) may have a predisposition to perceive food as somewhat more rewarding than in men, and may perhaps relate to why women generally reported less weight loss compared to men following exercise interventions (Donnelly & Smith 2005; Donnelly *et al.* 2003). In addition, there appears to be a trend for diminished hunger in women, lean, and non-restraint individuals with exercise. The lower hunger response observed in women was somewhat in contrast with previously published studies in women that reported no change in appetite or subjective feelings of hunger and fullness in response to exercise (Unick *et al.* 2010; Hagobian *et al.* 2009; Whybrow *et al.* 2008; Lluch *et al.* 2000). One of the likely explanations for this finding can be due to the fact that only 2 out of the 13 women

were classified as overweight, and there is evidence that lean individuals have been shown to regulate their appetite with exercise better than those with higher BMI (George & Morganstein 2003; Kissileff *et al.* 1990). The lack of decrease in hunger observed in the overweight individuals suggest that they may be less sensitive to the anorexigenic effects of exercise, which could possibly pose an unfavorable implications for weight control. In contrast to non-restrained eaters, restrained eaters have been shown to display no changes in the physiological feelings of hunger with exercise (Harris & George 2008; Lluch *et al.* 2000) and therefore, exercise has been shown to be effective in creating a negative energy balance in this particular group (Martins *et al.* 2008a).

Finally, one novel finding of the pilot investigation was the evidence that loss aversion scores correlated highly with prospective ideal portion size and food liking. Loss aversion describes the widespread behavioral avoidance of choices that can lead to losses, even when accompanied by equal or much larger gains (Martino *et al.* 2010) and has been shown to influence decision making in a wide variety of domains, including investments, politics, and health (Kermer *et al.* 2006). The association between loss aversion and prospective portion size and food liking can be likened to that of avoidance of financial losses, in which loss aversive individuals are rather motivated to seek for food and eat than experiencing hunger or energy deficit. The processes that involve in the decision-making of monetary gain and losses occur in the reward-processing center of the brain (Camara *et al.* 2008), therefore it may be possible that the components of food reward involved in appetite regulation can be influenced by this trait.

The findings of this study should be considered in the context of certain limitations. The restricted sample size limited the opportunity to control for numerous background variables. A larger sample will provide more power to detect differences between various groups/categories and to evaluate interactions. Participants were only monitored for only 2 h post-exercise, thus limiting the understanding of how exercise may influence these parameters beyond this period of time. With the computer-based approach, we are unable to claim with certainty that planned ideal portion size will actually influence actual food intake. However, there is good reason to believe that these two measures correspond closely, as serving size, whether determined by the individual or not, has been shown as an excellent predictor of the amount of food consumed (Wasink *et al.* 2005, Rolls *et al.* 2002). Another issue is the usage of visual stimuli in assessing the hedonic evaluation of food, as non-attractive images can influence participants' responses therefore masking a true response. Nevertheless, this pilot investigation demonstrates that the application of

this computer-based assessment in evaluating the exercise-induced appetite-related measures is particularly convenient in assessing several responses simultaneously and is a useful adjunct to measures of actual food and energy intake.

6.5 Summary

In consistence with previous reports demonstrating exercise-induced anorexia, the primary finding of this study was that an acute bout of exercise induced hunger suppression and prospective food intake. The outcomes of this pilot investigation also highlight the role of behavioural aspects that may influence food intake in relation to exercise and body weight control. In particular, the sex differences in food utility value has implications in the understanding of food reward perceptions between men and women. Also, further research is needed to explore the loss aversion trait in influencing feeding behavior in response to exercise or energy deficit.

CHAPTER 7

General Discussion

Free-living humans consume food throughout the day (*i.e.* ~ 3 meals and snacks in between), thus, most humans find themselves in the postprandial state for the majority of a 24-h period, perhaps with the exception of the early morning hours. The food that we eat and the metabolic responses that ensue influence risks for vascular and metabolic diseases, as well as obesity. Exercise reduces these risks (Gill & Cooper 2008; Nocon *et al.* 2008), and this is partly mediated by the effects of exercise on postprandial metabolism (*e.g.* lowering postprandial lipaemia and insulinaemia, increasing fat oxidation). However, to gain better insights into the role of feeding and exercise on metabolic and obesity risks, it is important to understand how these factors interact in a 'real-life' setting. Thus, the 'real-life' effect of exercise on metabolism need to take into account the potential effects of exercise on subsequent food intake. This is of particular interest because eating is almost inevitable after exercise, therefore, the benefits of acute exercise on postprandial metabolism may be overstated when studies do not account for any subsequent increases in food intake.

