Effects of Fasting and Meal Composition on Appetite and the Metabolic Responses to Exercise

by

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Copyright Statement

Abstract

Dietary restriction and regular exercise are traditional strategies which independently demonstrate success in managing body weight and improving markers of metabolic health in the short term, but often lack long-term success. As a result, even 'best-case scenario' predictions suggest that most of the English population will be at increased risk of disease because of excess body weight until at least the year 2035. Incorporating specified periods of fasting within a dietary regime, commonly referred to as 'intermittent fasting', has gained popularity as an alternative method of dietary restriction. Interestingly, fasting may also augment some of the benefits that are attained from exercise, possibly due to mechanisms related to increased fat oxidation. This thesis investigated the acute effects of two methods of increasing fat oxidation during exercise: fasting and carbohydrate restriction, before examining the utility of a novel meal containing virtually no energy, as an alternative method to mitigate against some of the challenges associated with fasting-based regimes. To maximise adherence and long-term success, it is crucial that exercise and nutrition interventions can be conveniently embedded into lifestyles. Therefore, this programme of work considered exercise timing as an important factor in study design and implementation.

Firstly, common exercise timing behaviours, opportunities, and preferences were surveyed (**Chapter 4**). Results of this survey showed that, despite most people preferring to exercise in the morning (08:00–11:59), there was a lack of opportunity to engage in morning exercise during the week, likely determined by a working lifestyle. As a result, the early evening (16:00–19:59) was the most common time for exercise during the week.

Informed by these findings, the acute metabolic, appetite, energy intake, and performance responses to a bout of fasted evening exercise were examined (**Chapter 5**). Fasting for 7 h before evening cycling exercise (18:30) increased fat oxidation and reduced net energy intake compared to exercising 2 h after a meal. Fasted evening exercise, however, was associated with increased appetite and reduced motivation to exercise, exercise enjoyment, and voluntary exercise performance, highlighting potential difficulties with adopting a fasted evening exercise regime.

Chapter 6 examined evening exercise after a low-carbohydrate, high-protein lunch, based on findings that carbohydrate consumption suppresses fat oxidation, but protein consumption may not. Consuming a low-carbohydrate, high-protein meal 3 h before evening cycling exercise

(16:15) increased fat oxidation compared to a high-carbohydrate meal, but by a lesser extent than an 8 h fast. Importantly, the low-carbohydrate, high-protein meal also suppressed subjective and hormonal markers (PYY and GLP-1) of appetite and reduced *ad-libitum* energy intake in the evening compared to the high-carbohydrate meal and fasting, thus potentially offering an alternative method of increasing fat oxidation whilst also mitigating the challenges associated with fasted evening exercise.

An alternative strategy to manage fasting-induced elevations in appetite without providing energy was examined in **Chapter 7**. In this study, a very low-energy, viscous 'placebo' meal reduced subjective appetite compared to fasting, although this response was shorter-lived (~1 h) compared to that following a more typically consumed, whole-food meal (~2 h). The transient suppression of subjective appetite following a very low-energy, placebo meal could increase the efficacy of fasting-based interventions, without providing calorie-containing nutrients which would interrupt the fasted metabolic state and offset the energy deficit created by fasting.

Overall, the findings within this thesis suggest that the metabolic benefits of overnight-fasted morning exercise might also be attained during fasted evening exercise, but challenges such as elevated appetite, reduced voluntary exercise performance, and reduced motivation to exercise and exercise enjoyment may preclude its success in the long term. A low-carbohydrate, high-protein meal and a very low-energy, viscous 'placebo' meal may offer alternative strategies to offset the challenges associated with fasting, although longer-term studies are required to assess the chronic effects of these interventions on indices of body weight/composition and metabolic health.

List of Key Abbreviations

AMPK: AMP-activated protein kinase

ANOVA: Analysis of variance

AUC: Area under the curve

BMI: Body mass index

CPT-1: Carnitine palmitoyltransferase 1

DIT: Dietary induced thermogenesis

DTE: Desire to eat

EER: Estimated energy requirements

ELISA: Enzyme-linked immunosorbent assay

ES: Effect size

FAT/CD36: Fatty acid translocase/cluster of differentiation 36

FFM: Fat-free mass

GIP: Glucose-dependent insulinotropic polypeptide

GLP-1: Glucagon-like peptide-1

GLUT2: Glucose transporter type 2

GLUT4: Glucose transporter type 4

HR: Heart rate

HSL: Hormone sensitive lipase

IMTG: Intramuscular triglyceride

LPL: Lipoprotein lipase

mmHg: Millimetres of mercury

NaOH: Sodium hydroxide

NEAT: Non-exercise activity thermogenesis

NEFA: Non-esterified fatty acids

PAEE: Physical activity energy expenditure

PAL: Physical activity level

PBS: Potassium phosphate buffer

PFC: Prospective food consumption

PGC-1α: Peroxisome proliferator-activated receptor-γ coactivator-1α

PHMB: P-hydroxymercuribenzonic acid

PYY: Peptide tyrosine-tyrosine

RER: Respiratory exchange ratio

RMR: Resting metabolic rate

RPE: Rating of perceived exertion

RQ: Respiratory quotient

SD: Standard deviation

SEM: Standard error of the mean

TAG: Triglyceride

TDEE: Total daily energy expenditure

VAS: Visual analogue scale

$\dot{V}O_{2max}$: Maximal oxygen uptake

$\dot{V}O_{2peak}$: Peak oxygen uptake

OGTT: Oral glucose tolerance test

HbA1c: Glycated haemoglobin

DLW: Doubly-labelled water

DXA: Dual-energy X-ray absorptiometry

SCN: Suprachiasmatic nucleus

ATP: Adenosine triphosphate

mRNA: Messenger ribonucleic acid

LDL: Low-density lipoprotein

VLDL: Very low-density lipoprotein

$\dot{V}O_2$: Oxygen uptake

$\dot{V}CO_2$: Carbon dioxide production

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Preface

Unless otherwise indicated by reference to published resources, the work presented in this thesis is that of the author and has not been previously submitted for another degree to this or any other University.

Some of the work in this thesis has been published in peer-reviewed journals and/or presented at conferences:

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Table of Contents

List of Tables

List of Figures

Chapter 1 – General Introduction

Obesity is defined by the World Health Organisation (WHO, 2021) as abnormal or excessive fat accumulation that may impair health and is considered a worldwide leading risk factor for several diseases, including type 2 diabetes and cardiovascular disease (Di Angelantonio et al., 2016; Swinburn et al., 2019). The rapid rise in obesity prevalence over the last 30–40 years is well documented (Hales et al., 2018; Ng et al., 2014; NHS Digital, 2020; WHO, 2021), and in 2018, 63% of the English population were estimated to be living with overweight or obesity (NHS Digital, 2020). Although evidence suggests that this rising prevalence may be slowing in some developed countries (Abarca-Gomez et al., 2017), the modelling of future trajectories has revealed a continued upwards trend (Cobiac & Scarborough, 2021). It is predicted that 11 million more adults will be living with obesity in the UK by 2030, with associated medical costs anticipated to rise by ~£2 billion per year (Wang et al., 2011). Even in the best-case scenario, most of the English population is predicted to be at increased risk of disease because of excess body weight until at least the year 2035 (Cobiac & Scarborough, 2021). Physiological changes occur in individuals with obesity which can independently reduce insulin sensitivity (Kahn et al., 2006) – a key driver in the development of type 2 diabetes (Reaven, 1988) – although poor management of blood glucose levels, even in non-obese individuals, can increase the future risk of developing cardiovascular disease (Ceriello et al., 2008; Levitan et al., 2004).

Dietary restriction and regular exercise are traditional strategies implicated in weight and health management, and in the short term, are independently successful in managing body weight and improving markers of metabolic health (Borghouts & Keizer, 2000; Donnelly et al., 2009; Most et al., 2017). Within today's obesogenic environment which is characterised by sedentary lifestyles and 24-h availability of energy-dense foods, the chronic success of dietary restriction interventions is often impeded by poor adherence (Dansinger et al., 2005) and unsuccessful long-term weight maintenance (Maclean et al., 2015). Additionally, exercise interventions for weight management are often less effective than predicted (Church et al., 2009; Martin et al., 2019), with some evidence of inter-individual variability in the metabolic responses (de Lannoy et al., 2017). There is a well-established bidirectional relationship between nutrition and exercise, in that alterations in one of these elements can interact with the responses to the other. Therefore, whilst often studied and implemented in isolation, interventions which integrate both nutrition and exercise have the potential to exploit their synergistic effects and optimise the benefits that are achieved.

Incorporating specified periods of fasting within a dietary regime has gained popularity as an alternative method of energy restriction (Johnstone, 2015; Patterson & Sears, 2017). More commonly grouped under the umbrella term 'intermittent fasting', several studies have shown these methods to offer comparable benefits to traditional daily energy restriction for managing weight and enhancing metabolic health (Barnosky et al., 2014; Cioffi et al., 2018; Rynders et al., 2019), but without the need to reduce portion sizes and count calories, which are common downfalls associated with traditional daily energy restriction (Alajmi et al., 2016; Das et al., 2007). It is also suggested that the act of fasting itself may exert benefits to metabolic health, independent of energy restriction (Hatori et al., 2012).

Fasting may also potentiate some of the benefits that are attained from exercise. For example, exercising after an overnight fast reduces daily energy intake compared to exercise after eating, and also increases fat oxidation (Edinburgh et al., 2019; Gonzelez et al., 2013). If performed regularly, fasted exercise may drive adaptations to increase fat oxidative capacity (De Bock et al., 2008; Van Proeyen et al., 2010; Van Proeyen et al., 2011), which is associated with improved markers of metabolic health (Robinson et al., 2015*a*). Accordingly, fasted exercise training has been shown to enhance improvements in insulin sensitivity without the need to increase volume or intensity (Edinburgh et al., 2020; Van Proeyen et al., 2010). This is important given that a perceived lack of time is a common barrier to performing more exercise (Reichert et al., 2007; Trost et al., 2002). Interestingly, the metabolic benefits of fasted exercise may be driven by carbohydrate restriction, rather than fasting *per se*, potentially representing an alternative method of increasing fat oxidation during exercise, without the need to endure a period of fasting.

Whilst these combined nutrition-exercise interventions hold promise, most research has been undertaken in the morning, likely because the overnight period offers a practical opportunity to achieve a fasted state. Morning exercise, however, might be not convenient or possible for many because of several logistical challenges and/or preferences. Due to circadian (24-h) fluctuations in metabolism, appetite, and behaviour (Smith & Betts, 2022), findings from morning exercise studies might not directly apply to exercise performed later in the day. Therefore, future nutrition-exercise interventions should be developed with an appreciation of their timing, thus allowing for their convenient incorporation within the daily lives of the largest proportion of the population. Information regarding the exercise timing behaviours and preferences of the population would be a vital tool in facilitating the development of such interventions, although these data are lacking.

Chapter 2 – Literature Review

2.1 Energy Balance

2.1.1 Components of Energy Balance

Energy balance describes the interplay between energy intake, energy expenditure, and energy storage. The first law of thermodynamics states that energy cannot be created or destroyed, only transferred from one form to another, and it is to this law that human physiology conforms with regards to the regulation of body weight. Fundamentally, a sustained surplus of energy intake over energy expenditure materialises as an increase in stored energy (Hall et al., 2012; Hill et al., 2012). On the contrary, stored energy will be utilised when expenditure is in excess of intake.

In order to fuel metabolic processes within the body, the energy derived from consuming carbohydrate, fat, and less so, protein, is used to resynthesise adenosine triphosphate (ATP). As energy intake occurs intermittently, the flux of energy consumed at an eating occasion is likely to exceed that required for metabolic processes at that given time. Therefore, the surplus of energy will be stored for future use as a metabolic substrate during the intervals between meals. Carbohydrate is stored intracellularly within both the skeletal muscle and the liver in its polymeric form – glycogen (Frayn, 2010). The skeletal muscle is by far the larger reserve of carbohydrate storage, with typical glycogen stores ranging from ~350–700 g depending on diet, muscle fibre type composition, sex, body weight, and training status (Knuiman et al., 2015). Carbohydrate storage capacity of the liver is limited by its anatomical size (~1.5 kg), and normally stores ~80–120 g of glycogen (Frayn, 2010; Knuiman et al., 2015). Due to its hydrophilic nature, 1 g of glycogen is associated with ~3 g of water, imposing finite limits on the amount of energy that can be conveniently stored as glycogen (Flatt, 1995).

On the contrary, triglycerides, which are the storage molecules of fat, are hydrophobic, highlighting a considerable weight advantage in storing excess energy as fat (Frayn, 2010). Triglycerides – compounds consisting of three fatty-acids, each linked by an ester bond to a molecule of glycerol (Frayn, 2010) – are stored primarily within adipocytes, which collectively form adipose tissue. Smaller quantities of triglycerides are present as lipid droplets within muscle fibres, particularly near the mitochondria (van Loon, 2004). In an ~70 kg male without obesity, endogenous fat stores would typically range between ~9–15 kg, corresponding to a total energy storage of ~80,000–140,000 kcal (van Loon, 2004). The capacity to store energy

as fat is virtually infinite, and thus a fine regulation of fat balance is not necessary. Day-to-day fluctuations in energy balance are consequently reflected as changes in fat balance, and not carbohydrate or protein balance (Abbott et al., 1988). Adipose tissue, therefore, appears to act as the buffering tissue for fluctuations in energy storage, with positive and negative balance being reflected as changes in adiposity (Galgani & Ravussin, 2008).

Chemical energy is obtained via the consumption of food and beverages, which is comprised of three major macronutrient groups: carbohydrate, fat, and protein. Whilst not considered a key macronutrient, energy can also be obtained from alcohol. Complexities arise in the process of calculating energy intake, which is less straightforward than the simple summation of the total energy contained within the foods consumed. Not all energy contained within food is metabolically available, and faecal losses are estimated to account for ~2–10% of gross energy intake (Hall et al., 2012). Additionally, food preparation processes (Burton & Lightowler, 2008), the structural components of foods consumed (Southgate & Durnin, 1970), and the action of gut microbes (Krajmalnik-Brown et al., 2012), all influence the eventual energy absorption from food. After taking into consideration faecal and urinary losses, the metabolisable energy density of the major macronutrient groups are typically described as follows: carbohydrate (4 $kcal \cdot g^{-1}$, 17 $kJ \cdot g^{-1}$), fat (9 $kcal \cdot g^{-1}$, 38 $kJ \cdot g^{-1}$), protein (4 $kcal \cdot g^{-1}$, 17 $kJ \cdot g^{-1}$), and alcohol (7 $kcal \cdot g^{-1}$, 29 $kJ \cdot g^{-1}$) (Hall et al., 2012).

Energy expenditure consists of three distinct elements: resting metabolic rate (RMR), dietary-induced thermogenesis (DIT), and physical activity energy expenditure (PAEE). RMR can be defined as 'the energy expended at rest by a fasted individual in a thermo-neutral environment' (Hills et al., 2014). RMR forms the largest contribution to total daily energy expenditure (TDEE), accounting for ~60–75% of gross expenditure (Poehlman, 1989). The energy expended via this avenue fuels the essential daily biological functions of the human body. Large variations in RMR exist between individuals, but also within individuals across the lifespan. As well as individual characteristics such as age, sex, physical fitness, and body size/composition (Hills et al., 2014), external factors including caffeine intake (Poehlman et al., 1985) and ambient temperature (Compher et al., 2006) can contribute to variations in RMR. In early studies, obesity was erroneously attributed to a low RMR due to inappropriately dividing RMR by total body weight (Hall et al., 2012), however, fat-free mass (FFM) is more metabolically active than fat mass, and contributes significantly to RMR (Nelson et al., 1992; Ravussin et al., 1986). This error results in an underestimation of RMR in individuals with increased relative body fatness (James et al., 1978).

DIT is determined by the energy content and macronutrient composition of the diet and refers to the energy expended in the process of digesting and assimilating nutrients. DIT can be estimated by dividing the increase in energy expenditure above RMR by the energy content of the food, and is often reported as a percentage (Westerterp, 2004). A hierarchy in DIT exists between nutrients, and reported values are 0–3% for fat, 5–10% for carbohydrate, 20–30% for protein, and 10–30% for alcohol (Westerterp, 2004). An isoenergetic diet proportionally higher in protein and carbohydrate compared to fat, therefore, results in a greater thermic response due to the differences in efficiency of gaining energy from these macronutrients (Westerterp-Plantenga et al., 1999). DIT for an individual consuming a mixed-macronutrient meal is generally considered to be ~10% of gross energy intake (Westerterp, 2004).

PAEE is the largest and most variable component of total energy expenditure and is defined as 'any bodily movement produced by skeletal muscles that results in energy expenditure' (Caspersen et al., 1985). PAEE can be divided into energy that is expended specifically during exercise, and non-exercise specific energy expenditure *i.e.*, non-exercise activity thermogenesis (NEAT). Exercise is considered to be 'planned, structured, and repetitive movement with the intention of promoting or maintaining one or more components of physical fitness' (Caspersen et al., 1985). Like RMR, exercise energy expenditure is strongly influenced by, amongst other factors, body size and body composition (Westerterp, 2013). Besides the physical and physiological characteristics of the individual, the resultant energy expenditure is a product of the modality, intensity, and duration of the exercise bout (Howley, 2001). NEAT comprises activities of daily living other than exercise such as sitting, standing, walking, and fidgeting (Levine et al., 2000).