7.1 Exercise and Postprandial Metabolism

The first experimental study of this thesis (Chapter 3) showed that a single as well as three consecutive exercise sessions reduced postprandial TG and insulin responses, and increased postprandial fat oxidation in response to *ad libitum* feeding. The magnitude of reduction postprandial lipaemia demonstrated in this study is comparable to other published reports in the overweight cohorts (Burton *et al.* 2008; Miyashita 2008). These findings indicate that the ability of aerobic exercise to attenuate postprandial lipaemia does extend beyond the laboratory and into daily life setting, where food intake is not externally controlled. This study also showed that 3 days of consecutive exercise did not lead to greater changes in postprandial metabolism than a single exercise session. The next study (Chapter 5) dealt with the issue of timing of exercise relative to meal ingestion. The findings showed that exercising before or after a breakfast meal had the same overall effect on postprandial insulin responses and fat oxidation. However, only exercise before breakfast significantly attenuated postprandial TG over the 9-h observation period of the

study. To the author's knowledge, there is no published evidence on comparing the effects of timing of exercise relative to meal ingestion on postprandial metabolism, particularly in the context of *ad libitum* feeding, therefore these findings can be considered pertinent to real life setting where food intake and exercise are often interspersed with each other.

The findings of these two studies also highlight the importance of negative energy deficit in mediating the exercise-induced enhancement in fat oxidation. Chapter 3 showed that exercise (single and consecutive sessions) increased whole-body fat oxidation during following day, whereas this effect was not evident in the study reported in Chapter 5. Although it seemed that total fat oxidation was greater in both exercise trials compared to control in Chapter 5, this greater amount of fat oxidised was primarily contributed by the enhanced fat oxidation during the exercise period, and not brought about by increased whole-body fat oxidation in the post-exercise period. Thus, there seems to be an apparent paradox on the effects of exercise on fat oxidation observed in these two studies. Melanson *et al.* (2009b) in his recent review summarised that exercise does not result in negative 24-h fat balance when 24-h energy balance is maintained, independent of training status or obesity. Against this background, it seems reasonable to postulate that lack of post-exercise elevation in fat oxidation observed in Chapter 5 can be off-set by carbohydrate consumption and participants being in a positive energy balance state. On the contrary, the enhanced whole-body fat oxidation despite feeding *ad libitum* observed in Chapter 3 may be indicative of their negative energy balance state, considering that they expended a substantial amount of energy during the exercise sessions (~700-2100 kcal) compared to the 400-kcal session in Chapter 5. But because 24-h energy balance was not measured, therefore we can only assume that this is the case.

7.2 Exercise, Appetite Responses and Feeding Behaviour

The study in Chapter 4 showed that a single exercise session (~700 kcal) did not result in a significant compensatory response in energy intake. However, there was a significant dietary compensation following 3 consecutive days of exercise (total ~2100 kcal), with the increase in energy intake reflecting 24% of the energy expended in exercise. While food intake does not appear to match elevated levels of energy expenditure in the short term (Hopkins *et al.* 2010), these findings suggests that a partial compensation is evident in the longer term. This study also showed no change in gut peptide responses (*i.e.*

acylated ghrelin and PYY_{3-36}) over the course of a day following either 1 or 3 days of prior exercise. Findings from Chapter 5 demonstrated that timing of exercise relative to breakfast does not influence energy intake in subsequent meals over the course of a day. Exercise also lead to a negative fat balance and lower carbohydrate and energy balances relative to a no-exercise control over the course of a day, and this was not influenced by timing of exercise relative to breakfast.

Published findings on exercise and appetite seems to be pointing in the direction that acute exercise does not stimulate appetite and food intake, however this does not seem to fit with mounting evidence recently concerning the limited efficacy of exercise in inducing weight loss. It appears that not all individuals who undertake long term exercise will lose weight under conditions of *ad libitum* feeding (Hopkins *et al.* 2010). Indeed, in an environment characterised by caloric-dense and palatable foods, dietary compensation can occur due to behavioural factors rather than homeostatic mechanisms linking energy expenditure and intake, therefore these elements need to be explored (Berthoud 2006; Hill *et al.* 1995). In the final experimental study (Chapter 6), a pilot study was designed to address the effects of exercise on non-metabolic factors related to appetite (*i.e.* food liking, food utility, ideal portion size) using a computer-based assessment. The findings showed that an acute bout of moderate intensity exercise produced the 'anorectic' effect, manifested by diminished hunger and lower prospective food intake (ideal portion size) compared to no exercise, which is in accordance with current literature. Although not a primary aim, this study discovered a novel association between loss aversion and prospective food intake and food liking.

7.3 Strengths, Limitations and Future Directions

A major distinction of the studies in this thesis compared to published literature involving exercise and postprandial metabolism was the inclusion of *ad-libitum* meals. An alternative to the typical high-fat test meals used in many previous studies, *ad libitum* consumption creates 'real world' setting to the experiments in determining the relevance of exercise for general population in everyday living. Mestek (2010) in his commentary in the November 2010 issue of the Medicine and Science in Sport and Exercise journal, commended the findings of Chapter 3 of this thesis (also published in the same November issue, Farah *et al.* 2010) as being '*the most compelling results to date'* from a public

health perspective. As the consideration for exercise prescription continue to expand, the interest for the public, especially among the obese population, lies in maximising the periods of acute fat balance across daily meals and exercise periods. This thesis also addressed the issue of exercise timing around meal ingestion and has shown that timing of exercise does not influence the beneficial effects of exercise, even with *ad libitum* feeding throughout the day. The findings of this thesis are also in agreement with the large body of evidence supporting the beneficial role of exercise on appetite regulation, by demonstrating that moderate intensity exercise (~400-700 kcal) does not induce compensation in energy intake and hunger in overweight individuals, thus providing clinical relevance to the role of exercise in the prevention of obesity and weight management.

Findings from Chapter 3 showed that repeated exercise sessions (~2100 kcal) did not further augment the attenuation in postprandial lipaemia and the enhanced fat oxidation compared to a single exercise bout (~700 kcal). This could be due to the short-lived effects of exercise on postprandial metabolism, thus the effects observed could be a result of exercise from the previous day. It is also important to consider the fact that the energy cost of each exercise session was substantially large (~700 kcal per session), therefore it is uncertain if an energy expenditure threshold exists for maximising the TG-lowering effect of exercise. Perhaps performing smaller doses (*e.g.* 250 – 350 kcal) of exercise on consecutive days would elicit an 'additive' effect, however, this warrants further investigation. One might argue that the energy cost of the exercise session in this particular study may be too substantial and therefore is not feasible for most sedentary and overweight/obese individuals. Although the study subjects managed to perform the exercise without any difficulty, it is worthwhile to acknowledge that other investigators have shown that smaller energy expenditures (~200-250 kcal) are just as effective in reducing postprandial lipaemia in both lean (Miyashita *et al.* 2008) and obese individuals (Miyashita 2008).

The observed compensatory response in energy intake in Chapter 4 suggests the possibility of delayed compensatory response in energy intake to exercise-induced energy deficit, hence why many acute exercise studies with a relatively short follow-up (1-2 d) reported no change in energy intake (King *et al.* 2010a, King *et al.* 2010b; Unick *et al.* 2010; Harris & George 2008; Imbeault *et al.* 1997). Further longer term studies are needed to confirm this, such as extending the observation period over a number of days to see if increases in food intake do continue to track energy balance. In order to create a

more ‘free-living’ condition, subjects can be allowed to maintain their usual daily activities and report to the laboratory for daily meals, instead of being confined to the laboratory during the whole observation period. Assessment of total daily energy expenditure using the doubly labelled water method will allow for the accurate determination of energy balance, although this method can be costly. An important issue to consider in evaluating the compensatory responses to exercise is the inter-individual variability. In comparing energy intake in response to exercise interventions, Stubbs *et al.* (2004) introduced the identification of ‘compensators’ and ‘non-compensators’. Compensators were labeled as those who exhibited a statistically significant increase in energy intake, whereas non-compensators did not increase energy intake. The large inter-individual variability in body weight and fat mass changes to a 12-wk exercise intervention as demonstrated by King *et al.* (2008) indicates that general exercise prescription is no longer a ‘one size fits all’ situation (Caudwell *et al.* 2009). In future studies, expressing the data individually or exploring them in subgroups, instead of reporting the mean group response, will avoid overlooking the issue of individual variability and help identify the physiological and behavioural mechanisms that mediate compensatory responses to exercise.

The findings that exercise did not alter gut peptide responses could be suggesting that appetite hormones may not be responsive to exercise-induced energy deficits. Hubert *et al.* (1998) in his study demonstrated that consuming a meal of reduced energy resulted in elevated hunger and energy intake at the next opportunity, while the same energy deficit but expended through exercise, did not produce any compensatory effect. This finding was later supported by a very recent study by King *et al.* (2011b) who demonstrated that 9-h AUC for acylated ghrelin concentrations increased, whereas PYY_{3-36} decreased, in response to energy deficit imposed by food restriction, but not by exercise. Thus, it is possible that diet-induced energy deficit has a far greater effect on appetite hormone responses than exercise-induced energy deficit, which may explain why long term success of weight loss is usually poor with dieting (Aronne *et al.* 2009). Furthermore, there are a number of peptide candidates responsible for modulating satiety and food intake, therefore measuring only one or two peptides may not always guarantee a definitive relationship. On the other hand, measurement of gut peptides can be subjected to limitations such as costs, special conditions sampling, technical methods (Delzenne *et al.* 2010), and the highly-unstable nature of some of the peptides (Hosoda *et al.* 2004).

Concerning the issue of exercise timing relative to meal ingestion, the reduction in postprandial TG response to exercise after breakfast was not significant but this 10.7% reduction is likely to be a real effect not detected due to low statistical power (0.35). It is also noteworthy to consider that the breakfast provided in the study was a test meal, with the energy content tailored to each participant's body mass (average energy content ~430 kcal). It might be that providing smaller or larger breakfast meals may lead to differences in postprandial responses and macronutrient balances in the post-exercise period. Thus this remains to be elucidated.

Studies on dopaminergic systems and aspects of food motivation in humans underscore the potential relevance of the "liking" versus "wanting" differentiation in relation to food intake and obesity (Mela 2006). Thus, it would be certainly useful to include measures of 'implicit wanting' in the computer-based assessment described in the pilot study, as it has been recently shown that individuals who do not respond to weight-reducing effect of exercise exhibited enhanced wanting for food after exercise interventions (Finlayson *et al.* 2011; Finlayson *et al.* 2009). Additionally, the computer-based approach could be used to investigate the effects of exercise-induced and diet-induced energy deficits on non-metabolic factors associated with feeding behaviour, as well as identifying the characteristics of those individuals who are most likely to respond to the weight-reducing effects of regular exercise, the 'responders'; and the individuals who do not, the 'non-responders'. This could help to better understand the factors other than physiological ones that drive compensation in food intake via exercise.