Although the theoretical basis underlying weight management in humans appears relatively simple – *i.e.*, change in weight is equal to the difference between energy intake and energy expenditure – the application of these principles to the intricate human physiological system fails to consider some complexities. Evidence supports the existence of a physiological control mechanism, which, as first proposed by Kennedy (1953), strives to maintain energy balance through the harmonious regulation of the components involved. For instance, energy balance is a dynamic process, oscillating between states of positive and negative balance as a result of intermittent eating occurrences and sporadic changes in physical activity across the day. However, a sustained period of energy imbalance must occur in order to drive body mass change, and large daily fluctuations in body mass are not observed as would be expected if energy balance were subject only to behavioural mechanisms controlling food intake and

volitional energy expenditure (Hill et al., 2012). Furthering this, changes in body weight may drive compensatory changes in RMR, DIT, and PAEE, which act to defend body energy stores (Hill et al., 2012). For example, maintaining a reduced energy intake can cause gradual reductions in TDEE by lowering RMR (due to reduced FFM), DIT (due to reduced energy intake), and PAEE (due to reduced energy cost of moving a lower body mass) (Hall et al., 2012). This ultimately leads to a restoration of energy balance at a lower body weight.

These compensatory processes were illustrated in a study by Leibel et al. (1995). Subjects were either under- or over-fed to elicit a ≥10% body weight. Maintenance of a body weight at a level ≥10% above initial weight was accompanied by a significant increase in total energy expenditure, with suppressed energy expenditure being observed in the group maintaining a reduced body weight. Therefore, the application of the static energy balance equation to humans appears inadequate, and obesity cannot be considered a problem in a single component of energy balance, but a product of the interactions that exist between the components (Hall et al., 2011; Hill et al., 2012). It is hypothesised that these processes evolved as a defence mechanism against malnutrition, therefore, the energy balance system appears to defend more strongly against weight loss than weight gain (Cummings et al., 2004*a*; Hill et al., 2012). When applying this biased system to today's obesogenic environment conducive to sedentary lifestyles and 24-h availability of energy-dense foods, it is perhaps no surprise that the prevalence of obesity continues to escalate.

2.1.2 Measuring Components of Energy Balance

Energy Expenditure

As previously summarised, energy expenditure consists of RMR, DIT, and PAEE. Several laboratory and free-living measurement tools exist for the assessment of energy expenditure (Hills et al., 2014), each with their own strengths and weaknesses. Some of the most commonly used tools will be briefly discussed below.

Laboratory Assessment of Energy Expenditure

Producing energy via the combustion of carbohydrate, fat, protein, and alcohol requires the consumption of oxygen, and produces carbon dioxide (Hills et al., 2014). The quantity of oxygen consumed, and carbon dioxide produced in the oxidation of each macronutrient is known (Frayn, 1983), therefore, by measuring the inspiration and expiration of these respective

gasses, it is possible to accurately estimate energy expenditure. This process is known as 'indirect calorimetry' (Douglas, 1911; Frayn, 1983). The ratio of carbon dioxide production to oxygen consumption (respiratory exchange ratio (RER)), also provides a useful index for determining the type of fuel being used for metabolism (*i.e.*, carbohydrate, fat, or protein) (Frayn, 1983). These principles and protocols are outlined in further detail in **Chapter 3**.

Evidence-based guidelines exist for the accurate determination of RMR by indirect calorimetry (Betts & Thompson, 2012; Compher et al., 2006). Subjects should be tested within controlled environmental conditions in a rested, steady state, following a period of fasting. Whole-room metabolic chambers can accurately measure RMR over periods ranging from hours to days, however, the constraints imposed by their small size make for erroneous estimates of PAEE (Hills et al., 2014; Levine, 2005). Alternatively, Douglas bags (Douglas, 1911; Levine, 2005) and online breath-by-breath and portable systems (Ainslie et al., 2003) are less restricted by space and allow for measurements during defined activities within both the laboratory and the field. Nevertheless, these approaches to indirect calorimetry are not appropriate for use within free-living settings due to impracticalities involved with transporting Douglas bags/metabolic carts (Levine, 2005), and the finite battery life of portable systems (Ainslie et al., 2003).

Free-Living Assessment of Energy Expenditure

A simple method of estimating energy expenditure is the use of predictive equations. Popular examples include the Mifflin-St Jeor (Mifflin et al., 1990), the Harris-Benedict (Harris & Benedict, 1919), the Owen (Owen et al. 1986; Owen et al. 1987) and the Schofield (Schofield, 1985). The Mifflin-St Jeor equation is considered the most accurate of these equations when compared to objectively measured energy expenditure (Frankenfield et al., 2005). To estimate RMR, the formula requires the input of height, weight, and biological sex. The RMR value is then multiplied by a physical activity level, dependent upon the subject's habitual activity level. The Food and Agricultural Organization (FAO)/WHO (2004) define 3 physical activity level categories: 1.40–1.69 (sedentary lifestyle), 1.70–1.99 (moderately active lifestyle), and 2.00–2.40 (vigorously active lifestyle). **In Chapters 5, 6, and 7** in this thesis, the Mifflin-St Jeor equation (Mifflin et al., 1990) was used to estimate subjects' energy requirements for the prescription of standardised test meals (see **Chapter 3**).

The doubly-labelled water (DLW) technique was developed by Lifson et al. (1955) and was subsequently validated for use in humans (Schoeller & van Santen, 1982). The DLW technique is an objective, non-invasive method for accurately assessing free-living TDEE over periods

of up to 2 weeks (Schoeller, 2002). DLW is considered the 'gold-standard' approach of assessing TDEE (Ainslie et al., 2003; Hills et al., 2014). The principle of the method is that after a loading dose of water labelled with stable isotopes of ^{2}H (deuterium) and ^{18}O, the deuterium is eliminated from the body as water, while ^{18}O is eliminated as water and carbon dioxide. The difference between these two elimination rates is reflective of carbon dioxide production (Schoeller, 1990; Schoeller, 2002), which can then be used to estimate energy expenditure. In spite of the accuracy and precision of the DLW technique, it is expensive, requires sophisticated laboratory-based equipment, and cannot distinguish between the individual components of energy expenditure (Ainslie et al., 2003; Hills et al., 2014).

Wearable sensors are an alternative, cheaper option to estimate free-living energy expenditure. Firstly, heart rate monitoring is based on the assumption that a linear relationship exists between heart rate and energy expenditure (Christensen et al., 1983). An individual regression line of heart rate to energy expenditure can be determined in the laboratory (Hills et al., 2014) and subsequently used to estimate energy expenditure based on heart rate data obtained in free-living conditions. This method has demonstrated good agreement with energy expenditure measured by DLW (Livingstone et al., 1990). Accelerometers provide estimates of energy expenditure by detecting the frequency, intensity, and duration of bodily movements, typically in the vertical, horizontal, and mediolateral planes (Ainslie et al., 2003). However, accelerometers cannot detect non-accelerating activities such as cycling/rowing, or the extra energy expended during arm movements, uphill walking, and carrying heavy objects (Crouter et al., 2006). In attempts to overcome the shortfalls of these wearable sensors, some researchers have turned to their combined use (Rennie et al., 2000). Estimates of PAEE resulting from the simultaneous measurement of heart rate and acceleration using monitors such as the Actiheart™ have been shown to produce measurements in agreement with those obtained using DLW (Brage et al., 2015).

Energy Intake

Energy intake is a complex phenomenon influenced by physiological, cognitive, sensory, and environmental factors, making it difficult to measure (Blundell, 2010). Laboratory measures allow for objective assessment of energy intake, with a great degree of control and internal validity. In spite of this, laboratory approaches lack ecological validity and fail to acknowledge the array of variables which influence eating behaviour in the real world (de Castro, 2000).

Free-living measures to assess energy intake overcome issues related to ecological validity, but do not come without their own shortfalls.

Laboratory Assessment of Energy Intake

The most commonly employed laboratory measure of energy intake is the preload-test meal paradigm (Blundell, 2010). This protocol typically involves providing subjects with a precisely prepared preload meal (independent variable), and allowing a standardised time period to pass, before allowing them access to a test meal which they can consume *ad-libitum*. The test meal is experimenter-weighed before and after the eating period to quantify energy intake. The only variance between trials should be the independent variable, as the preload-test meal time interval (Rolls et al., 1991), knowledge about the time until the next meal (de Graaf et al., 1999), and the awareness of experimenter observation (Robinson et al., 2015*b*), can alter energy intake. If adequate control is imposed, *ad-libitum* meals are reproducible within the same individual (Gregersen et al., 2008). This methodology has also been employed in the assessment of meal omission (Chowdhury et al., 2015; Chowdhury et al., 2016*a*; Clayton et al., 2015) and exercise (Bachman et al., 2016; Deighton et al., 2012; Edinburgh et al., 2019; Gonzalez et al., 2013).

The test meal typically takes one of two forms: a homogenous, single-course test meal, or a multi-item buffet style meal. The single-course meal involves providing subjects with *ad-libitum* access to a homogenous meal and is used when the hypothesis being tested is specifically concerned with changes total energy intake, as opposed to food choices and macronutrient preferences (Blundell, 2010). The meal should be selected based upon cultural norms and typical mealtimes and should be closely matched between experimental trials to eliminate the confounding effects of sensory cues (Wadhera & Capaldi-Phillips, 2014). Alternatively, a multi-item buffet meal allows assessment of food choices and macronutrient preferences, even in the absence of changes in total energy intake (Blundell, 2010). In such studies, a large variety of foods are provided to subjects to consume *ad-libitum*. However, the wide variety of food items can delay satiation and promote overconsumption (Hetherington et al., 2006), making this method prone to considerable variance. Consequently, it is advised that this approach should be limited to studies specifically examining food choices (Blundell, 2010). In the work presented in **Chapters 5, 6 and 7** of this thesis, the single-item approach was selected.

Free-Living Assessment of Energy Intake

An accurate, albeit expensive and labour-intensive, means of estimating free-living energy intake over extended periods is to use the 'intake-balance method' (Sanghvi et al., 2015). This involves measuring energy expenditure using DLW at several instances over the period of interest, alongside measurements of body energy stores using dual-energy X-ray absorptiometry (DXA) scans (Racette et al., 2012). Using the principles of energy balance, mean energy intake can be inferred from differences between measured energy expenditure and changes in body energy stores.

A cheaper option of estimating free-living energy intake is to use either retrospective or prospective self-reported methods. Retrospective methods involve subjects reciting typical dietary habits over weeks, months, and years (Burke, 1947), or the details of all food and beverages consumed over the last 24 h (Shim et al., 2014). These methods are reliant upon not only the subjects' memory, but also estimated portion sizes, which can lead to considerable error (Almiron-Roig et al., 2013). Additionally, the 24-h recall method is only representative of very recent intakes, which likely vary day-to-day, making multiple recalls necessary to gauge intake over an extended period (Shim et al., 2014). Prospective methods involve subjects weighing and recording all food and beverages consumed over a specified period, which reduces error arising from recall bias and portion size estimates. Typically, a monitoring period of 3–7 days is used (de Castro, 1991; Whybrow et al., 2008), although 3 days is often considered preferable, as extended periods of monitoring are tedious and can reduce compliance (Young & Trulson, 1960). Despite eliminating memory-related errors, the awareness of having dietary habits monitored can influence dietary behaviours (Stubbs et al., 2014), and self-reported food intake is prone to misreporting, particularly the under-reporting of energy (Stubbs et al., 2014; Whybrow et al., 2020) and/or macronutrient intake (Livingstone & Black, 2003).

Using the same principles as the 'intake-balance' method, self-reported energy intakes can be validated against objectively measured energy expenditure (Schoeller & van Santen, 1982; Schoeller, 1990). Studies comparing self-reported energy intake with energy expenditure assessed by DLW typically show an under-reporting of energy intake (Hill & Davies, 2001), possibly by ~20% (de Castro, 2000). This has led some to advise against the use of such measures altogether (Dhurandhar et al., 2015), although the intra-individual consistency in reporting error (de Castro, 1994) highlights that self-reported measures of energy intake should

not be disregarded for use within studies employing a repeated-measures design interested in within-subject responses (de Castro, 1994).

2.2 Regulation of Appetite

Daily energy intake is ultimately determined by satiation (process that leads to the termination of eating *i.e.*, intra-meal satiety) and satiety (process that leads to inhibition of further eating *i.e.*, inter-meal satiety). These concepts are complex and influenced by several sensory and cognitive factors including the palatability of a food, and the learned responses which drive expectations of the satiating effects of a food (Blundell, 2010). Environmentally determined factors such as food availability and contextual influences also play a role in eating behaviour, and the obesogenic environment characteristic of today's modern Western society is a likely contributor to the increasing rates of obesity. The 'Satiety Cascade', initially constructed by Blundell et al. (1987), and more recently adapted by Mela (2006), highlights the complexities of appetite regulation, which is subject to homeostatic, hedonic, and behavioural cues. Studies within this thesis will focus primarily on the homeostatic domain of appetite regulation, which involves the coordinated responses of central and peripheral signals (Badman & Flier, 2005). The hypothalamus (in particular the arcuate nucleus) and the brainstem receive neural and hormonal signals from the periphery, which transmit information reflective of the acute nutritional state and adiposity of the body (Murphy & Bloom, 2006). Of these homeostatic mechanisms, gut-to-brain communication is increasingly recognised as playing an important role in regulating energy intake. The remarkable success of various types of bariatric surgeries in reversing type 2 diabetes and obesity have been attributed, in part, to changes in the release patterns of various gastrointestinal hormones (Cummings et al., 2004*a*; Ochner et al., 2007). As discussed in **Chapters 2.4.2 and 2.5.3**, acute nutrition and exercise interventions can alter appetite regulatory hormone profiles (Clayton & James, 2016; Dorling et al., 2018), therefore, it seems imperative to monitor this aspect of appetite regulation in response to an intervention.

Peptide hormones are released from peripheral tissues including the gut, pancreas, and adipose tissue, and are subsequently transported to the appetite-regulatory centres within the brain to communicate information regarding the acute and chronic energy status of the body (Wynne et al., 2005). Hormones that reflect the chronic energy storage of the body are generally referred to as 'tonic' hormones, and those implicated in the acute regulation of appetite and energy balance, as 'episodic' hormones. The former comprises of insulin and the adipose tissue-

derived hormone leptin, whereas the latter comprises several gut peptides including ghrelin, peptide tyrosine-tyrosine (PYY), and glucagon-like peptide-1 (GLP-1).

2.2.1 Tonic Hormones

Leptin

An 'adiposity negative-feedback' model of energy homeostasis was first introduced by Kennedy (1953), who proposed that circulating signals inform the brain of changes in body fat reserves, resulting in adaptive alterations in energy balance to re-establish fat stores. Support for this concept came in the form of the discovery of leptin (Zhang et al., 1994). Leptin is a peptide hormone secreted from adipose tissue, with circulating concentrations that correlate with body fat stores (Considine et al., 1996). Leptin is transported in the blood to the brain, where it crosses the blood-brain barrier via a saturable process and exerts its effects in the arcuate nucleus of the hypothalamus (Wynne et al., 2005). Leptin suppresses appetite by inhibiting orexigenic neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons and stimulating anorexigenic pro-opiomelanocortin (POMC) neurons (Cowley et al., 2001). A period of excessive energy consumption to elicit body weight gain concomitantly increases leptin concentrations to inhibit food intake (Kolaczynski et al., 1996), and vice versa with weight loss (Rosenbaum et al., 2008). In addition to its responsiveness to feeding occasions, leptin displays a strong diurnal rhythm, peaking in the late evening with a nadir in the morning (Schoeller et al., 1997; Templeman et al., 2021*a*).

Leptin resistance is a term used to describe the paradoxical relationship between leptin's role as an anorexigenic hormone and its elevated levels within obese individuals (Oswal & Yeo, 2010). Chronic exposure to elevated leptin concentrations decreases the transport of leptin into the central nervous system and impairs the signalling properties of leptin receptors (Oswal & Yeo, 2010). This resistive effect negates the use of leptin as a therapeutic remedy for long-term weight management.

Insulin

Similar to leptin, circulating concentrations of insulin are proportional to body fat stores (Woods et al., 1998), supporting its role within an adiposity negative-feedback model. Insulin is also secreted episodically by pancreatic β-cells in response to individual feeding occasions, and exhibits, in addition to its anorexigenic effects, a range of crucial functions relating to the

assimilation and metabolism of ingested nutrients. The role of insulin in this aspect of metabolism will be discussed in more detail in **Chapter 2.3**. Through a receptor-mediated transport process, insulin penetrates the blood-brain barrier (Woods et al., 2003), and acts on receptors in the arcuate nucleus of the hypothalamus to control energy homeostasis, much like leptin does (Wynne et al., 2005). Specifically, insulin inhibits (NPY/AgRP) neurons and stimulates POMC neurons. Intracerebral infusion of insulin in primates can reduce food intake and body weight (Woods et al., 1979), with comparable observations being made in men following intranasal insulin administration (Hallschmid et al., 2004).