Despite the wealth of studies being published in the literature with regards to the effects of exercise on the regulation of appetite in particular, the mechanisms involved are not fully understood and conclusions are have yet to be drawn. Methodological differences such as exercise-induced energy expenditures, exercise intensities, energy state, gender, BMI, and the time interval between exercise and meal consumptions are likely to explain for the inconsistencies in literature findings. As such, the findings reported in this thesis are limited to responses in overweight/obese, otherwise healthy white men, and may not represent the responses for lean individuals, women, and people of other ethnicity or with metabolic diseases. Further research in different groups of people is warranted to provide greater insight into how exercise might regulate postprandial metabolism, appetite control and feeding behaviour.

6.4 Conclusion

In summary, the findings of this thesis present strong evidence that the effects of exercise on postprandial metabolism when food is consumed *ad libitum* is very similar to when food intake is externally controlled. Furthermore, timing of exercise relative to meal ingestion is not of significant importance when the metabolic effects are concerned. Finally, there is a loose coupling between moderate-intensity exercise and energy intake on an acute level (*i.e.* immediately to 1-2 days post-exercise). Ultimately, these findings should encourage the public, especially the overweight/obese population to take part in exercise without the need to worry about specific timing of exercise or altering food intake in order to elicit the beneficial effects. In light of the recently emerging concern regarding the ineffectiveness of exercise in weight control, the author strongly feels that the ultimate goal of exercise should not be directed towards weight loss *per se*, but rather to achieve overall health and maintain the quality of life.

"There is more to health than the numbers on the bathroom scale"

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Appendix A

Health Screen for Study Volunteers

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (i) their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

Please complete this brief questionnaire to confirm fitness to participate:

At present, do you have any health problem for which you are:

(a) on medication, prescribed or otherwise	yes [ ]	no [ ]
(b) attending your general practitioner	yes [ ]	no [ ]
(c) on a hospital waiting list	yes [ ]	no [ ]

In the past two years, have you had any illness which required you to:

(a) consult your GP	yes [ ]	no [ ]
(b) attend a hospital outpatient department	yes [ ]	no [ ]
(c) be admitted to hospital	yes [ ]	no [ ]

Have you ever had any of the following:

(a) Convulsions/epilepsy	yes [ ]	no [ ]
(b) Asthma	yes [ ]	no [ ]
(c) Eczema	yes [ ]	no [ ]
(d) Diabetes	yes [ ]	no [ ]
(e) A blood disorder	yes [ ]	no [ ]
(f) Head injury	yes [ ]	no [ ]
(g) Digestive problems	yes [ ]	no [ ]
(h) Hearing problems	yes [ ]	no [ ]
(i) Problems with bones or joints	yes [ ]	no [ ]
(j) Disturbance of balance/co-ordination	yes [ ]	no [ ]
(k) Numbness in hands or feet	yes [ ]	no [ ]
(l) Disturbance of vision	yes [ ]	no [ ]
(m) Thyroid problems	yes [ ]	no [ ]
(n) Kidney or liver problems	yes [ ]	no [ ]
(o) Chest pain or heart problems	yes [ ]	no [ ]

(p) Any other health problems yes [ ] no [ ]

4. **Have any of your family** (parents, grandparents, brothers, sisters, children, aunts, uncles, cousins) ever had any of the following: (if yes please give details including age of first diagnosis)
   (a) Any heart problems yes [ ] no [ ]
   (b) Diabetes yes [ ] no [ ]
   (c) Stroke yes [ ] no [ ]
   (d) Any other family illnesses yes [ ] no [ ]

5. Do you currently smoke yes [ ] no [ ]
   Have you ever smoked yes [ ] no [ ]

   If so, for how long did you smoke and when did you stop? …….

6. Are you currently dieting or consuming specialised diet? yes [ ] no [ ]
7. How many units of alcohol do you typically drink in a week? ……

If YES to any question, please describe briefly if you wish (e.g. to confirm whether problem was short-lived, insignificant or well controlled.)

……

……

……

……

……

……

THANK YOU

Appendix B

Three Factor Eating Questionnaire (TFEQ)

PART I : Answer the following questions by circling <u>true</u> (T) OR <u>false</u> (F) whichever is appropriate to you.

1. When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.	**T**	**F**
2. I usually eat too much at social occasions, e.g. parties and picnics.	**T**	**F**
3. I am usually so hungry that I eat more than three times a day.	**T**	**F**
4. When I have eaten my quota of calories, I am usually good about not eating anymore.	**T**	**F**
5. Dieting is so hard for me because I just get too hungry.	**T**	**F**
6. I deliberately take small helpings as a means of controlling my weight.	**T**	**F**
7. Sometimes things just taste so good that I keep on eating even when I am no longer hungry.	**T**	**F**
8. Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat.	**T**	**F**
9. When I feel anxious, I find myself eating.	**T**	**F**
10. Life is too short to worry about dieting.	**T**	**F**
11. Since my weight goes up and down, I have gone on reducing diets more that once.	**T**	**F**
12. I often feel hungry that I just have to eat something.	**T**	**F**
13. When I am with someone who is overeating, I usually overeat too.	**T**	**F**
14. I have a pretty good idea of the number of calories in common food.	**T**	**F**
15. Sometimes when I start eating, I just can't seem to stop.	**T**	**F**
16. It is not difficult for me to leave something on my plate.	**T**	**F**
17. At certain times of the day, I get hungry because I get used to eating then.	**T**	**F**
18. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it.	**T**	**F**
19. Being with someone who is eating often makes me hungry enough to eat.	**T**	**F**

20. When I feel depressed, I often overeat. **T** **F**

21. I enjoy eating too much to spoil it by counting calories or watching my weight. **T** **F**

22. When I see a real delicacy, I often get so hungry that I have to eat right away. **T** **F**

23. I often stop eating when I am not really full as a conscious means of limiting the mount I eat. **T** **F**

24. I get so hungry that my stomach often seems like a bottomless pit. **T** **F**

25. My weight has hardly changed at all in the last ten years. **T** **F**

26. I am always hungry so it is hard for me to stop eating before I finish the food on my plate. **T** **F**

27. When I feel lonely, I console myself by eating. **T** **F**

28. I consciously hold back at meals in order not to gain weight. **T** **F**

29. I sometimes get very hungry late in the evening or at night. **T** **F**

30. I eat anything I want, any time I want. **T** **F**

31. Without even thinking about it, I take a long time to eat. **T** **F**

32. I count calories as a conscious means of controlling my weight. **T** **F**

33. I do not eat some foods because they make me fat. **T** **F**

34. I am always hungry enough to eat at any time. **T** **F**

35. I pay a great deal of attention to changes in my figure. **T** **F**

36. While on a diet, if I eat a food that is not allowed, I often splurge and eat other high calorie foods. **T** **F**

Next page...

PART II : Answer the following questions by circling the <u>number</u> next to the response that is appropriate to you.

37. How often are you dieting in a conscious effort to control your weight?

1	**2**	**3**	**4**
rarely	sometimes	usually	always

38. Would a weight fluctuation of 5 lbs. affect the way you live your life?

1	**2**	**3**	**4**
not at all	slightly	moderately	very much

39. How often do you feel hungry?

1	**2**	**3**	**4**
only at mealtimes	sometimes between meals	often between meals	almost always

40. Do your feelings of guilt about overeating help you to control your food intake?

1	**2**	**3**	**4**
never	rarely	often	always

41. How difficult would it be fro your to stop eating halfway through dinner and not eat for the next four hours?

1	**2**	**3**	**4**
easy	slightly difficult	moderately difficult	very difficult

42. How conscious are you of what are you eating?

1	**2**	**3**	**4**
not at all	slightly	moderately	extremely

43. How frequently do you avoid ‘stocking up’ on tempting foods?

1	**2**	**3**	**4**
almost never	seldom	usually	almost always

44. How likely are you to shop for low calorie foods?

1	**2**	**3**	**4**
unlikely	slightly unlikely	moderately likely	very likely

45. Do you eat sensibly in front of others and splurge alone?

1	**2**	**3**	**4**
never	rarely	often	always

46. How likely are you to consciously eat slowly in order to cut down on how much you eat?

1	**2**	**3**	**4**
unlikely	slightly unlikely	moderately likely	very likely

47. How frequently do you skip dessert because you are no longer hungry?

1	**2**	**3**	**4**
almost never	seldom	at least once a week	almost everyday

48. How likely are you to consciously eat less than you want?

1	**2**	**3**	**4**
unlikely	slightly unlikely	moderately likely	very likely

49. Do you go on eating binges though you are not hungry?

1	**2**	**3**	**4**
never	rarely	often	at least once a week

50. On a scale of 0 to 5, where 0 means no restraint (eating whenever you want, whatever you want), and 5 means total restraint (constantly limiting food intake and never 'giving' in), what number would you give yourself?

0	eat whatever you want, whenever you want it
1	usually eat whatever you want, whenever you want it
2	often eat whatever you want, whenever you want it
3	often limit food intake, but often 'give in'
4	usually limit food intake, rarely 'give in'
5	constantly limiting food intake, never 'giving in'

51. To what extent does this statement describe your eating behaviour?
"I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising to start dieting again tomorrow."

1	**2**	**3**	**4**
not like me	little like me	pretty good description of me	describes me perfectly

The End

Appendix C

Dutch Eating Behaviour Questionnaire (DEBQ)

PART I : Answer the following questions by circling the corresponding <u>number</u> which describes you best.