Peripheral insulin sensitivity is influenced by both total body fat stores and fat distribution, with visceral adiposity in particular acting as a key determinant of whole-body insulin sensitivity (Porte et al., 2002). In line with this, the relationship between increased insulin concentrations and reduced appetite and energy intake which has been reported in lean individuals is disrupted in individuals with obesity (Flint et al., 2007). In accordance with the relationship between adiposity and peripheral insulin resistance, it is proposed that the lack of insulin induced appetite modulation in those with obesity results from a dampened central insulin sensitivity at the level of the hypothalamus (De Souza et al., 2005).

2.2.2 Episodic Hormones

Ghrelin

Ghrelin is a 28-chain amino-acid peptide which is primarily secreted from the oxyntic cells of the stomach (Kojima et al., 1999). Unlike the other episodic hormonal regulators of appetite, which exhibit negative feedback functions to reduce food intake in response to adequate energy stores, ghrelin is unique, in that it possesses a feed-forward function (Badman & Flier, 2005) by stimulating the orexigenic NPY/AgRP neurons (Wynne et al., 2005). This effect is illustrated by the release patterns of ghrelin in humans, which entail surges in circulating concentrations during periods of fasting, and rapid reductions after eating, which occur in proportion to the caloric load of the meal consumed (Callahan et al., 2004; Cummings et al., 2001). Such release patterns have been observed even when meals are initiated voluntarily, thus eliminating the effects of time- and food-related cues (Cummings et al., 2004*b*). In this study (Cummings et al., 2004*b*), peripherally measured ghrelin concentrations and subjective hunger scores showed similar temporal profiles, which is consistent with a role for ghrelin in meal initiation. Nevertheless, the meal-initiating role of ghrelin has been subject to

considerable debate, as some evidence suggests that the pre-meal rise in ghrelin is an entrained anticipatory response to habitual meal patterns (Frecka & Mattes, 2008). Ghrelin concentrations show a strong diurnal variation, in likeness with that observed for leptin. Under conditions of fasting (Espelund et al., 2005) and during conventional meal patterns (Cummings et al., 2001) ghrelin concentrations peak in the late evening, with a nadir in the early morning upon waking. However, during a recent study which adopted a semi-constant routine (hourly feeding throughout waking hours, and sleeping permitted), the secretion profile of ghrelin was shown to be approximately antiphasic with that of subjective hunger, peaking shortly after waking (Templeman et al., 2021*a*). This supports the notion that sleep-wake cycles and feeding patterns influence the secretory profile of ghrelin.

In addition to its potential role in meal initiation, ghrelin appears to influence energy intake. This was first demonstrated in free-living rats who, following both central and peripheral administration of ghrelin, increased total 24-h food intake in a dose-dependent manner, which led to increased body weight (Tschöp et al., 2000). Subsequent studies have extended these findings to humans (Druce et al., 2005; Wren et al., 2001). In the first of these studies, Wren et al. (2001) examined the appetite and energy intake responses of 9 healthy males and females following intravenous infusion of ghrelin at a rate of 5 $pmol \cdot kg^{-1} \cdot min^{-1}$ for 4.5 h. Compared to the infusion of saline, ghrelin significantly increased subjective appetite and consequently increased energy intake at an *ad-libitum* meal in all subjects, with a mean increase of 28%. These findings imply a central role of ghrelin in appetite and energy intake regulation, but it should be noted that the administration of ghrelin in these studies resulted in supraphysiological plasma ghrelin concentrations. Ghrelin is also proposed to influence chronic energy homeostasis, as circulating concentrations are decreased in individuals with obesity (Tschöp et al., 2001). This initially appears paradoxical, however, despite reduced plasma concentrations, the postprandial fall in circulating ghrelin concentrations is less pronounced in obese individuals (English et al., 2002).

Within the circulation, ghrelin is found in either its acylated or unacylated form. In order for it to exhibit its biological functions, ghrelin must cross the blood-brain barrier and bind with the growth-hormone-secretagogue-receptor (GHS-R) (Kojima & Kangawa, 2005). For this to occur, the acylation of ghrelin is necessary, and involves the linking of octanoic acid to its third amino acid residue (serine) under the enzymatic regulation of ghrelin O acyltransferase (GOAT) (Yang et al, 2008). Despite unacylated ghrelin previously being considered solely as an inert degradation product of acylated ghrelin, evidence suggests that it may in fact have its

own distinct functions as a separate hormone (Delhanty et al., 2012). Specifically, unacylated ghrelin has been shown to inhibit the effects of acylated ghrelin in humans (Brogoli et al., 2004; Gauna et al., 2004) and may improve glucose metabolism (Benso et al., 2012).

Peptide tyrosine-tyrosine (PYY)

PYY is a gut-derived hormone of the PP-fold group of peptides, which also includes pancreatic polypeptide (PP) and neuropeptide YY (NPY) (Badman & Flier, 2005). PYY is initially produced as a 36-chain amino-acid peptide (PYY_{1-36}) by L-cells throughout the length of the gut with concentrations increasing distally (Adrian et al., 1985). The 34-chain amino-acid form of PYY (PYY_{3-36}) is created by cleavage of the N-terminal tyrosine and proline residues by the dipeptidyl peptidase IV (DPP-IV) (Eberlein et al., 1989). In the circulation, total PYY concentrations consist of ~65% PYY_{3-36} and 35% PYY_{1-36} (Batterham et al., 2006), and a strong correlation between changes in total PYY and PYY_{3-36} has been observed (Tsilchorozidou et al., 2008). PYY_{3-36} crosses the blood-brain barrier freely from the circulation and exerts anorexigenic actions through binding with the Y2 receptors of the hypothalamus and ultimately inhibiting NPY/AgRP neurons and activating POMC neurons (Batterham et al., 2002; Batterham et al., 2003).

Plasma concentrations of PYY increase rapidly in response to feeding, peaking 1–2 h after nutrient ingestion, and remaining elevated for up to 6 h (Adrian et al., 1985). The extent of the rise in the circulation appears to reflect both the size, and the macronutrient profile of the foods ingested (Adrian et al., 1985). For example, when compared to isocaloric doses of carbohydrate and fat, dietary protein intake exerts the greatest release of PYY (Batterham et al., 2006). Furthermore, fasting reduces circulating PYY concentrations (Chan et al., 2006). Such release patterns are in agreement with a role of PYY in appetite regulation.

The suppressive effect of PYY_{3-36} on energy intake was first observed in rats, as following peripheral injection of PYY_{3-36}, food intake was significantly reduced (Batterham et al., 2002). The same research group confirmed these findings in lean men and women by infusing PYY_{3-36} at a rate of 0.8 $pmol \cdot kg^{-1} \cdot min^{-1}$ for 90 min, which ultimately reduced *ad-libitum* energy intake by 36% 2 h later (Batterham et al., 2002). Much like the ghrelin-infusion studies, this study elicited supraphysiological plasma PYY_{3-36} concentrations, and food intake was not suppressed in lean and obese men following infusion of PYY_{3-36} within a normal physiological range (Degen et al., 2005). Despite this, studies have shown strong correlations between postprandial

increases in PYY_{3-36} concentrations and reduced appetite (Guo et al., 2006; Stoeckel et al., 2008).

Humans with obesity have lower fasting concentrations of PYY_{3-36}, however, these subjects appear to retain their sensitivity to exogenous PYY_{3-36} administration (Batterham et al., 2003). Therefore, unlike the resistance that develops for leptin and insulin, the aetiology of obesity is unlikely facilitated by PYY_{3-36} resistance, possibly making PYY_{3-36} an appealing therapeutic target for the treatment of obesity.

Glucagon-like peptide-1 (GLP-1)

GLP-1 is rapidly secreted from intestinal L-cells in response to nutrient ingestion and is primarily recognised as an incretin hormone. As an incretin hormone, GLP-1 enhances glucose-stimulated insulin secretion in response to nutrient ingestion (Baggio & Drucker, 2007). Two biologically active forms of GLP-1 exist: $GLP\text{-}1_{7-37}$ and $GLP\text{-}1_{7-36}$, with the latter demonstrating the greatest abundance in human circulation (Ørskov et al., 1994). Circulating $GLP\text{-}1_{7-36}$ is quickly degraded by the serum enzyme dipeptidyl peptidase IV (DPP-IV), permitting it to a half-life as short as 2 min (Kieffer et al., 1995). GLP-1 is secreted in proportion to the energy content of a meal, but may also be mediated by nutrient composition, with protein seemingly enhancing the response (Belza et al., 2013; Lejeune et al., 2006).

In a meta-analysis examining the effect of $GLP\text{-}1_{7-36}$ infusion on subsequent food intake in lean and obese humans, a mean decrease in energy intake of 11.7% was reported compared to when saline was infused (Verdich et al., 2001*a*). A significant reduction in gastric emptying rate was also observed, and this has been presented as a potential mediator (amongst other central mechanisms) of the satiating effects of $GLP\text{-}1_{7-36}$ (van Bloemendaal et al., 2014). When infused in a physiological dose, the appetite suppressing effects of $GLP\text{-}1_{7-36}$ appeared to persist, although actual energy intake was unaffected (Flint et al., 2001). Despite lean and obese individuals responding similarly to the anorexigenic effects of infused $GLP\text{-}1_{7-36}$ (Verdich et al., 2001*a*), postprandial $GLP\text{-}1_{7-36}$ concentrations following a meal were significantly lower in obese individuals, which was largely normalised after weight loss (Verdich et al,. 2001*b*). In addition to their independent influences on appetite, a combined infusion of $GLP\text{-}1_{7-36}$ and PYY_{3-36} has been shown to inhibit food intake to a greater extent than either of these hormones in isolation (Neary et al., 2005). Thus, the anorexigenic effects of $GLP\text{-}1_{7-36}$ may occur through simultaneous interactions with other gut hormones.

2.2.3 Measurement of Subjective Appetite

Although measuring hormonal markers provides some insight into an individual's appetite, perceived appetite is a subjective construct, and therefore, the measurement of appetite should include some subjective component (Mattes et al., 2005). In research, one of the most common methods of measuring subjective appetite is the use of visual analogue scales (VAS; Stubbs et al., 2000).

VAS typically consist of a 100- or 150-mm straight line, with opposing phrases printed at opposite ends such as 'not at all hungry' and 'extremely hungry' (Blundell et al., 2010). In practice, the subject places a mark on the scale using a pen before the researcher measures the distance between the mark and the leftmost end of the scale using a ruler. To encompass the multidimensional nature of appetite, it is recommended that several different sensations should be measured including hunger, fullness, desire to eat, prospective food consumption, and satiety (Blundell et al., 2010). Consistently using these recommended scales and anchor descriptions appears to be a valid and sensitive method for use in research (Blundell et al., 2010). For example, Flint et al. (2000) examined the reproducibility of VAS for the aforementioned appetite sensations over a 4.5 h postprandial period following a standardised breakfast meal. Scales were shown to be reproducible, and it was proposed that in a paired research design, a sample size of between 8–11 subjects would be sufficient to detect differences in mean postprandial appetite scores. It should be noted that energy intake does not always correspond with subjective appetite, as humans can choose to eat without feeling hungry, or contrastingly refrain from food intake despite feeling the sensation of hunger (Mattes, 1990). Accordingly, VAS are often more sensitive to a dietary manipulation than are changes in energy intake (Johnstone et al., 1996; Stubbs et al., 2000).

The introduction of electronic appetite rating systems was made over two decades ago (Stubbs et al., 2001) and have several advantages over the traditional pen-and-paper methods. For example, responses to questionnaires are dated and timestamped, confirming that questionnaires were completed by subjects at desired timepoints (Mattes et al., 2005). Additionally, electronic scales may be less time consuming and therefore less prone to human error (Mattes et al., 2005). Due to obvious differences such as a varying screen, and therefore line size, electronic methods should not be used interchangeably with pen-and-paper methods (Stubbs et al., 2000). More recently, the idea of utilising personal mobile phones to administer VAS was examined (Holliday et al., 2021). In this study, measurements of subjective appetite

were made using the traditional pen-and-paper method and then on a mobile phone upon waking and every hour thereafter for 12 h. The following day, measurements were repeated but with the order of measurement tools reversed. Measurements on the mobile phone were reliable, and importantly, 90% of subjects preferred this method due to reasons including greater accessibility, a simplified process, and easier/quicker use. In summary, providing methods are used appropriately, both pen-and-paper, and electronic VAS can be effective measurement tools for subjective appetite in research. In this thesis, subjective appetite was measured using the traditional, pen-and-paper VAS in **Chapter 7**, whereas electronic VAS were used in **Chapters 5 and 6**.

2.3 Fasting

2.3.1 The Postabsorptive (Fasted) State

The postabsorptive state is the term given to the early stage of fasting, which begins once all ingested nutrients from the last meal have been absorbed from the small intestine, *e.g.*, after an overnight fast (Frayn, 2010; Maughan et al., 2010). The timing of the onset of this stage is dependent on the size and composition of the preceding meal and may occur within as little as 3–4 h, or as long as 7–8 h (Maughan et al., 2010). In the early postabsorptive state, plasma glucose concentrations are maintained by roughly equal contributions of hepatic glycogen breakdown (glycogenolysis) and hepatic gluconeogenesis (the production of glucose from primarily lactate, alanine, and glycerol) (Frayn, 2010). Therefore, following an overnight fast, liver glycogen stores are considerably reduced (Nilsson & Hultman, 1973), whereas muscle glycogen content remains largely unchanged (Knapik et al., 1988). When the fasting duration is extended further (*i.e.*, >40 h), gluconeogenesis can contribute as much as 90% to glucose production (Katz & Tayek, 1998). The metabolic environment during this state is characterised by low plasma concentrations of insulin, with concomitantly elevated concentrations of glucagon and catecholamines, which in turn promotes lipolysis – the mobilisation of NEFA and glycerol from adipose tissue (Duncan et al., 2007). Adipose tissue lipolysis is facilitated by the actions of both hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) (Zimmermann et al., 2004). The subsequent increase in available NEFA competes with glucose for oxidation, and the result of these metabolic changes is a shift in whole body metabolism away from carbohydrate oxidation towards fatty acid oxidation (Randle et al., 1963), ultimately sparing the body's finite carbohydrate reserves for glucose dependent tissues such as the brain

and red blood cells (Cahill et al., 1966). Additionally, the mobilised glycerol is a fundamental precursor for hepatic gluconeogenesis, also contributing as a source of fuel for the dependent tissues (Maughan et al., 2010).

2.3.2 The Postprandial (Fed) State

The metabolic response to feeding begins even before food enters the mouth, driven by a set of responses which comprise the 'cephalic phase'. The brain's anticipation of food begins with sight and smell, which is further reinforced by the taste and texture of food in the mouth (Frayn, 2010). Cephalic stimulation then drives several food-anticipatory processes, such as parasympathetic stimulation of saliva flow, gastric juice secretion, and the cephalic-phase insulin secretion (Frayn, 2010). Once food is ingested, the typical response to a mixed macronutrient meal involves a sharp rise in plasma concentrations of glucose and lactate (peaking within 60 min), alongside reductions in circulating NEFA and glycerol (nadir between 60–120 min) (Coppack et al., 1990; Frayn et al., 1993). The observation that, in healthy subjects, plasma concentrations of glucose are tightly regulated within ~60% of baseline values (rise from ~4–5 $mmol \cdot L^{-1}$ to ~7–8 $mmol \cdot L^{-1}$) following relatively large intakes of carbohydrate, highlights the effectiveness of the physiological systems that regulate glucose appearance and disappearance (Frayn, 1997).

The increased concentrations of glucose and amino acids (particularly leucine and arginine) within the circulation following a meal directly stimulate the release of insulin from the pancreatic β-cells (Wilcox, 2005). The first-phase insulin response is facilitated by the activation of glucose transporter type 2 (GLUT2), which is present within glucose-sensing cells of the hepatic portal vein region (Thorens, 2015). GLUT2 is also responsible for insulin-independent glucose uptake in the liver (Adeva-Andany et al., 2016). Additionally, the release of insulin is stimulated indirectly via the actions of intestinal incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and GLP-1, which are rapidly secreted in response to nutrient ingestion (Baggio & Drucker, 2007). The secretion of insulin into the circulation occurs in proportion to the plasma glucose concentrations following the meal, in that a greater glucose concentration will induce a greater insulin response (Frayn, 2010).

The meal-induced rise in plasma glucose and insulin concentrations initially inhibits glucagon secretion from the pancreatic α-cells, profoundly altering the insulin to glucagon ratio (Gerich, 1993). This altered ratio, in addition to the increased hepatic glucose concentrations due to

GLUT2 activity, suppresses hepatic glycogen breakdown and promotes glycogen synthesis, ultimately dampening the postprandial rise in plasma glucose concentrations (Saltiel & Khan, 2001). Insulin also stimulates the translocation of glucose transporter type 4 (GLUT4) from intracellular sites to the cell membrane, which increases glucose uptake (Saltiel & Khan, 2001). The majority of glucose disposal occurs within the skeletal muscle, with adipose tissue accounting for only a small proportion (Gerich, 1993). Following the initial rapid rise in plasma glucose concentrations, a net increase in glucose disposal over appearance prevents glucotoxicity and replenishes endogenous glycogen stores.