1 = never; 2 = seldom; 3 = sometimes; 4 = often; 5 = very often

1. If you have put on weight, do you eat less that you usually do?	**1**	**2**	**3**	**4**	**5**
2. Do you try to eat less at mealtimes than you would like to eat?	**1**	**2**	**3**	**4**	**5**
3. How often do you refuse food or drink offered because you are concerned about your weight?	**1**	**2**	**3**	**4**	**5**
4. Do you watch exactly what you eat?	**1**	**2**	**3**	**4**	**5**
5. Do you deliberately eat foods that are slimming?	**1**	**2**	**3**	**4**	**5**
6. When you have eaten too much, do you eat less than usual the following days?	**1**	**2**	**3**	**4**	**5**
7. Do you deliberately eat less in order not to become heavier?	**1**	**2**	**3**	**4**	**5**
8. How often do you try not to eat between meals because you are watching your weight?	**1**	**2**	**3**	**4**	**5**
9. How often in the evening do you try not to eat because you are watching your weight?	**1**	**2**	**3**	**4**	**5**
10. Do you take into account your weight with what you eat?	**1**	**2**	**3**	**4**	**5**
11. Do you have the desire to eat when you are irritated?	**1**	**2**	**3**	**4**	**5**
12. Do you have a desire to eat when you are depressed or discouraged?	**1**	**2**	**3**	**4**	**5**
13. Do you have the desire to eat when you are cross?	**1**	**2**	**3**	**4**	**5**
14. Do you have a desire to eat when you are approaching something unpleasant to happen?	**1**	**2**	**3**	**4**	**5**
15. Do you have a desire to eat when things are going against you or when things have gone wrong?	**1**	**2**	**3**	**4**	**5**
16. Do you have a desire to eat when you are frightened?	**1**	**2**	**3**	**4**	**5**
17. Do you have a desire to eat when you are disappointed?	**1**	**2**	**3**	**4**	**5**
18. Do you have a desire to eat when you are emotionally upset?	**1**	**2**	**3**	**4**	**5**
19. Do you have a desire to eat when you have nothing to do?	**1**	**2**	**3**	**4**	**5**

20. Do you have a desire to eat when you are feeling lonely?	**1**	**2**	**3**	**4**	**5**
21. Do you have a desire to eat when somebody lets you down?	**1**	**2**	**3**	**4**	**5**
22. Do you have a desire to eat when you are bored or restless?	**1**	**2**	**3**	**4**	**5**
23. If food tastes good to you, do you eat more than usual?	**1**	**2**	**3**	**4**	**5**
24. If food smells and looks good, do you eat more than usual?	**1**	**2**	**3**	**4**	**5**
25. If you see or smell something delicious, do you have desire to eat it?	**1**	**2**	**3**	**4**	**5**
26. If you have something delicious to eat, do you eat it straight away?	**1**	**2**	**3**	**4**	**5**
27. If you walk past the baker do you have the desire to buy something delicious?	**1**	**2**	**3**	**4**	**5**
28. If you walk past a snackbar or a café, do you have the desire to buy something delicious?	**1**	**2**	**3**	**4**	**5**
29. If you see others eating, do you also have the desire to eat?	**1**	**2**	**3**	**4**	**5**
30. Can you resist eating delicious foods?	**1**	**2**	**3**	**4**	**5**
31. Do you eat more than usual, when you see others eating?	**1**	**2**	**3**	**4**	**5**
32. When preparing a meal, are you inclined to eat something?	**1**	**2**	**3**	**4**	**5**

The End

Appendix D

Appetite Questionnaire

Please answer the following questions by placing a vertical mark through the line for each question. Regard the end of each line as indicating the most extreme sensation you have ever felt and mark how you feel **NOW.**

Time: _____

1. How **hungry** do you feel (now)?

I am not hungry ——————————— **Never been hungrier**

2. How **satisfied** do you feel (now)?

I am not satisfied at all ——————————— **I cannot eat another bite**

3. How **full** do you feel (now)?

Not at all full ——————————— **Totally full**

4. How **much** do you think you **can eat** (now)?

A lot ——————————— **Nothing at all**

5. How strong is your **desire to eat** (now)?

Not at all ——————————— **Very**

Appendix E

Experiences in Close Relationship Questionnaire

Below are a number of statements regarding how people feel and behave in their closest relationships. Please respond according to how you feel and behave ***generally*** in these relationships. Please indicate the extent to which you agree with the following statements. Do this by placing a number from the scale below (1 – 7) in front of each state. **All information are strictly confidential.**

Strongly Disagree **Neutral** **Strongly Agree**

1……..……2…..……...3……..…..…..4…..……..…5……..…..…..6……..7

1. I prefer not to show people close to me how I feel deep down.
2. I worry about being abandoned.
3. I am very comfortable being close to others.
4. I worry a lot about my relationships.
5. Just when people start to get close to me I feel myself pulling away.
6. I worry that people won't care about me as much as I care about them.
7. I get uncomfortable when people want to be very close.
8. I worry a fair amount about losing my relationships.
9. I don't feel comfortable opening up to others.
10. I often wish that my loved ones' feelings for me were as strong as my feelings for them.
11. I want to get close to others but they keep pulling away.
12. I often want to merge completely with others, and this sometimes scares them away.
13. I am nervous when others get too close to me.
14. I worry about being alone.
15. I feel comfortable sharing my thoughts and feelings with those I am close to.
16. My desire to be close sometimes scares others away.
17. I try to avoid getting too close to others.
18. I need a lot of reassurance that I am loved by those close to me.
19. I find it relatively easy to get close to others.
20. Sometimes I feel that I force others to show more feeling, more commitment.
21. I find it difficult to allow myself to depend on others.
22. I do not often worry about being abandoned.
23. I prefer not to be close to others.
24. If I can't get those close to me to show interest in me, I get upset or angry.
25. I tell those close to me just about everything.

26. I find that others don't want to get as close as I would like.
27. I usually discuss my problems and concerns with those close to me.
28. When I'm involved in a relationship, I feel somewhat anxious and insecure.
29. I feel comfortable depending on others.
30. I get frustrated when those I am close to aren't around me as much as I would like.
31. I don't mind asking others for comfort, advice, or help.
32. I get frustrated when those close to me are not available when I need them.
33. It helps to turn to others in times of need.
34. When those close to me disapprove of me, I feel really bad about myself.
35. I turn to others for many things, including comfort and reassurance.
36. I resent it when those I am close to spend time away from me.

The End

Appendix F

Active Ghrelin (GHRA-88HK)

Manufacturer: Linco Research Inc., St. Charles, MO, USA

Active Ghrelin Standard Preparation

1. Active Ghrelin Standard was reconstituted with 2 ml of deionised water.

2. Serial dilutions were performed by adding 0.5 ml of the reconstituted standard to tubes (1-8) containing 0.5 ml of Assay Buffer (refer **Table 1**).

Table 1. Standard preparation

Tube no.	Volume of Assay Buffer	Volume of Standard	Standard concentration (pg/ml)
1	0.5 ml	0.5 ml of standard	x/2
2	0.5 ml	0.5 ml of tube 1	x/4
3	0.5 ml	0.5 ml of tube 2	x/8
4	0.5 ml	0.5 ml of tube 3	x/16
5	0.5 ml	0.5 ml of tube 4	x/32
6	0.5 ml	0.5 ml of tube 5	x/64
7	0.5 ml	0.5 ml of tube 6	x/128
8	0.5 ml	0.5 ml of tube 7	x/256

Assay Procedure

1) Day 1

1. Assay Buffer (300 µl) was pipetted to the Non-Specific Binding (NSB) tubes (3-4). 200 µl of Assay Buffer was pippeted in the Reference tubes (5-6). 100 µl of Assay Buffer was pipetted to tubes (7) through the end of the assay.

2. Standards, Quality Controls, and samples of 100 µl each were pipetted in duplicate into respective tubes.

3. 100 µl of Ghrelin Antibody was added to all tubes except Total Count tubes (1-2) and NSB tubes (3-4) (refer Table 2).

4. All tubes were vortexed, covered, and incubated overnight (20-24 hrs) at 4ºC.

2) Day 2

1. ^{125}I-Ghrelin lyophilized tracer was hydrated with 13.5 ml of Label Hydrating Buffer containing 0.025% Triton-X 100 and guinea pig IgG as a carrier. Solution was mixed gently before 100 µl of the mixture was pipetted to all tubes.

2. All tubes were vortexed, covered, and incubated overnight (20-24 hrs) at 4ºC.

3) Day 3

1. 1.0 ml of cold (4ºC) Precipitating Reagent containing goat anti-IgG serum, was added to all tubes except Total Count tubes (1-2).

2. Tubes were vortexed and incubated at 4ºC.

3. After incubation, tubes were centrifuged at 4ºC for 20 minutes at 2000-3000 xg.

4. Supernatant from all centrifuged tubes except Total Count tubes (1-2) were immediately decanted and drained.

5. Pellet was counted using the gamma counter.

Table 2. Assay procedure flow chart

DAY ONE					DAY TWO		DAY THREE	
Set-up	**Step 1**	**Step 2 & 3**	**Step 4**	**Step 5**	**Step 6**	**Step 7**	**Step 8**	**Step 9-11**
Tube no.	Add buffer assay	Add standard /QC / sample	Add Ghrelin antibody	Vortex, cover, and incubate at 4°C	Add ^{125}I-ghrelin tracer	Vortex, cover, and incubate at 4°C	Add precipitating reagent	Incubate for 20 min at 4°C, centrifuge at 4°C for 20 min. Decant and count.
1,2	-	-	-		100 µl		-	
3,4	300 µl	-	-		100 µl		1.0 ml	
5,6	300 µl	-	100 µl		100 µl		1.0 ml	
7,8	300 µl	100 µl of tube 8	100 µl		100 µl		1.0 ml	
9,10	300 µl	100 µl of tube 7	100 µl		100 µl		1.0 ml	
11,12	300 µl	100 µl of tube 6	100 µl		100 µl		1.0 ml	
13,14	300 µl	100 µl of tube 5	100 µl		100 µl		1.0 ml	
15,16	300 µl	100 µl of tube 4	100 µl		100 µl		1.0 ml	
17,18	300 µl	100 µl of tube 3	100 µl		100 µl		1.0 ml	
19,20	300 µl	100 µl of tube 2	100 µl		100 µl		1.0 ml	
21,22	300 µl	100 µl of tube 1	100 µl		100 µl		1.0 ml	
23,24	300 µl	100 µl of standard	100 µl		100 µl		1.0 ml	
25,26	300 µl	100 µl of QC 1	100 µl		100 µl		1.0 ml	
27,28	300 µl	100 µl of QC 2	100 µl		100 µl		1.0 ml	
29,30	300 µl	100 µl of sample	100 µl		100 µl		1.0 ml	

Appendix G

PYY_{3-36} (PYY-67HK)

Manufacturer: Linco Research Inc., St. Charles, MO, USA

Active Ghrelin Standard Preparation

3. PYY Standard was reconstituted with 2 ml of deionised water.

4. Serial dilutions were performed by adding 0.5 ml of the reconstituted standard to tubes (1-6) containing 0.5 ml of Assay Buffer (refer **Table 1**).