As well as replenishing glycogen stores, a large proportion of the glucose taken up by the tissues is oxidised (Gerich, 1993). As such, in the postprandial state, metabolism in the skeletal muscle is shifted in favour of glucose, rather than fatty acid oxidation (Frayn, 2010). This shift in metabolism is facilitated by the actions of insulin, which inhibits the rate of long-chain fatty acid entrance into the mitochondria for β-oxidation (Sidossis et al., 1996), enhances fatty acid re-esterification in adipose tissue (Enevoldsen et al., 2004; Frayn et al., 1995), and inhibits HSL and ATGL activity (Spriet, 2014). These actions ultimately reduce the circulating concentrations of NEFA and lessen the substrate competition for oxidation, allowing for the preferential metabolism of glucose (Randle et al., 1963).

The majority of dietary fat is ingested in the form of triacylglycerol (TAG), with smaller proportions being made up of cholesterol and phospholipids. TAG is absorbed in the small intestine before being processed in the enterocytes to form chylomicron particles, which are subsequently released into the lymphatic system and then the bloodstream (Frayn, 2010). Plasma concentrations of TAG typically peak at ~1.0–1.5 $mmol \cdot L^{-1}$ approximately 4 h after consuming a meal containing ~30 g of fat (Coppack et al., 1990), and may reach up to ~2 $mmol \cdot L^{-1}$ following larger (~80 g) volumes (Griffiths et al., 1994). Chylomicron TAG is transported to adipose tissue, where it is hydrolysed via the actions of enzyme lipoprotein lipase (LPL) into its fatty acid and glycerol subcomponents (Zechner, 1997). These fatty acids then enter the adipocyte in a process facilitated by Cluster of Differentiation 36 (CD36) (Goldberg et al., 2009), where they are stored as TAG following their re-esterification (Frayn, 2010). Not all fatty acids released from chylomicron TAG are taken up by the tissues, leading to the release of NEFA into the blood in a process termed ‘spillover’ (Lambert & Parks, 2012). The quantity of ingested fat seemingly influences the extent of spillover (Barrows et al., 2005), which can contribute between 5–35% of fatty acids to the plasma NEFA pool following a meal (Lambert & Parks, 2012). Although not specific to the postprandial period, TAG is also secreted into the

bloodstream by the liver as very low-density lipoprotein-TAG (VLDL-TAG). Chylomicron TAG and VLDL-TAG compete for hydrolysis by LPL, however, likely due to their larger size, there is a preferential hydrolysis of chylomicrons, leading to their faster clearance. As such, the later postprandial period is characterised by elevated VLDL-TAG concentrations (Frayn, 2010).

If fat is consumed as part of a mixed-macronutrient meal, then insulin will also play a role in the clearance of circulating TAG by promoting its uptake into the tissues for storage via the upregulation of LPL (Zechner, 1997). Interestingly, insulin appears to exert its effects on LPL in a tissue-specific manner, specifically by stimulating LPL activity in adipose tissue, but suppressing it in skeletal muscle (Zechner, 1997). This ultimately leads to the postprandial redirection of TAG away from skeletal muscle towards its storage within adipose tissue (Farese et al., 1991). Despite this, skeletal muscle can still contribute substantially to TAG clearance (Horton et al., 2002), and excessive storage of these intramuscular triglycerides (IMTG) is associated with insulin resistance (Ebeling et al., 1998; Pan et al., 1997).

After ingesting large mixed-macronutrient meal, concentrations of plasma glucose typically return to baseline within 3–4 h (Frayn, 2010). However, due to the slower absorption process, TAG concentrations do not peak until around this timepoint and remain considerably elevated even after 6 h (Coppack et al., 1990). Therefore, within the paradigm of the typical Western meal pattern of three or more evenly spaced meals during the day (Yates & Warde, 2015), it is likely that another meal will be consumed before the postabsorptive state has been reached. Indeed, in these circumstances, the postprandial response to the first will have some bearing on that of the subsequent meal. For instance, although the timing of the peak is largely similar to that of the first meal, the rise in plasma glucose concentrations following the subsequent meal is attenuated, either with (Chowdhury et al., 2015), or without (Jovanovic et al., 2009), a concomitant reduction in insulin – a paradigm known as the 'second meal effect' (Hamman & Hirschman, 1919; Staub, 1921). There are a number of possible mechanisms mediating the second meal effect, for example, the recent prior exposure of skeletal muscle to insulin may act to 'prime' the cells for glucose uptake via the translocation of more GLUT4 to the cell membrane (Geiger et al., 2006). Additional mechanisms include a delayed rate of gastric emptying (Ma et al., 2009), possibly due to elevated GLP-1 concentrations at the second meal (Meier et al., 2006), as well as prior insulin release acting to prime the pancreatic β cells to potentiate early phase insulinaemia at the second meal (Lee et al., 2011). Finally, the upregulation of lipolysis and elevated plasma NEFA concentrations in response to a prolonged

fast can inhibit the insulin response (Grill & Qvigstad 2000). Therefore, the insulinaemic response to the first meal of the day which suppresses NEFA concentrations may permit for greater insulin-stimulated glucose uptake into the tissues at the second meal (Jovanovic et al., 2009).

With regards to the lipaemic response, if the subsequent meal is consumed within 6 h of the first, as is common in Western cultures, the peak in TAG concentrations from the first meal is likely to occur shortly after the ingestion of the second meal. The second meal induces a larger, more rapid peak in plasma TAG concentrations, likely due to the appearance of additional chylomicron TAG from the previous meal (Lambert & Parks, 2012). This cumulative TAG response ensues over the course of the day following each subsequent meal. Thus, given Western meal patterns typically involve several frequent meals interspersed with short fasting intervals, it is likely that most people spend the majority of the waking day in the postprandial state, which produces a lipogenic environment conducive to fat mass accretion (Saponaro et al., 2015). The storage of excess adiposity causes metabolic changes that are associated with the risk of developing several chronic diseases, including type 2 diabetes (Kahn et al., 2006), cardiovascular disease (Van Gaal et al., 2006), and some forms of cancer (Dobbins et al., 2013). Therefore, any strategies which can effectively increase the mobilisation and oxidation of endogenous triglyceride stores, either by increasing the proportion of time spent in the postabsorptive state through prolonging fasting periods, or by other means such as exercise (or a combination of the two), may have clinical relevance for the prevention and treatment of obesity and obesity-related diseases.

2.3.3 Metabolic Consequences of Excess Adiposity

Adipose tissue is typically the storage site of excess energy (Galgani & Ravussin, 2008), but crucially also acts as an endocrine organ, secreting proteins (adipokines) including interleukin (IL)-6, IL-1β, tumour-necrosis factor (TNF)-α, leptin, and adiponectin (McArdle et al., 2013). During sustained periods of energy surplus, adipocytes initially increase in size (hypertrophy), which then triggers signalling for increased adipocyte number (hyperplasia) (Arner et al., 2010). This continued adipocyte hypertrophy can result in adipocyte dysfunction, which holds serious metabolic consequences.

Many of the detrimental health consequences of being overweight or obese are due to the insulin resistance that accompanies excess adiposity. In fact, insulin resistance is thought to be

the first measurable symptom in individuals with increased risk of type 2 diabetes and cardiovascular disease (Petersen et al., 2007). Chronic adipocyte hypertrophy results in increased non-esterified fatty acid (NEFA) release from the adipose tissue into the circulation (Rutkowski et al., 2015). Increased circulating NEFAs compete with glucose for substrate oxidation and can inhibit enzymes involved in glucose metabolism such as pyruvate dehydrogenase and phosphofructokinase (Kahn et al., 2006; Randle et al., 1963). This lipid 'spillover' can also lead to the accumulation of ectopic fat storage of within non-adipose tissues such as the liver and skeletal muscle, increasing the intracellular content of fatty acid metabolites such as diacylglycerol (DAG), fatty acyl-coenzyme A (fatty acyl-CoA), and ceramides, ultimately reducing insulin sensitivity within these peripheral tissues (Kahn et al., 2006; Rutkowski et al., 2015). Accordingly, a sustained excessive increase in circulating NEFAs is associated with the insulin resistance akin to both obesity and type 2 diabetes (Reaven et al., 1988).

Adipocyte hypertrophy is also associated with pro-inflammatory cytokine secretion and immune cell infiltration, likely mediated by the development of adipocyte hypoxia (Ye, 2009), ultimately inducing a state of chronic low-grade inflammation (McArdle et al., 2013). In this hypoxic state, adipose tissue increases its production of pro-inflammatory mediators such as TNF-α and IL-6, which have downstream effects on a number of pathways that can lead to insulin resistance (McArdle et al., 2013). Furthermore, circulating leptin concentrations are positively correlated with increased adiposity (Considine et al., 1996), which may be an important trigger for the pro-inflammatory immune response in those with obesity (Wensveen et al., 2015). Adiponectin, on the other hand, has insulin sensitising effects within several tissues via the stimulation of fatty acid oxidation, and is reduced in obesity (Kahn et al., 2006).

This evidence provides a causal link between excess adiposity and the development of several chronic diseases such as type 2 diabetes. Given the ever-increasing prevalence of obesity-related diseases and their associated costs (Cobiac & Scarborough, 2021; Wang et al., 2011), interventions targeted at the management of body weight, and specifically excess adiposity, should be a public health priority.

2.3.4 Potential Benefits of Fasting

Traditional dietary weight management strategies typically involve making small daily reductions in energy intake (*i.e.,* continuous energy restriction), and have shown success in reducing body weight and improving markers of metabolic health (Most et al., 2017). Despite short-term success, these traditional interventions are often confounded by poor long-term adherence and weight maintenance (Dansinger et al., 2005; Maclean et al., 2015). Interestingly, issues relating to adherence have been attributed, in part, to difficulties associated with limiting energy intake across the entire day or at specific mealtimes, which may be more challenging than abstaining from food intake completely (Templeman et al. 2020). The absence of satiety after eating smaller meals (Alajmi et al., 2016), and difficulties with counting calories (Das et al., 2007), may be considerable barriers to traditional energy restriction for some individuals, meaning dietary interventions requiring complete abstinence from energy intake may offer a practical solution (Johnstone, 2015; Parr et al., 2020*a*).

Intermittent fasting is an umbrella term for dietary strategies requiring the complete abstinence from consuming energy-containing foods and beverages for a defined period of time (Anton et al., 2018; Patterson & Sears, 2017; Templeman et al., 2020). Several iterations of intermittent fasting exist, including alternate-day fasting, modified alternate-day fasting, time-restricted eating, and 5:2 (Anton et al., 2018; Patterson & Sears, 2017; Templeman et al., 2020). Although findings from randomised, controlled trials have shown comparable benefits from intermittent fasting and continuous energy restriction for managing weight and enhancing metabolic health (Barnosky et al., 2014; Cioffi et al., 2018; Rynders et al., 2019), most long-term studies >8 weeks have examined versions of intermittent fasting which allow for small energy intakes (400–600 kcal) to be consumed within 'fasting windows', such as 5:2 and alternate-day modified fasting (Carter et al., 2018; Trepanowski et al., 2017). Whilst this is unlikely to have a meaningful effect on energy balance, terminating the fasting period might impede potential fasting-specific benefits which extend beyond simple energy restriction (Anton et al., 2018). For example, fasting daily for 16 h induced several health benefits in rodents, including improved insulin sensitivity, reduced hyperlipidaemia, reduced inflammation, and reduced body weight, without the need to restrict energy intake (Hatori et al., 2012). As such, interventions which impose a fasting duration long enough to elicit a postprandial metabolic state may have independent benefits.

Complete alternate-day fasting is one example of such an intervention and involves total abstinence from energy intake on fasting days, thus initiating the elevated mobilisation and oxidation of endogenous lipid-derived substrates characteristic of the postprandial state (Maughan et al., 2010; Templeman et al., 2020). In perhaps the most comprehensive study to date, Templeman et al. (2021*b*) aimed to isolate the independent and combined effects of energy restriction and fasting *per se* on measures of body composition, energy balance, and metabolic health by utilising an alternate-day fasting protocol. In this study, subjects were randomised to one of three groups for 20 days: 1) prescribed daily energy restriction by consuming 75% habitual energy intake every day; 2) a matched degree of energy restriction but with alternating 24 h periods of complete fasting and consuming 150% habitual energy intake; and 3) intermittent fasting without energy restriction by alternating between 24 h periods of fasting and consuming 200% habitual energy intake. Traditional daily energy restriction reduced body mass (-1.91 kg), which was attributed almost entirely to reductions in fat mass (-1.75 kg). Alternate-day fasting with calorie restriction also reduced body mass (-1.60 kg), but reductions in fat mass were attenuated (-0.74 kg), suggesting a greater loss of fat-free mass. Fasting without energy restriction did not significantly reduce body mass (-0.52 kg) or fat mass (-0.12 kg). The greater loss of fat-free mass following the combined alternate-day fasting and calorie restriction intervention may have, at least partly, been mediated by the observed compensatory reductions in spontaneous light- and moderate-intensity physical activity, which were not observed in the other two groups. Reduced physical activity levels in response to extended periods of fasting have also been shown previously (Betts et al., 2014; Chowdhury et al., 2016*b*). Postprandial markers of cardiometabolic health, gut hormones, and adipose tissue gene expression did not respond differently between the interventions. Therefore, this recent study suggests that alternate-day fasting leads to less favourable changes in body composition compared to daily energy restriction and does not elicit fasting-specific effects on metabolic regulation or cardiovascular heath (Templeman et al., 2021*b*).

Extending the naturally occurring overnight fast by skipping breakfast is another method of achieving a postprandial metabolic state, and has received considerable research attention (Betts et al., 2016; Clayton & James, 2016). Skipping breakfast is generally advocated against, partly due to the theory that it will increase appetite and lead to subsequent overconsumption of energy later in the day (Garaulet & Gómez-Abellán, 2014). In line with this theory, acute, laboratory-controlled studies have shown that skipping breakfast elevates appetite during the morning, compared to when breakfast is consumed (Astbury et al., 2011; Chowdhury et al.,

2015; Chowdhury et al., 2016*a*; Clayton et al., 2015; Clayton et al, 2016*a*; Gonzalez et al., 2013; Levitsky & Pacanowski, 2013). Breakfast omission also results in changes in the circulating profile of appetite-regulatory hormones which correspond with these differences in subjective appetite. Specifically, breakfast omission increases circulating concentrations of the appetite-stimulating hormone ghrelin, alongside concomitant reductions in concentrations of appetite-suppressing hormones such as PYY and GLP-1 (Chowdhury et al., 2015; Chowdhury et al., 2016*a*). As such, breakfast omission typically results in increased energy intake at the first meal after breakfast (*i.e.,* lunch) (Astbury et al., 2011; Chowdhury et al., 2015; Clayton et al., 2015; Levitsky & Pacanowski, 2013).

Interestingly, with the exception of one study (Astbury et al., 2011), the increase in lunch energy intake rarely fully compensates for the energy omitted at breakfast. Breakfast omission, therefore, generally reduces daily energy intake compared to breakfast consumption (Chowdhury et al., 2015; Chowdhury et al., 2016*a* Clayton et al., 2015; Gonzalez et al., 2013; Levitsky & Pacanowski, 2013). Whilst it is possible that further energy intake compensation may occur beyond lunch, the differences in subjective appetite and/or appetite regulatory hormones which are apparent during the morning appear to be abolished upon consumption of lunch (Astbury et al., 2011; Chowdhury et al., 2015; Chowdhury et al., 2016*a*; Clayton et al., 2015; Clayton et al, 2016*a*; Levitsky & Pacanowski, 2013). Accordingly, studies examining energy intake at subsequent meals have revealed no such difference after breakfast omission, compared to consumption (Clayton et al., 2015; Levitsky & Pacanowski, 2013). Collectively, these studies suggest that the effects of breakfast omission on energy intake are largely constrained to lunch.

Despite breakfast omission acutely reducing energy intake, longer-term studies implementing breakfast omission for periods ranging from 6–16 weeks have failed to report superior improvements in body mass or composition in both lean adults (Betts et al., 2014; Tinsley et al., 2019), and adults living with obesity (Chowdhury et al., 2016*b*; Schlundt et al., 1992). These findings may be partly due to compensatory alterations in components of energy balance which serve to undo the energy deficit created by skipping breakfast. For example, compared to the acute response, the energy intake responses to chronic breakfast omission are considerably more variable. Multiple exposures to breakfast omission over periods ranging from 1–6 weeks have been shown to increase (Farshchi et al., 2005*a*), decrease (Betts et al., 2014; Reeves et al., 2014), and not effect (Chowdhury et al., 2016*b*; Halsey et al., 2012) daily energy intake, compared to breakfast consumption. Aside from energy intake, breakfast

omission has also been shown to reduce physical activity energy expenditure when implemented over 6 weeks in both lean adults (Betts et al., 2014) and adults living with obesity (Chowdhury et al., 2016*b*). These findings are in line with those of Templeman et al. (2021*b*), who showed reduced physical activity energy expenditure in lean adults undergoing 20 days of alternate-day fasting.

Research has also explored the effects of chronic breakfast omission or consumption on metabolic health outcomes. Under isocaloric conditions, 8 weeks of extended morning fasting (meals consumed at 13:00, 16:00 and 20:00) reduced fasting glucose and insulin concentrations compared to a control trial (meals consumed at 08:00, 13:00 and 20:00) (Moro et al., 2016). In contrast, Farshchi et al. (2005*a*) showed that fasting until 11:00 for 2 weeks reduced insulin sensitivity in response to a test drink, compared to an isocaloric trial where the first meal was consumed at 08:00. Additionally, Betts et al. (2014) showed that 6 weeks of skipping breakfast under free-living conditions in lean adults resulted in higher glucose variability in the evening compared to daily breakfast consumption. Data from the same research group also suggests that 6 weeks of breakfast omission may impair insulin sensitivity in adults with obesity (Chowdhury et al., 2016*b*). Other than some evidence of altered adipose tissue gene expression in response to 6 weeks of daily breakfast skipping in lean adults (Gonzalez et al., 2018), there appears to be little effect of chronic breakfast omission on markers of metabolic adaptation (Chowdhury et al., 2018; Chowdhury et al., 2019).

Taken collectively, the studies discussed above suggest that, at least acutely, extending the overnight fast by skipping breakfast may reduce daily energy intake, but the long-term effects on weight management outcomes may be impaired by compensatory elevations in appetite and energy intake and reductions in physical activity energy expenditure. To date, there is also little evidence of a causal link between chronic breakfast omission and improvements in metabolic health outcomes. Because exploiting the naturally occurring overnight fast may be a particularly feasible method of achieving an extended fasting period sufficient to elicit a postabsorptive state, strategies designed alleviate these compensatory responses could be efficacious.

2.4 Fasting and Exercise

Exercise is a well-established strategy with clear benefits for weight and health management, and as such, is frequently ingrained within public health guidelines (National Health Service, 2021). Through increasing energy expenditure, exercise interventions can successfully aid weight management (Donnelly et al., 2009; Volek et al., 2005). Exercise also improves insulin sensitivity and metabolic health via a plethora of acute physiological responses and chronic adaptations which are reviewed extensively elsewhere (see: Bird & Hawley, 2017; Bourghouts & Kezier, 2000; Sylow & Richter, 2019). Despite this, long-term exercise interventions often result in disappointing weight management outcomes compared to predictions (Church et al., 2009; Martin et al., 2019), which may be partly attributed to compensatory reductions in non-exercise energy expenditure and/or increases in energy intake (Donnelly et al., 2009; King et al., 2008; Thompson et al., 2014). Additionally, there is some evidence to suggest that there exists some inter-individual variability in metabolic responses to exercise (de Lannoy et al., 2017).

Whilst government guidelines provide advice with regards to the duration, intensity, and type of exercise that individuals should engage in (National Health Service, 2021), they neglect recommendations relating to the timing of exercise. Furthermore, studies implementing supervised exercise interventions also rarely consider the timing of exercise as an important factor. Interestingly, there is now emerging evidence that the timing of exercise, whether that be the timing around meals (*i.e.*, fed or fasted exercise), or the time of day, can modulate adherence to (Schumacher et al., 2019; Schumacher et al., 2020) and the outcomes of (Blankenship et al., 2021; Edinburgh et al., 2022; Fillon et al., 2020; Heden & Kanaley, 2019; Mancilla et al., 2020; Wallis & Gonzalez, 2019) exercise interventions. Exercise timing may, therefore, represent a variable which may be deliberately manipulated to optimise the responses to exercise. In the following sections, the effects of meal-exercise timing will be reviewed in relation to metabolic and energy balance responses before the effects of time-of-day are discussed in **Chapter 2.6**.

2.4.1 Substrate Utilisation

The increased demand for energy to fuel muscular contractions when shifting from rest to exercise must be met by an increase in the production of ATP (Spriet, 2014). Carbohydrate and fatty acids are the dominant fuel sources metabolised by the skeletal muscles during exercise, and although amino acids can be oxidised, their contribution to energy production is generally minimal, even in conditions of prolonged exercise and fasting (Jeukendrup & Wallis, 2005; Wagenmakers, 1989).

During exercise, there is an increased uptake of circulating glucose into the skeletal muscles, which is matched by increased hepatic glucose production (Ahlborg et al., 1974). Glucose production by the liver is sufficient to maintain plasma glucose concentrations during the early stages of aerobic exercise, but when exercise is prolonged (>60–90 min), peripheral glucose uptake supersedes hepatic output, and blood glucose concentrations begin to decline (Ahlborg et al., 1974), unless exogenous carbohydrate is consumed (Coyle et al., 1983). In the absence of exogenous carbohydrate provision, blood glucose concentrations are maintained by hepatic gluconeogenesis – the production of glucose from primarily lactate, alanine, and glycerol (Frayn, 2010). Circulating glucose enters the muscle via the GLUT4 transport proteins and is oxidised alongside muscle glycogen. As discussed in **Chapter 2.1**, muscle glycogen stores are considerably larger than hepatic stores, ranging from ~350–700 g (Knuiman et al., 2015), and are readily available for oxidation without the transport requirements of hepatically-derived glucose. The utilisation of muscle glycogen increases with exercise intensity, and accounts for the largest proportion of energy contribution during exercise greater than ~65% $\dot{V}O_{2max}$ (Romjin et al., 1993; van Loon et al., 2001).

Following the onset of exercise, concentrations of catecholamines are increased and insulin reduced. These conditions subsequently stimulate adipose tissue lipolysis (Enevoldsen et al., 2004). As such, exercise increases fatty acid availability within the circulation (Romjin et al., 1993), which is further exacerbated by a concomitant reduction in fatty acid re-esterification within adipose tissue (Wolfe et al., 1990). The increase in circulating fatty acid concentrations, plus increased transport of fatty acids away from adipose tissue towards skeletal muscle, is responsible for the increase in fat oxidation typically seen when shifting from rest to moderate-intensity exercise (Achten & Jeukendrup, 2004). Fatty acids stored within the skeletal muscle in the form of IMTG are also oxidised during exercise (van Loon, 2004). IMTG utilisation is typically greatest in the early stages of exercise due to their close proximity to the mitochondria,

allowing for immediate oxidation before adipose tissue-derived fatty acids are mobilised and delivered (Romjin et al., 1993; van Loon, 2004). The utilisation of IMTG as a substrate appears to be more pronounced in individuals with an increased training status (Gemmink et al., 2020).

The oxidation of carbohydrate and fat contribute simultaneously to energy production during exercise; however, their relative contributions vary in response to a number of factors such as diet, exercise intensity and duration, environmental conditions, biological sex, and training status (Hargreaves & Spriet, 2020). The pre-exercise nutritional state can also profoundly influence the contributions of carbohydrate and fat to energy production (Vieira et al., 2016). Exercising in the fasted state is characterised by elevated rates of fat oxidation and reduced rates of carbohydrate oxidation compared to exercise performed after consuming a carbohydrate-containing meal (Bergman & Brooks, 1999; Coyle et al., 1985; Coyle et al., 1997; Edinburgh et al., 2018; Enevoldsen et al., 2004; Farah & Gill, 2013; Gonzalez et al., 2013; Horowitz et al., 1997). As discussed in **Chapter 2.1**, the fasted state is characterised by decreased hepatic glycogen availability and a lipolytic hormonal environment including reduced plasma insulin concentrations and elevated catecholamine concentrations (Duncan et al., 2007). In this state, fatty acids and glycerol are mobilised from adipose tissue (Duncan et al., 2007), leading to the preferential oxidation of fat as a substrate during fasted exercise (Coyle et al., 1997; Vieira et al., 2016). In addition to increased oxidation of circulating free-fatty acids, previous research has shown that increased fat oxidation during overnight-fasted exercise is supported by type 1 skeletal muscle fibre IMTG utilisation in lean individuals (Coyle et al., 1997; De Bock et al., 2005). The ingestion of a carbohydrate-containing meal before exercise ultimately leads to elevated plasma glucose and insulin concentrations which can inhibit lipolysis (Spriet, 2014) and enhance fatty acid re-esterification in adipose tissue (Enevoldsen et al., 2004; Frayn et al., 1995), reducing the availability of circulating fatty acids as a fuel source for the muscles (Horowitz et al., 1997). Furthermore, elevated insulin concentrations inhibit the transport of long-chain fatty acids into the mitochondria for β-oxidation (Coyle et al., 1997; Sidossis et al., 1996).

Whilst pre-exercise meal consumption clearly reduces fat oxidation during exercise, the time interval between carbohydrate ingestion and exercise may influence this response. Montain et al. (1991) provided a high-carbohydrate meal (2 g·kg body mass^{-1} carbohydrate) to a sample of trained and untrained men who, after an interval of 2, 4, 6, 8, and 12 h, cycled for 30 min at 70% $\dot{V}O_{2max}$. Carbohydrate oxidation was increased during exercise performed 2 and 4 h after the meal, compared to when the meal-exercise interval was extended to 6, 8 and 12 h.

Interestingly, despite glycerol concentrations increasing linearly with increasing fasting duration (indicating increased lipolysis), carbohydrate oxidation rates were not different during exercise performed 6, 8, or 12 h following the meal, suggesting that the effects of pre-exercise carbohydrate consumption on substrate utilisation last up to 6 h. In line with these findings, Maffucci & McMurray (2000) showed that, in a sample of active women, 30 min of steady-state running followed by an incremental test to exhaustion resulted in a lower RER when performed 6 h, compared to 3 h after consuming a carbohydrate-containing meal (~9.6 kcal·kg body mass^{-1}, 55% carbohydrate). Finally, Dumortier et al. (2005) observed higher fat oxidation rates in postmenopausal women with overweight and obesity during exercise performed 3 h, compared to 1 h after a meal containing 65 g carbohydrate. It can be concluded that the length of the pre-exercise fast profoundly influences metabolism, and that consumption of a carbohydrate-containing meal within the prior ~6 h suppresses fat oxidation and elevates carbohydrate oxidation during exercise.

In addition to diet, the intensity and duration of exercise are major factors that determine substrate oxidation during exercise. The influence of exercise intensity on the relative contribution of carbohydrate and fat to energy expenditure is described neatly within the 'crossover' concept (Brooks & Mercier, 1994). Briefly, this concept describes a gradual decline in the relative contribution of fat to energy expenditure as exercise intensity increases from rest. At the same time, the relative contribution of carbohydrate follows an inverse pattern until the crossover point is reached, which describes the exercise intensity at which carbohydrate becomes the dominant substrate contributing to energy expenditure. Further increases in exercise intensity continue to increase the contribution of carbohydrate and reduce the contribution of fat (Brooks & Mercier, 1994). In absolute terms, the rate of fat oxidation during exercise follows an 'inverted U' pattern. Fat oxidation rates rise progressively from rest until they peak at an exercise intensity of ~65% $\dot{V}O_{2max}$, which has been termed Fat_{max} (Achten et al., 2002; Venables et al., 2005). Further increases in exercise intensity beyond Fat_{max} reduce fat oxidation rates and exercising at intensities ~90% $\dot{V}O_{2max}$ results in negligible contributions of fat to energy expenditure (Achten et al., 2002; Venables et al., 2005). In contrast, carbohydrate oxidation rates increase progressively with exercise intensity from rest to maximal exercise, becoming the dominant substrate at approximately 50–60% $\dot{V}O_{2max}$ (Romjin et al., 1993; van Loon et al., 2001). In accordance with this, the differences in substrate oxidation between fed and fasted exercise described above are less apparent when exercise intensity is increased, and carbohydrate oxidation dominates (Bergman & Brooks, 1999).

Regarding exercise duration, when exercise intensity is held constant, there is a gradual decline in carbohydrate oxidation and concomitant rise in fat oxidation as exercise duration progresses (Ahlborg et al., 1974; Romjin et al., 1993), possibly due to a progressive reduction in muscle glycogen concentrations during prolonged exercise (Coyle et al., 1986).

To summarise, elevated energy demands during exercise are met by the simultaneous oxidation of carbohydrate and fat which resynthesise ATP and allow muscular contractions to occur. The relative contribution of carbohydrate and fat to energy expenditure can be profoundly altered by the consumption of a pre-exercise meal. Specifically, exercising after a period of fasting favours fat oxidation, whereas exercise performed in closer proximity to a carbohydrate-containing meal favours carbohydrate oxidation.

2.4.2 Appetite and Energy Balance

The effects of acute exercise on subjective appetite, appetite regulatory hormones, and subsequent energy intake have been reviewed extensively in several articles (Deighton & Stensel, 2014; King et al., 2013*a*; Schubert et al., 2013; Schubert et al., 2014). A brief summary of this literature is provided below for context, before a more thorough discussion of the modulating role of fasting.

Exercise directly influences energy balance by increasing energy expenditure, but it may also modulate appetite and energy intake (Hubert et al., 1998). Historically, a widely held belief was that the energy expended during exercise leads to a compensatory increase in hunger and energy intake to eradicate the exercise-induced energy deficit (Blundell & King, 1998). Despite this, mounting evidence has shown that a single bout of aerobic exercise (>60% $\dot{V}O_{2peak}$) suppresses appetite during and immediately after the bout in a process known as 'exercise-induced anorexia' (King et al., 1994). Appetite typically returns to control values within 30–60 min of the cessation of running, cycling, and swimming exercise (Deighton & Stensel, 2014), with similar post-exercise appetite responses being reported in males and females (Alajmi et al., 2016; Hagobian et al., 2013; Hazel et al., 2017; Panissa et al., 2016). Because of this, exercise does not typically alter subsequent energy intake on the day that it is performed (Deighton & Stensel, 2014), but it may delay the onset of eating following exercise (King et al., 2013*b*). It should be acknowledged that findings are not unequivocal, as some studies have shown both an acute increase (Laan et al. 2010; Martins et al. 2007) and decrease (Ueda et al. 2009; Westerterp-Plantenga et al. 1997) in post-exercise energy intake. Nevertheless, it has

been suggested that exercise remains an effective method of inducing a short-term energy deficit (Schubert et al., 2013). Most research has measured appetite and energy intake within close proximity to the termination of exercise. Whilst energy intake compensation may not occur immediately after exercise, it is possible that some compensation may occur in the hours and days succeeding exercise (Rocha et al., 2013). Regarding appetite regulatory hormones, research has typically focussed on the orexigenic hormone acylated ghrelin, and anorexigenic hormones PYY, GLP-1, and PP (Schubert et al., 2014). Aerobic exercise performed ≥60% $\dot{V}O_{2peak}$ suppresses circulating concentrations of acylated ghrelin, and concomitantly increases circulating concentrations of PYY, GLP-1, and PP (Schubert et al., 2014), but as with subjective appetite, changes in appetite regulatory hormones are typically short-lived (Broom et al., 2007; King et al., 2010; Martins et al., 2007).

Studies have also compared the appetite and energy intake responses to fed and fasted exercise as a means of optimising the effectiveness of exercise as a weight management strategy. These studies typically explore responses to exercise by either manipulating meal-exercise sequencing (*i.e.*, consuming a single meal before or after exercise) (Borer et al., 2005; Broad et al., 2020; Cheng et al., 2009; Deighton et al., 2012; Farah & Gill, 2013), or by omitting the pre-exercise meal completely (Bachman et al., 2016; Edinburgh et al., 2019; Gonzalez et al., 2013; Griffiths et al., 2020; McIver et al., 2019*a*, McIver et al., 2019*b*; Rothschild et al., 2021; Veasey et al., 2015). Studies which compare fed and fasted exercise using the meal omission design are limited by the fact that experimental conditions are not energy matched, making it difficult to establish whether the outcome has been influenced by fasted exercise *per se*, or just the omission of a standardised meal. However, the energy-matched, meal-exercise sequence study design is limited by differences in the timing of the standardised meal relative to the appetite and energy intake assessments.

Meal-Exercise Sequence Studies

In a sample of post-menopausal women, reductions in hunger, capacity to eat, and desire to eat following breakfast consumption were attenuated when exercise was performed in the fasted state prior to the meal, compared to 1 h after the meal (Borer et al., 2005). These findings suggest that the appetite suppressing effects of a meal may be extended when exercise is performed in the postprandial period. Using a similar approach, Cheng et al. (2009) examined appetite responses over 7 h following a single, high-fat breakfast which was consumed either 2 h before, or immediately after, exercise (cycling for 50 min at 60% $\dot{V}O_{2max}$). Despite exercise

in the 12 h overnight-fasted state suppressing pre-breakfast hunger, there was a greater and more prolonged suppression of post-breakfast hunger when exercise was performed 2 h after the meal. This extended postprandial suppression of hunger was associated with a prolonged elevation of plasma PYY concentrations following fed-state exercise.

Three studies have been conducted to elucidate whether the differences in acute appetite responses to fed and fasted exercise materialise as a change in subsequent energy intake (Broad et al., 2020; Deighton et al., 2012; Farah & Gill, 2013). In the study by Deighton et al. (2012), healthy males performed 60 min running at 70% $\dot{V}O_{2max}$ either before breakfast following an overnight fast, or 2.5 h after breakfast. In accordance with findings from Borer et al. (2005) and Cheng et al. (2009), fed exercise resulted in a greater and more prolonged suppression of appetite following breakfast. However, this did not result in any differences in energy intake between trials at *ad-libitum* lunch and dinner meals, or during the overnight period. Farah & Gill (2013) also reported no differences in lunch energy intake following aerobic exercise performed before or after breakfast. Similar responses have also been shown in response to high-intensity, sprint interval training (Broad et al., 2020). In this study, sprint interval exercise performed 1 h after a meal reduced appetite sensations compared to the same exercise performed after an overnight fast, but again, subsequent energy intake was not different between trials. Therefore, despite apparent differences in appetite following a meal that is consumed either before or after exercise, these do not appear to translate to differences in subsequent energy intake. This ultimately suggests that regardless of nutrient-exercise timing, exercise is likely to reduce relative energy intake compared to a non-exercising control.