Table 1. Standard preparation

Tube no.	Volume of Assay Buffer	Volume of Standard	Standard concentration (pg/ml)
1	0.5 ml	0.5 ml of standard	x/2
2	0.5 ml	0.5 ml of tube 1	x/4
3	0.5 ml	0.5 ml of tube 2	x/8
4	0.5 ml	0.5 ml of tube 3	x/16
5	0.5 ml	0.5 ml of tube 4	x/32
6	0.5 ml	0.5 ml of tube 5	x/64

Assay Procedure

3) Day 1

5. Assay Buffer (200 µl) was pipetted to the Non-Specific Binding (NSB) tubes (3-4). 100 µl of Assay Buffer was pippeted in the Reference tubes (5-6) and sample tubes 25 through the end of the assay. Assay Buffer was not added to standard and QC tubes.

6. 100 µl of Matrix Solution containing treated human serum was added to the NSB tubes (3-4), Reference tubes (5-6), and Standard tubes (7-20) and Quality Control tubes (21-24).

7. Standards and Quality Controls of 100 µl each were pipetted into tubes (7-20) and (21-24) respectively, and 100 µl of samples into tubes (25-)

8. 100 µl of PYY_{3-36} Antibody was added to all tubes except Total Count tubes (1-2) and NSB tubes (3-4) (refer Table 2).

9. All tubes were vortexed, covered, and incubated overnight (20-24 hrs) at 4ºC.

4) Day 2

3. ^{125}I-PYY lyophilized tracer was hydrated with 13.5 ml of Assay Buffer. Solution was mixed gently before 100 µl of the mixture was pipetted to all tubes.

4. All tubes were vortexed, covered, and incubated overnight (20-24 hrs) at 4ºC.

3) Day 3

6. 10 µl of Guinea Pig carrier was added to all tubes except Total Count tubes (1-2).

7. 1.0 ml of cold (4ºC) Precipitating Reagent containing goat anti-IgG serum, was added to all tubes except Total Count tubes (1-2).

8. Tubes were vortexed and incubated at 4ºC.

9. After incubation, tubes were centrifuged at 4ºC for 20 minutes at 2000-3000 xg.

10. Supernatant from all centrifuged tubes except Total Count tubes (1-2) were immediately decanted and drained.

11. Pellet was counted using the gamma counter.

Table 2. Assay procedure flow chart

DAY 1					DAY 2		
Set-up	**Step 1**	**Step 2**	**Step 3 & 4**	**Step 5**	**Step 6**	**Step 7**	**Step 8**
Tube no.	Add buffer assay	Add Matrix Solution	Add standard/ QC/ sample	Add PYY_{3-36} antibody	Vortex, cover, and incubate at 4°C	Add ^{125}I-PYY tracer	Vortex, cover, and incubate at 4°C
1,2	-	-	-	-		100 µl	
3,4	200 µl	100 µl	-	-		100 µl	
5,6	100 µl	100 µl	-	100 µl		100 µl	
7,8	-	100 µl	100 µl of tube 6	100 µl		100 µl	
9,10	-	100 µl	100 µl of tube 5	100 µl		100 µl	
11,12	-	100 µl	100 µl of tube 4	100 µl		100 µl	
13,14	-	100 µl	100 µl of tube 3	100 µl		100 µl	
15,16	-	100 µl	100 µl of tube 2	100 µl		100 µl	
17,18	-	100 µl	100 µl of tube 1	100 µl		100 µl	
19,20	-	100 µl	100 µl of standard	100 µl		100 µl	
21,22	-	100 µl	100 µl of QC 1	100 µl		100 µl	
23,24	-	100 µl	100 µl of QC 2	100 µl		100 µl	
25,n	-	100 µl	100 µl of sample	100 µl		100 µl	

	DAY 3		
Set-up	**Step 9**	**Step 10**	**Step 11**
Tube no.	Add guinea pig carrier	Add precipitating reagent	Incubate for 20 min at 4°C, centrifuge at 4°C for 20 min. Decant and count.
1,2	-	-	
3,4	10 µl	1.0 ml	
5,6	10 µl	1.0 ml	
7,8	10 µl	1.0 ml	
9,10	10 µl	1.0 ml	
11,12	10 µl	1.0 ml	
13,14	10 µl	1.0 ml	
15,16	10 µl	1.0 ml	
17,18	10 µl	1.0 ml	
19,20	10 µl	1.0 ml	
21,22	10 µl	1.0 ml	
23,24	10 µl	1.0 ml	
25,n	10 µl	1.0 ml	