Pre-Exercise Meal Omission Studies

As well as manipulating the meal-exercise sequence (exercise before versus after breakfast), studies have examined appetite and energy intake following fasted exercise by omitting the breakfast meal completely. From an energy balance perspective, this approach has the potential to be particularly effective, given that in the absence of exercise, breakfast omission is unlikely to result in complete energy compensation at later meals (Chowdhury et al., 2015, Chowdhury et al., 2016*a*; Clayton et al., 2015; Levitsky & Pacanowski, 2013). Therefore, the addition of exercise to this paradigm may further protect against energy compensation following meal omission by increasing the magnitude of the energy deficit.

This was first explored in a study by Gonzalez et al. (2013), in which healthy males completed 1 h of treadmill running at ~60% $\dot{V}O_{2peak}$ in the overnight-fasted state or 2 h after breakfast.

Unlike the meal-exercise sequence studies, breakfast was not provided after exercise, meaning energy provision was less in the fasted trial. *Ad-libitum* energy intake measured 90 min after exercise was similar between trials, resulting in an overall reduction in energy intake and a more negative energy balance when exercise was performed after an overnight fast compared to after breakfast. Griffiths et al. (2020) also showed similar lunch energy intakes following 1 h treadmill walking performed 1 h after breakfast and following a >12 h overnight fast, despite lower appetite ratings in the fed exercise trial. In this study, acylated ghrelin concentrations were suppressed when breakfast was consumed before exercise, but no differences in acylated ghrelin concentrations remained between trials following exercise. These findings are corroborated by McIver et al. (2019*a*) who showed no differences in acylated ghrelin or PYY concentrations following a bout of fed or fasted walking. It is possible that the low physiological demand of walking exercise in these studies is partly responsible for the lack of difference in appetite regulatory hormones following fed and fasted exercise. For example, previous studies detecting differences in post-exercise appetite-regulatory hormone profiles typically use intensities ≥60% $\dot{V}O_{2peak}$ (Schubert et al., 2014).

The effects of overnight-fasted exercise on energy intake have also been examined beyond a single meal over the course of a day (Bachman et al., 2016; McIver et al., 2019*b*). In the study by Bachman and Colleagues (2016), healthy, active males ran for 1 h at 60% $\dot{V}O_{2max}$ in the overnight-fasted state or 2 h after breakfast. As well as assessing energy intake at lunch, subjects were provided with take-home food packages which could be consumed *ad-libitum* over the remainder of the day. Despite fasted exercise elevating hunger after exercise and before lunch, there were no differences in lunch energy intake between trials, meaning energy intake over the entire day was lower following fasted exercise. Interestingly, reduced energy intake over the day was not solely a product of omitting breakfast, but also because energy intake was greater later in the day following fed-state exercise, suggesting a prolonged effect of fasted exercise on energy intake. In the only study to examine the responses to fasted exercise at a time of day other than the morning, McIver et al. (2019*b*) showed similar 24 h energy intakes following fed and fasted walking which commenced at either ~09:00 or ~16:00, despite fasted exercise increasing subjective appetite at both times of day.

The studies discussed above focussed exclusively on energy intake following fasted exercise. Edinburgh et al. (2019) examined the effect of 1 h cycling in the overnight-fasted versus fed-state on subsequent 24 h energy expenditure via heart rate accelerometer, in addition to energy intake. Despite a slightly greater lunch intake following fasted exercise compared to fed

exercise, this was not enough to fully compensate for the energy omitted at breakfast. Additionally, compared to a non-exercising control group, energy intake was increased later in the day following fed exercise only, providing support to the concept of fasted exercise providing an extended effect on energy intake initially reported by Bachman et al. (2016). The novel finding of this study was that free-living energy expenditure was not different following fed or fasted exercise or in the non-exercising control trial, meaning that fasted exercise resulted in a lower 24 h energy balance compared to the other trials.

The finding that fasted morning exercise reduces evening energy intake reported by both Bachman et al. (2016) and Edinburgh et al. (2019) has been linked with the shift in substrate oxidation towards fat during fasted exercise (Gonzalez et al., 2019; Hopkins et al., 2019). Because humans possess a finite capacity to store carbohydrate, it has been suggested that depleted endogenous carbohydrate stores following exercise drive appetite and energy intake as a means of restoring carbohydrate balance (Flatt et al., 1996; Flatt et al., 2001). Therefore, the reduced reliance on carbohydrate for oxidation during fasted exercise may preserve endogenous stores and subsequently negate compensatory elevations in appetite and energy intake. In accordance with this 'glycogenostatic theory' of energy intake compensation, individuals demonstrating higher rates of carbohydrate oxidation at rest and during exercise appear to have higher *ad-libitum* energy intakes (Almeras et al., 1995; Burton et al., 2010; Hopkins et al., 2014; Pannacciulli et al., 2007; Snitker et al., 1997). Advances in non-invasive research techniques have allowed for the tissue-specific (liver and muscle) relationship between carbohydrate utilisation and energy intake to be examined (Edinburgh et al., 2019). By infusing 6, 6-$^{2}H_2$ glucose, Edinburgh et al. (2019) showed that post-exercise compensatory energy intake was correlated with hepatic glycogen utilisation, but not muscle glycogen utilisation, whole-body fat utilisation, or energy expenditure. These findings have led to the hypothesis that hepatic carbohydrate availability specifically may contribute to appetite control (Gonzalez et al., 2019; Hopkins, 2019), and may be a mechanism through which fasted exercise could aid in the creation of an energy deficit.

In summary, the satiating effects of a meal may be extended by performing exercise after, compared to before, but this is unlikely to affect subsequent energy intake. However, the complete omission of a meal before exercise is not fully compensated for over the subsequent 24 h by either increased energy intake or reduced energy expenditure, ultimately producing a more negative energy balance when the pre-exercise meal is skipped, compared to consumed. Most research on fasted exercise has been undertaken in the morning (other than McIver et al.,

2019*b*), likely because the overnight fast offers a practical and convenient opportunity to achieve a fasted state without the need to skip meals. As such, the response to fasted exercise at other times of day is not well researched and it is possible that responses to exercise interventions differ based on the time of day in which exercise is performed. Additionally, these acute reductions in 24 h energy intake following fasted exercise may not necessarily translate to chronic reductions in energy balance and improved weight management. Studies which have compared fed and fasted exercise during periods ranging from 4–12 weeks are reviewed in the following section.

2.4.3 Responses to Fasted Exercise Training

Several authors have reviewed the acute postprandial glucose and insulin responses to a single meal consumed before (fed) or after (fasted) exercise. The consensus amongst this literature is that exercise performed in the postprandial period following the consumption of a meal attenuates the glycaemic response to that meal (Chacko, 2014; Chacko, 2017; Haxhi et al. 2013; Heden & Kanaley, 2019). This is likely due to the insulin- and non-insulin-dependent uptake of ingested carbohydrates into the skeletal muscle and their subsequent oxidation (Heden & Kanaley, 2019). However, it should not be assumed that these acute glycaemic responses translate to chronic changes in glycaemic control. For example, as nicely described by Edinburgh and Colleagues (2022), if an individual performed exercise three times per week and consumed three meals per day, then the glucose lowering effects of exercise would only apply to 3 of the 21 meals (14%) consumed over that week. Furthermore, although fasted exercise induces a more negative energy balance than fed exercise (**Chapter 2.4.2**), caution should be taken when extrapolating these findings to long-term changes in body weight and/or composition due to the well-known compensatory alterations in energy intake and expenditure which often attenuate exercise-induced weight management efforts (King et al., 2008; Thompson et al., 2014). Therefore, it is important to discuss the chronic effects of fasted, compared to fed, exercise training on long-term glycaemic control and body weight/composition outcomes.

In a seminal study, lean and healthy males consumed a hypercaloric (≥30% kcal above habitual intake), fat-rich (50% of kcal) diet over 6 weeks whilst completing endurance exercise on 4 days per week, totalling 300 min per week (Van Proeyen et al., 2010). Subjects either completed the exercise sessions after an overnight fast, or after consuming a carbohydrate-rich

breakfast (675 kcal, 70% carbohydrate, 15% fat, 15% protein) ~90 min before exercise. Subjects consumed an additional 1 g·kg body mass^{-1} carbohydrate (maltodextrin) per hour during exercise in the fed, but not fasted trial. To ensure energy balance between trials, the breakfast and maltodextrin was provided to subjects in the mid-afternoon in the fasted trial. Compared with a non-exercising control group, area under the curve (AUC) for glucose in response to a 75 g oral glucose tolerance test (OGTT) was reduced following 6 weeks of exercise training in the fasted, but not fed state. Postprandial insulin concentrations were not different between groups, meaning the Matsuda insulin sensitivity index was improved only after fasted exercise.

Fasted exercise also negated the increase in body weight that was observed in the fed trial (+1.4 ± 0.4 versus +0.7 ± 0.4 kg), despite energy intake being prescribed by the researchers (Van Proeyen et al., 2010). In two other studies from the same laboratory, lean and healthy males completed similar 6-week exercise training interventions, but alongside an isocaloric, rather than hypercaloric, diet (De Bock et al., 2008; Van Proeyen et al., 2011). In these studies, no changes in body weight from baseline were shown following either fed or fasted exercise training. Finally, healthy females following a 4-week aerobic exercise intervention (3 x 1 h sessions per week) and adhering to a hypocaloric diet (500 kcal below estimated energy requirements) experienced similar reductions in body weight and fat mass from baseline, irrespective of whether exercise sessions were performed after an overnight fast or following a 250-kcal breakfast (Schoenfeld et al., 2014). Therefore, in lean individuals, fasted exercise training may benefit glycaemic control, but most of the evidence suggests no advantage of fasted exercise over fed exercise for changes in body weight or composition.

Because feeding and fasting can induce divergent physiological responses in lean individuals and individuals living with obesity (Gonzalez et al., 2018), the effects of fasted exercise training have also been examined in individuals classified as overweight or obese (Edinburgh et al., 2020). For 6 weeks, aerobic cycling exercise was performed 3 times per week either 2 h before, or 2 h after the provision of a high carbohydrate breakfast (1.3 g·kg body mass^{-1} carbohydrate). Other than the timing of the standardised breakfast on exercise days, no other dietary restrictions were imposed, and subjects were permitted to eat freely for the duration of the intervention. Body weight and waist-to-hip ratio did not change from baseline following either exercise intervention. Whilst the postprandial plasma glucose response to an OGTT was also not different to baseline following either trial, plasma insulin concentrations were reduced following fasted, but not fed exercise training. As such, increases in oral glucose insulin

sensitivity were observed only after fasted exercise training, and these improvements were correlated with increased whole-body lipid oxidation during exercise. It is important to highlight that in this study, carbohydrate was not consumed during exercise, as was done in the study in lean males (Van Proeyen et al., 2010). Therefore, simply consuming a high-carbohydrate meal before exercise sessions may impede exercise training-induced metabolic health improvements in individuals with overweight or obesity.

In females with overweight or obesity, 6 weeks of high-intensity interval training failed to induce any changes in postprandial glucose and insulin concentrations in response to an OGTT (Gillen et al., 2013). These responses were the same, regardless of whether exercise sessions were performed 1 h before (in the overnight fasted state), or 1 h after a carbohydrate-rich breakfast (439 kcal, 74% carbohydrate, 12% protein, and 14% fat). Differences in substrate oxidation during high-intensity exercise are less pronounced, with carbohydrate acting as the primary fuel source regardless of whether exercise is performed fed or fasted (Bergman & Brooks, 1999). As discussed subsequently, increased fat oxidation may be an important mediator of the superior improvements in insulin sensitivity following fasted exercise, so the high intensity of exercise sessions may help to explain the lack of difference between fed and fasted exercise in the Gillen et al. (2013) study. Exercise training did not alter body weight, but did reduce whole-body fat mass, as well as increase fat-free mass in the leg region. These changes in body composition were similar following both fed and fasted exercise training.

The final two studies of this discussion compared responses to fed and fasted exercise training in individuals living with type 2 diabetes. In the first of these, Brinkmann et al. (2019) showed similar reductions in glycated haemoglobin (HbA1c) and serum insulin concentrations following an 8-week combined endurance/strength training program (3 sessions per week), regardless of whether exercise was performed before or after breakfast. Body fat mass (-1.9 kg) and fat-free mass (+1.7 kg) were similarly altered following both fed and fasted exercise training. In the second study, a 12-week endurance exercise intervention (3 sessions per week) significantly reduced whole-body fat mass (-1.6 kg) and HbA1c concentrations, but the improvements in HbA1c were enhanced when exercise sessions were performed after, rather than before breakfast (Verboven et al. 2020). It should be noted that HbA1c is not necessarily a measure of glycaemic control, but an estimate of blood glucose concentrations over an extended, 2–3-month period (Bennet et al., 2007). Given that these studies employed parallel-groups designs, and pre-exercise breakfast meals were self-selected by subjects to increase ecological validity, it is possible that the HbA1c results reflected differences in the glycaemic

load of the meals selected by the subjects, rather than glycaemic control *per se.* In support of this, the self-selected breakfast in the fed exercise trial (375 ± 72 kcal, 60 ± 4% carbohydrate) was smaller than in the fasted exercise trial (479 ± 73 kcal, 58 ± 4% carbohydrate), possibly contributing to the greater reductions in HbA1c (Verboven et al., 2020).

In summary, aerobic exercise performed in the overnight fasted state may enhance metabolic adaptations in individuals classified as lean, overweight, or obese. These findings, however, do not appear to translate to individuals diagnosed with type 2 diabetes, for whom there is no clear evidence supporting an enhanced efficacy of fasted exercise for improving metabolic health. Although few studies have set out to address this question, evidence in both metabolically healthy individuals and individuals with type 2 diabetes does not appear to support any preferential effects of either fed or fasted exercise for training-induced changes in body mass or composition. However, several of these studies prescribed a diet with a fixed energy intake (De Bock et al., 2008; Schoenfeld et al., 2014; Van Proeyen et al., 2010; Van Proeyen et al., 2011), which do not allow for any dietary compensation to occur, potentially masking any differential changes in body weight or composition that may occur outside of dietary control.

2.4.4 Mechanisms for Improved Glycaemic Control with Fasted Exercise Training

Although the exact mechanisms mediating improvements in insulin sensitivity following fasted exercise training are unknown, several candidate mechanisms have been proposed and will be discussed in the following section.

Firstly, exercising in a fasted state upregulates fat oxidation and concomitantly reduces carbohydrate oxidation (see **Chapter 2.4.1**). To facilitate increased rates of fat oxidation, plasma concentrations of free fatty acids and glycerol are elevated as a result of increased adipose-tissue lipolysis (Enevoldsen et al., 2004). Elevated concentrations of plasma free fatty acids can act as ligands for peroxisome proliferator-activated receptor (PPARs), which are key regulators of fatty acid oxidation (Wang, 2010). Elevating plasma free fatty acid concentrations through dietary interventions (Garcia-Roves et al., 2007) and exercising with low carbohydrate availability (Philp et al., 2013) has been successful in raising PPAR activity in rodent models. Importantly, PPARs can upregulate the expression of proteins involved in fat metabolism such as carnitine palmitoyltransferase (CPT-1) (Dressel et al., 2003; Garcia-Roves et al., 2007). An increased capacity to oxidise endogenous fat stores during exercise could be beneficial for adaptations relating to insulin sensitivity (Gemmink et al., 2020). For example, observational

evidence appears to link a reduced capacity to oxidise fat with the development of insulin resistance (Blaak et al., 2000; Kelley & Simoneau, 1994; Kelley et al., 1999), and a high resting RER (indicative of low rates of fat oxidation) is linked to increased metabolic risk (Rosenkilde et al., 2010). Even in lean and healthy males, an increased capacity to oxidise fat during exercise has been associated with elevated 24 h fat oxidation and improved markers of metabolic health (Robinson et al., 2015*a*). Accordingly, exercise interventions targeted at increasing fat oxidative capacity have shown success in improving insulin sensitivity in individuals with overweight/obesity (Edinburgh et al., 2020; Goodpaster et al., 2003; Venables & Jeukendrup, 2008). From the opposite perspective, pharmacologically inhibiting fat oxidation during exercise has been shown to blunt skeletal muscle signalling fundamental to exercise adaptation (Constantin-Teodosiu et al., 2012; Zbinden-Foncea et al., 2013).

If an increased capacity to oxidise fat is indeed a mechanism through which fasted exercise exerts its beneficial effects on insulin sensitivity, then this may help to explain the null findings in individuals with type 2 diabetes (Brinkmann et al., 2019; Verboven et al., 2020). Individuals with type 2 diabetes have impaired metabolic flexibility, meaning they have a reduced capacity to switch between oxidising carbohydrate and fat (Goodpaster & Sparks, 2017), and are, therefore, less likely to observe differences in fuel utilisation between fed and fasted exercise. Also, as was previously alluded to, this may help to explain why Gillen and Colleagues (2013) reported similar glycaemic control outcomes following 3 weeks of fed and fasted high-intensity interval training, which was likely fuelled predominantly by carbohydrate, irrespective of pre-exercise nutrition status (Berman & Brookes, 1999).

As well as the increased mobilisation and oxidation of adipose tissue-derived fatty acids, IMTG also contribute to the increased rates of fat oxidation during fasted exercise in lean individuals (De Bock et al., 2005; Van Proeyen et al., 2011) and individuals with overweight or obesity (Edinburgh et al., 2020). IMTG concentrations are elevated in insulin-resistant individuals (Ebeling et al., 1998; Pan et al., 1997), which led to the belief that IMTG promotes insulin resistance (Bergman et al., 2018). However, endurance-trained individuals have comparatively high IMTG concentrations, despite a lack of insulin resistance (Goodpaster et al., 2001; van Loon et al., 2004). It is now understood that lipid turnover and the lipid droplet characteristics are greater contributors to insulin sensitivity than total IMTG content (Gemmink et al., 2020; Zacharewicz et al., 2018). For example, exercise-induced improvements in insulin sensitivity in previously untrained, insulin-resistant individuals have been shown to correspond with reductions in lipid droplet size (He et al., 2012), reductions in subsarcolemmal lipid content,

and increases in intramyofibrillar lipid content (Nielsen et al., 2010; Samjoo et al., 2013), often independent of any changes in total IMTG content. Additionally, exercise training can reduce harmful lipid species within the muscle such as diacylglyceride and ceramide (Dubé et al., 2008; Shepherd et al., 2017), which are linked to insulin resistance (Hulver & Dohm, 2004). These changes in lipid droplet size and morphology more closely simulate the lipid droplet phenotype found in insulin-sensitive endurance athletes (Daemen et al., 2018). Therefore, it is possible that the increase in muscle-derived lipid turnover during fasted exercise may lead to greater IMTG remodelling towards this favourable phenotype (Gemmink et al., 2020; Zacharewicz et al., 2018).

Fasted exercise has the potential to further mediate exercise adaptations via AMP-activated protein kinase (AMPK) and its effects on downstream proteins. AMPK is a heterotrimetric protein consisting of three subunits (α, β, and γ) (Kjøbsted et al., 2018). During exercise, the increased turnover of ATP to fuel skeletal muscle contractions results in an elevated AMP:ATP ratio, which can stimulate AMPK via the γ subunit (Gowans & Hardie, 2014). Exercise-stimulated AMPK activity can elicit insulin-independent glucose uptake by promoting GLUT4 translocation to the cell membrane by phosphorylating downstream proteins TBC1D1 and TBC1D4 (Treebak et al., 2014). AMPK activity also regulates fatty acid oxidation, particularly in the post-exercise period, via the phosphorylation of acetyl coenzyme A carboxylase (Hardie, 1989). Recurrent exercise training and resultant stimulation of AMPK can facilitate chronic adaptations which may improve insulin sensitivity such as increased GLUT4 protein expression and mitochondrial biogenesis via the upregulation of mitochondrial transcription factors including peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) (Jørgensen et al., 2006). Such adaptations have clinical relevance for individuals with obesity and insulin resistance, who possess a low mitochondrial oxidative capacity (Kelley et al., 2002; Patti et al., 2003).

Commencing exercise in a fasted state increases AMPK activity compared to exercise performed after carbohydrate ingestion (Akerstrom et al., 2006; Civitarese et al., 2005). Due to the presence of a glycogen-binding site on the AMPK β subunit (McBride et al., 2009), the augmented AMPK response to fasted exercise may be mediated by lower muscle glycogen concentrations. Indeed, commencing exercise with lowered muscle glycogen has been shown to increase AMPK activation compared to exercise with higher muscle glycogen concentrations (Wojtaszewski et al., 2003; Yeo et al., 2010). Independent of muscle glycogen concentrations, AMPK can be activated by increased fatty acid availability (Watt et al., 2006), which activates

the β subunit and upregulates fat oxidation (Pinkosky et al., 2020). As fatty acid concentrations are elevated during fasted exercise, this may be an additional process through which AMPK is upregulated in this state.

The studies detailed earlier in this section which showed enhanced improvements in insulin sensitivity following 6 weeks of fasted exercise training in lean individuals (Van Proeyen et al., 2010) and individuals classified as overweight or obese (Edinburgh et al., 2020) also showed increases in AMPK. Van Proeyen et al. (2010) showed that fasted exercise training increased AMPK phosphorylation by 25% compared to baseline, whilst fed exercise training had no effects on AMPK phosphorylation. This was accompanied by a 28% increase in muscle GLUT4 protein content in the fasted trial only, as well as similar ~30% increases in mRNA levels of proteins involved in fatty acid transport (fatty acid translocate/cluster of differentiation 36, FAT/CD36 and CPT-1). These findings corroborate previous reports that fasted exercise training for 6–8 weeks increased the content/expression of FAT/CD36, CPT-1 (De Bock et al., 2008), and GLUT4 (Nybo et al., 2009). In individuals with overweight or obesity, fasted exercise training led to a ~3-fold increase in muscle AMPK levels, and a ~2-fold increase in muscle GLUT4 levels (Edinburgh et al., 2020), however no changes in fatty acid transport or oxidation proteins were observed in this study.

In summary, fasted exercise training may improve insulin sensitivity via the augmentation of skeletal muscle adaptations which promote an enhanced capacity to oxidise fat, increased turnover, and subsequent remodelling of intramuscular triglycerides, and/or increased GLUT4 protein levels allowing for greater glucose uptake and metabolism, potentially mediated by AMPK protein levels.

2.5 Carbohydrate Restriction and Exercise

Due to the large insulinemic response and subsequent suppression of lipolysis after consuming carbohydrate (Coyle et al., 1997), the shift from carbohydrate to fat metabolism during fasted exercise is likely a result of carbohydrate restriction, as opposed to fasting *per se.* Accordingly, the concept of restricting carbohydrate intake prior to exercise training, commonly referred to as ‘training low’, has received considerable research interest within the field of sports performance due its potential to enhance endurance training-induced metabolic adaptations (Bartlett et al., 2015; Impey et al., 2018). Dietary interventions which restrict carbohydrate before exercise may offer an alternative method of ameliorating the metabolic benefits of

exercise without the need to endure a period of fasting. To dissociate the effects of carbohydrate restriction from energy restriction, studies need to ensure that experimental trials are isocaloric. For this to occur, the reduction in energy intake via carbohydrate restriction must be compensated for by respective increases in energy intake from another macronutrient (*i.e.,* fat or protein). Therefore, carbohydrate-restricted exercise interventions must be considered in the context that more than one macronutrient has been manipulated.

2.5.1 Low-Carbohydrate, High-Fat

The acute consumption of a low-carbohydrate (<20 g), high-fat meal before exercise attenuates the postprandial rise in plasma insulin concentrations and increases fat oxidation compared to consuming a high-carbohydrate pre-exercise meal (Rowlands & Hopkins, 2002*a*). Furthermore, independent of carbohydrate, a high-fat meal increases circulating free fatty acid concentrations in the blood (Griffiths et al., 1994; Lambert & Parks, 2012; Okano et al., 1996; Whitley et al., 1998), which itself can increase fat oxidation during exercise (Hawley et al., 2000; Okano et al., 1996). Therefore, it is likely that the acutely upregulated fat oxidation rates following a low-carbohydrate, high-fat pre-exercise meal are sourced primarily from the increased availability of dietary fat, as opposed to the endogenous lipid stores (adipose tissue-derived fatty acids and IMTG), which as outlined in the previous section, may mediate some of the adaptations to fasted exercise relating to improved insulin sensitivity (Edinburgh et al., 2022; Gemmink et al., 2020; Zacharewicz et al., 2018).

Several studies have examined adaptations to consuming low-carbohydrate, high-fat diets alongside exercise training (for comprehensive reviews, see: Burke, 2015; Burke, 2021; Volek et al., 2015). Several iterations of low-carbohydrate, high-fat diets have been examined, such as ketogenic and non-ketogenic low-carbohydrate diets, and periodised carbohydrate availability (Burke et al., 2018). There is agreement amongst the literature that adaptation to a low-carbohydrate, high-fat diet elevates rates of fat oxidation during exercise (Burke, 2015; Burke et al., 2018; Burke, 2021; Volek et al., 2015). These shifts in substrate oxidation patterns have been observed in as little as 5 days of consuming a low-carbohydrate, high-fat diet (Burke et al., 2000; Burke et al., 2002; Goedeke et al., 1999) and persist even after carbohydrate restoration before exercise (Burke et al., 2000; Burke et al., 2002), indicating skeletal muscle adaptations which increase capacity for fat oxidation. Indeed, a low-carbohydrate, high-fat diet alongside exercise increases activity of skeletal muscle proteins such as HSL (Stellingwerff et

al., 2006) and CPT-1 (Goedecke et al. 1999), as well as increasing the expression of lipid transporter FAT/CD36 (Cameron-Smith et al., 2003), and signalling kinase AMPK (Yeo et al., 2008). Accordingly, such interventions have shown some success in improving fasting glucose and insulin concentrations (LaFountain et al., 2019), although this was likely due to large reductions in body mass (-7.7 kg). Longer-term exposure (>6 months) to a low-carbohydrate, high-fat diet alongside exercise has been shown to impair dynamic measures of insulin sensitivity in well-trained individuals (Webster et al., 2020), which corroborates previous findings of reduced glucose tolerance following low-carbohydrate, high-fat diets in the absence of energy restriction (Rosenbaum et al., 2019). It is not known whether this apparent reduction in glycaemic control following a low-carbohydrate, high-fat diet is pathological, or simply an adaptive response to reduced glucose availability.

2.5.2 Metabolic Responses to Low-Carbohydrate, High-Protein

An alternative method of maintaining energy intake whilst restricting carbohydrate intake is to replace dietary carbohydrate with protein. For example, consuming 0.45 g·kg body mass^{-1} of protein 30 min before a bout of aerobic cycling exercise resulted in greater rates of fat oxidation compared to an isocaloric high-carbohydrate meal (1 g·kg body mass^{-1} carbohydrate), and no differences in fat oxidation compared to the same exercise performed after an overnight fast (Rothschild et al., 2021). These findings agree with those of Impey et al. (2015) and Taylor et al. (2013), who showed comparable rates of fat oxidation during steady-state exercise performed after consuming 20–25 g of protein and after an overnight fast. Interestingly, the increase in fat oxidation during exercise after a low-carbohydrate, high-protein meal occurs despite elevated concentrations of circulating insulin compared to fasting (Impey et al., 2015; Rowlands & Hopkins, 2002*a*; Taylor et al., 2013), which is somewhat of a paradox given the antilipolytic effects of insulin (Spriet, 2014). However, it has been suggested that the elevated glucagon response to protein ingestion may stimulate lipolysis (Carlson et al., 1993; Perea et al., 1995; Rowlands & Hopkins, 2002*a*). As well as maintaining fat oxidation rates during exercise, consuming a low-carbohydrate, high-protein meal also does not appear to blunt the molecular adaptations which are commonly seen with fasted exercise (**Chapter 2.4.4**). For example, consuming 20 g of protein 45 min before steady-state cycling increased AMPK phosphorylation and PGC-1α mRNA transcription to a similar extent as overnight-fasted exercise (Taylor et al., 2013). In agreement with these findings, Larsen et al. (2020) showed

similar increases in PGC-1α mRNA expression following 90 min steady-state exercise performed after consuming either ~35 g protein or an energy-free placebo. More recently, consuming 0.33 g·kg body mass^{-1} protein before an acute bout of sprint interval exercise enhanced exercise-induced increases in FAT/CD36 compared to the same exercise performed after an overnight fast (Aird et al., 2021).

A limited number of studies have explored the metabolic responses to exercise after a low-carbohydrate, high-protein meal beyond a single exposure. In a recent study by Aird et al. (2021), healthy males completed 3 weeks of sprint interval training during which 3 x weekly training sessions were performed after consuming 0.33 g·kg body mass^{-1} of either concentrated or hydrolysed whey protein or following an overnight fast. After the intervention, muscle biopsies revealed that exercise training similarly upregulated CD36 mRNA expression when exercise was performed after an overnight fast and following concentrated whey protein, and CD36 mRNA expression was further increased when exercise was performed after hydrolysed whey protein. Therefore, it was concluded that pre-exercise protein consumption results in either similar or enhanced adaptations compared to fasted exercise training. Furthermore, under rested conditions, consuming a hypocaloric, low-carbohydrate, high-protein diet for 7 days increased AMPK mRNA expression (Furber et al., 2017).

In another study by Furber et al. (2021), healthy males consumed either a reduced-carbohydrate, high-protein diet (30% carbohydrate, 40% protein, and 30% fat), or an isocaloric high-carbohydrate, low-protein diet (60% carbohydrate, 10% protein, and 30% fat) for 7 days whilst maintaining their own exercise training program. Compared to baseline, 7 days of adherence to the reduced-carbohydrate, high-protein diet increased fat oxidation and concomitantly reduced carbohydrate oxidation during exercise, and also increased resting AMPK mRNA expression. However, both substrate oxidation and AMPK mRNA expression returned to pre-intervention levels after 7 days of reintroduction of the habitual diet, suggesting that robust cellular adaptations augmenting fat oxidation did not occur, or that 7 days was not long enough to facilitate such adaptations. Similar findings were reported by Leckey et al. (2018), in that 5 days of consuming a low-carbohydrate, high-protein diet (~18% carbohydrate, ~67% protein, and ~15% fat) alongside daily aerobic exercise training increased fat oxidation rates and reduced carbohydrate oxidation rates compared to 5 days of consuming a high-carbohydrate diet at baseline (~73% carbohydrate, ~14% protein, and ~13% fat). In this instance, substrate oxidation patterns returned to baseline after just 24 h of high dietary carbohydrate intake.

In summary, fat oxidation rates, along with the expression of skeletal muscle proteins such as AMPK and CD36, are increased when acute exercise is performed after a low-carbohydrate, high-protein meal, and whilst adhering to a low-carbohydrate, high-protein diet alongside exercise training. These responses may have implications for improved insulin sensitivity, but appear transient, as returning to habitual dietary consumption may reverse these responses. It is crucial to highlight that the metabolic responses to exercise following a low-carbohydrate, high-protein meal/diet have only been explored in a few studies, all employing heterogenous methodologies. It is possible that the duration of the dietary intervention, the carbohydrate content of the 'low-carbohydrate', high-protein meal/diet, and the frequency/intensity/modality of the exercise program could all have influenced the findings of these studies. Furthermore, studies typically use a low-carbohydrate, high-protein pre-exercise meal with a small energy content (~80–400 kcal) (Impey et al., 2015; Larsen et al., 2020; Oliveira et al., 2021; Rothschild et al., 2021; Taylor et al., 2013), which may lack ecological validity. Finally, only one study has directly compared metabolic responses to exercise performed after a low-carbohydrate, high-protein meal with exercise performed after a typical, high-carbohydrate meal and fasting (Rothschild et al., 2021).

2.5.3 Appetite and Energy Balance Responses to Low-Carbohydrate, High-Protein

A low-carbohydrate, high-protein meal before exercise may indirectly influence metabolic health and insulin sensitivity via its effects on energy balance. For example, increasing the protein content of a meal at the expense of other macronutrients reduces subsequent appetite (Hill & Blundell, 1986; Rolls et al., 1988; Stubbs et al., 1999) and/or energy intake (Barkeling et al., 1990; Booth et al., 1970; Latner & Schwartz, 1999). Compared to a high-carbohydrate meal, a high-protein meal may also result in a greater or more prolonged suppression of the orexigenic hormone acylated ghrelin (Blom et al., 2006; Foster-Schubert et al., 2008) and concomitant increases in anorexigenic hormones PYY (Batterham et al., 2006) and GLP-1 (Belza et al., 2013; van der Klaauw et al., 2013). Accordingly, energy-restricted diets with increased protein content have been shown to facilitate weight loss whilst maintaining fat-free mass, as well as improving body weight maintenance after weight loss (Leidy et al., 2015; Westerterp-Plantenga et al., 2004).

Despite evidence advocating a low-carbohydrate, high-protein diet for the purposes of weight management, there is little evidence comparing the appetite and energy intake responses to an

acute bout of exercise performed after high-carbohydrate and high-protein meals. In one study, healthy males and females consumed a high-protein meal replacement (30% carbohydrate, 43% protein, and 27% fat) or an isocaloric control breakfast (55% carbohydrate, 15% protein, and 30% fat) ~1.5 h before 40 min treadmill running (Oliveira et al., 2021). In this study, the post-exercise increase in hunger was attenuated when the high-protein meal replacement was consumed before exercise, compared to the control meal. This response was mirrored by increased concentrations of anorexigenic hormones PYY and GLP-1. Subjective appetite and appetite regulatory hormones were only measured once immediately after exercise, and subsequent energy intake was not measured in this study. Therefore, whether the high-protein meal replacement differentially influenced appetite or energy intake in the hours proceeding exercise is unknown. In the aforementioned study by Rothschild et al. (2021), subjective hunger was suppressed immediately following exercise, irrespective of whether exercise was performed after an overnight fast, or after a high-carbohydrate or high-protein breakfast. Again, the single snapshot measurement of appetite, and the lack of energy intake assessment in this study precludes conclusions from being drawn regarding the effects of a low-carbohydrate, high-protein pre-exercise meal on subsequent appetite and energy intake.

Pre-exercise protein consumption may also impact energy balance through its effects on energy expenditure. Dietary-induced thermogenesis for protein (20–30%) is greater than that for carbohydrate (5–10%), and fat (0–3%) (Westerterp, 2004). Therefore, energy expenditure in the postprandial period is greater following protein consumption compared to both carbohydrate and fat (Johnston et al., 2002; Karst et al., 1984). This increase in energy expenditure seems to persist in response to exercise. For example, Gieske et al. (2018) and Wingfield et al. (2015) showed elevated rates of energy expenditure for up to 60 min following exercise performed after consuming 25 g of protein compared to 25 g of carbohydrate. When examining responses beyond the first 60 min following exercise, elevated rates of energy expenditure have also been observed 24 h after resistance exercise performed after consuming protein, compared to carbohydrate (Hackney et al., 2010).

2.6 The Circadian System

Circadian rhythms are present within the majority of life-forms that exist on earth, ranging from single-celled organisms to multicellular human beings, and refer to oscillations in endogenous processes which occur over a time course corresponding closely to the duration of the 24-h solar day (Johnston, 2014; Morris et al., 2012). Such rhythms represent a mechanism which has evolved over hundreds of thousands of years as a selective advantage to the organism, allowing it to anticipate and respond to environmental cues in order to promote survival (Gerhart-Hines & Lazar, 2015). In mammals, the extent of control exerted by the circadian system is considerable, as up to 10% of all mRNA in a given tissue is shown to exhibit a circadian rhythm (Mohawk et al., 2012). As such, a broad range of biological processes are subject to circadian control, including fluctuations in core body temperature, heart rate, sleep, and hormone and neurotransmitter secretion (Hastings et al., 2008; Skene & Arendt, 2006; Smith & Betts, 2022). Of particular relevance to this body of work is the circadian variation that exist in several facets of metabolism such as glucose homeostasis (Kalsbeek et al., 2014; Van Cauter et al., 1989; Van Cauter et al., 1992; Van Cauter et al., 1997), lipid homeostasis (Dallmann et al., 2012; Morgan et al., 1999; Pan & Hussain, 2007; Zimmet et al., 1974), substrate oxidation (Rynders et al., 2020), and appetite regulation (Rynders et al., 2020; Templeman et al., 2021*a*).

In mammals, the circadian system is organised hierarchically, in that, despite almost every cell in the body containing its own cell-autonomous circadian clock 'machinery' (Mohawk et al., 2012), there exists a 'master' circadian clock within the suprachiasmatic nucleus (SCN) region of the brain which orchestrates the rhythms of 'peripheral clocks' which are located externally to the SCN (Johnston, 2014).

2.6.1 Molecular Clock Machinery

In mammals, the cell-autonomous clock machinery that is present in nearly every cell in the body is generated by coupled transcription/translation feedback loops (TTFL) which function synonymously to generate ~24-h rhythms of gene expression (Partch et al., 2014). A primary loop exists at the core of this TTFL model, in which transcriptional activators CLOCK and BMAL1 promote the transcription of a group of 3 Period (*Per*) and 2 Cryptochrome (*Cry*) repressor genes (Partch et al., 2014). These *Per* and *Cry* genes accumulate in the cytoplasm and dimerise to form a complex which then translocates to the nucleus to interact with CLOCK

and BMAL1, ultimately repressing their own transcription in an ~24-h cycle (Mohawk et al., 2012; Partch et al., 2014).

2.6.2 Central Pacemaker

Given the evidence alluding to the evolution of circadian rhythms as a means of allowing the organism to achieve temporal homeostasis between its behaviour and the external environment (Gerhart-Hines & Lazar, 2015; Partch et al., 2014), it makes logical sense for there to exist an internal mechanism for the sensing of environmental stimuli. Perhaps the most predicable environmental cue that displays a daily oscillation is the external light/dark cycle, however, molecular circadian clocks are present in cells within most peripheral tissues and are thus unlikely to be directly exposed to such environmental stimuli.

The SCN of the hypothalamus receives external photic information from the retina via synaptic transmission by axons of the retinohypothalamic tract, before transmitting this information to peripheral clocks via a variety of outputs (Dibner et al., 2010). Thus, within the hierarchical organisation of mammalian circadian rhythms, the SCN adopts the role of the 'central pacemaker', and the light/dark cycle acts as the main time-giver (or 'zeitgeber') to the SCN, allowing it to coordinate organism-wide circadian clocks with the external environment (Johnston et al., 2016). The anatomical location of the SCN in the anterior hypothalamus, directly above the optic chiasm, is in accordance with its proposed role (Dibner et al., 2010). Furthermore, in animal studies, SCN lesions have resulted in a loss of circadian rhythm (Moore & Eichler, 1972) and in SCN transplant studies, the recipient of the lesioned SCN demonstrates behavioural rhythms that reflect those of the SCN donor, rather than of the host (Ralph et al., 1990).

2.6.3 Synchronisation of Peripheral Clocks

Several peripheral tissues work in harmony in the regulation of human metabolism and consequently, endogenous circadian clocks must be synchronised to each other and to the environment for them to benefit the organism (Johnston et al., 2016). For example, diurnal humans have evolved a ~24-h circadian rhythm characterised by activity and food intake during the light phase, and a period of rest and fasting during the dark phase (Longo & Panda, 2016). As such, during fasting periods, the skeletal muscle, liver, and adipose tissue must

concomitantly alter their metabolism to maintain glucose homeostasis (Harfmann et al., 2015). Specifically, during fasting periods, plasma glucose concentrations are maintained by hepatic glucose production and gluconeogenesis, whilst a concomitant increase in free fatty acid mobilisation from adipose tissue results in greater rates of fat oxidation, ultimately sparing the body's limited carbohydrate stores (Frayn, 2010).

The SCN synchronises peripherally located cell-autonomous clocks via numerous mechanisms, namely via autonomic and endocrine signalling, regulation of body temperature, and local signals (Mohawk et al., 2012; Pickel & Sung, 2020). Examples of autonomic communication between the SCN and peripheral tissues come from the discovery that 24-h variations in plasma glucose and leptin concentrations are heavily determined by SCN-generated sympathetic innervation of the liver and adipose tissue, respectively (Cailotto et al., 2005; Kalsbeek et al., 2001). Relating to endocrine signalling, melatonin is a hormone secreted by the pineal gland – a gland that receives light signals through sympathetic innervation from the SCN (Astiz et al., 2019) – and is considered a key hormone involved in central-peripheral clock communication. Melatonin secretion displays a strong circadian rhythm, with concentrations elevated during the night (Templeman et al., 2021*a*). Melatonin is implicated in the conveyance of information about day length to the body, to coordinate various physiological functions that vary with season, such as reproduction, appetite, body weight, and sleep (Zawilska et al., 2009). The relative robustness of circulating melatonin concentrations makes its indirect measurement (in urine, blood, and saliva) a good biomarker of circadian dysregulation (Skene & Arendt, 2006). Cortisol is another example of a peripheral hormone which influences various metabolic functions and also displays a robust circadian rhythm (typically peaking in the morning immediately upon waking) (Krieger et al., 1971). Circadian rhythms in body temperature are a direct product of SCN influence and have a well-established role in the synchronisation of peripheral clocks in mammals (Brown et al., 2002). Finally, local signals refer to SCN-generated behavioural rhythms such as feeding and locomotor activity, which can also act to entrain peripheral clocks.

2.6.4 Peripheral Clocks

The identification of self-sustained peripheral clocks in mammals first came from animal studies, in which cultured cells from peripheral tissues exhibited the preservation of, albeit somewhat dampened, circadian oscillations when in isolation (Yamazaki et al., 2000; Yoo et al., 2004). In the context of nutrition and metabolism, circadian rhythmic gene expression has been detected in tissues including the liver, pancreas, adipose tissue, stomach, and skeletal muscle (Dibner et al., 2010). Interestingly, there appears to be little cross-over between rhythmic gene expression in different tissues (Storch et al., 2002), highlighting not only the importance of the role of the SCN in coordinating these clocks, but also the broad range of biological processes that are subject to circadian influence. Such processes include the timing of digestion, nutrient uptake and metabolism, hormonal and metabolite regulation, appetite, energy intake, and energy expenditure (Ruddick-Collins et al., 2018).

Advancing understanding of clock gene expression in humans has been impeded by the limited opportunities to obtain samples from specific tissues, with previous studies typically relying on easy-to-sample cells or post-mortem tissue (Cermakian & Boivin, 2009). Advances have been made in experimental procedures (Johnston, 2012), which have led to the discovery of clock genes in skin fibroblasts (Brown et al., 2005), adipose tissue (Otway et al., 2011), and pancreatic islet cells (Pulimeno et al., 2013) in humans. Of particular relevance here is the discovery of circadian clock genes in human skeletal muscle, whereby the basal secretion of several myokines (IL-6, IL-8 and monocyte chemotactic protein-1 (MCP-1)) displayed a circadian profile which was attenuated upon clock disruption (Perrin et al., 2015). In rodent studies, several metabolic functions implicated in glucose metabolism, such as insulin-stimulated glucose uptake, glucose oxidation, and insulin sensitivity are shown to be impaired following muscle-specific clock disruption (Gachon et al., 2017). Thus, the discovery of a circadian profile within human skeletal muscle provides a potential mechanism underpinning the diurnal variations in glucose tolerance in humans (Kalsbeek et al., 2014; Van Cauter et al., 1992). This was later confirmed in a study which performed high-throughput RNA sequencing in human skeletal muscle biopsies, as well as in cultured human skeletal muscle (Perrin et al., 2018). Findings were that transcript accumulation peaked at 16:00 for genes involved in muscle force production and mitochondrial activity, and 04:00 for genes involved in immune function and inflammation. Rhythmic expression of genes related to insulin secretion and lipid metabolism were also identified, suggesting that cell-autonomous skeletal muscle clocks are fundamental features of these processes (Perrin et al., 2018). Mitochondrial function has been

associated with skeletal muscle clocks previously, with mitochondria from human muscle biopsies exhibiting a day-night rhythm, with energy expenditure and mitochondrial oxidative capacity peaking later in the day (van Moorsel et al., 2016). This provides some mechanistic evidence underpinning the diurnal rhythms which have been observed in substrate oxidation (Capani et al., 1981; Rynders et al., 2020; Sensi & Capani, 1987).

Circadian rhythmicity has also been identified in the profile of several appetite regulatory hormones introduced in **Chapter 2.2** using constant, or semi-constant routine protocols with continuous feeding (Rynders et al., 2020; Templeman et al., 2021*a*). For example, using a semi-constant routine protocol (continuous feeding, but artificial light turned off at night to allow sleeping), Templeman et al. (2021*a*) identified a diurnal rhythm in leptin (peaking at 00:32) and unacylated ghrelin (peaking at 08:26) concentrations. This observed ghrelin response contrasts with studies adopting continuous fasting protocols, which have shown peaks in total ghrelin concentrations corresponding with habitual mealtimes (Espelund et al., 2005; Natalucci et al., 2005). Contrasting findings have also been reported under diurnal conditions, in studies adopting constant routine protocols (continuous feeding, wakefulness, and light exposure), and under conditions of forced desynchrony, all reporting peaks in ghrelin concentrations late in the evening (Cummings et al., 2001; Dzaja et al., 2004; Rynders et al., 2020). These findings highlight that the diurnal secretion of ghrelin is influenced by sleep-wake cycles and feeding patterns. Additionally, circadian rhythms have been identified for concentrations of PYY (peak ~14:00 and nadir ~04:00), GLP-1 (peak ~10:00 and nadir ~17:00), and PP (peak ~15:00 and nadir ~09:00) (Carnell et al., 2018; Galindo Muñoz et al., 2015; Hill et al., 2011; Johns et al., 2006; Rynders et al., 2020; Smith & Betts, 2022). Subjective hunger has, in several studies, been shown to peak in the evening (~19:00–21:00) and reduce to its nadir in the morning (~07:00–08:00), where satiety tends to be at its highest (Carnell et al., 2018; Rynders et al., 2020; Scheer et al., 2013; Templeman et al., 2021*a*).

It is clear that the metabolic responses to nutrition and exercise are intimately linked to circadian clocks, and this provides the foundations for the concept that there may exist an optimal time to eat and exercise for health (Gerhart-Hines & Lazar, 2015; Gabriel & Zierath, 2019).

2.6.5 Feeding and Exercise Influence Circadian Rhythms

The process of synchronising endogenous circadian clocks with each other and with the environment is termed ‘entrainment’ (Johnston et al., 2016). As discussed previously, light-dark cycles are the dominant zeitgeber for the SCN (Mohawk et al., 2012), which relays this temporal information to the periphery using autonomic and hormonal signalling, as well as through the regulation of body temperature. In addition to these pathways, the SCN indirectly regulates behavioural processes, namely feeding-fasting and activity-rest cycles, which normally correspond with the external light and dark phases (Longo & Panda, 2016). It has been established that both feeding (Damiola et al., 2000; Flanagan et al., 2021) and physical activity (Kemler et al., 2020; Wolff & Esser, 2012) possess the ability to entrain peripherally located clocks in an SCN-independent manner. Thus, indirect behaviour modification with regards to feeding and activity present as additional mechanisms by which endogenous circadian clocks are synchronised.

Daily feeding-fasting cycles are considered the dominant zeitgeber for several peripheral organs, including the liver, kidney, pancreas, and heart (Dibner et al., 2010). Several hormones which respond specifically to feeding and fasting such as insulin (Crosby et al., 2019; Tuvia et al., 2021) and glucagon (Ikeda et al., 2018; Sun et al., 2015), have also been shown to directly influence circadian gene expression. The superiority of feeding over light as a zeitgeber for peripheral clocks was demonstrated in a pioneering study by Damiola and Colleagues (2000). Mice (nocturnal animals) were kept under a 12 h light/12 h dark cycle and were fed for 9 days exclusively during either the dark phase (aligned) or light phase (misaligned). Fascinatingly, feeding during the light phase inverted circadian gene expression within the liver, pancreas, kidney, and heart to align with the new feeding schedule, whilst the temporal expression of the same genes in the SCN remained unaltered by the change of schedule. This observation has been confirmed in subsequent rodent studies (Hara et al., 2001; Stokkan et al., 2001). The timing of a bout of exercise has also been shown to shift circadian rhythmic gene expression in the liver, kidney, skeletal muscle, and lung tissues (Sasaki et al., 2016; Kemler et al., 2020; Wolff & Esser, 2012). Thus, in rodents, feeding and exercising out of sync with the SCN is able to independently entrain peripheral clocks and uncouple them from SCN control. These aforementioned findings in rodents have also been extended to human subjects, as alterations in both nutrient (Gu et al., 2020; Wehrens et al., 2017) and exercise (Kemler et al., 2020; Youngstedt et al., 2019) timing have induced shifts in markers of the circadian rhythm. These

findings highlight the relevance of the diurnal timing of eating and exercise for optimal circadian regulation, which may ultimately impact on physiological outcomes.

2.6.6 Metabolic Consequences of Circadian Disruption

The uncoupling of the light-entrained SCN rhythm from feeding- and activity-entrained peripheral rhythms is a phenomenon that is facilitated by factors common to modern society, such as artificial lighting and heating, sedentary lifestyles, and the 24-h availability of inexpensive, high-calorie foods (Gerhart-Hines & Lazar, 2015). Such circadian misalignment has been implicated in the development of obesity and metabolic disorders (Jiang & Turek, 2017; Stenvers et al., 2019). In a mouse model, mice that were fed a high-fat diet exclusively during the light phase (misaligned) gained more weight than mice fed a high-fat diet during the dark phase (aligned) over a 6-week period, despite no differences being observed in energy intake or locomotor activity between conditions (Arble et al., 2009). These findings are in accordance with observational data in humans, which suggest that shift workers are at a greater risk of developing obesity and metabolic disorders such as type 2 diabetes (Antunes et al., 2010; Gan et al., 2015). Night-shift workers have been shown to consume more energy during the dark phase, which is normally a period of sleep and fasting (Lennernäs et al., 1995). Eating out of sync with external circadian cues may, therefore, be a contributing factor in the increased incidence of obesity and metabolic complications associated with shift work, however, other factors common to this population, such as disturbed sleep, reduced physical activity, and high-energy snacking (Atkinson et al., 2008) make causal inferences currently difficult. Laboratory studies inducing simulated shift work in humans have been pivotal in our understanding of this association. One notable study by Scheer and Colleagues (2009) employed a 10-day, forced-desynchrony protocol with a recurring 28-h ‘day’. When eating and sleeping occurred ~12 h out of their habitual phase, subjects demonstrated decreased leptin concentrations (hormone related to satiety and body weight regulation), and increased glucose concentrations, despite insulin also being elevated. In 3 out of 8 subjects, postprandial plasma glucose concentrations exceeded a threshold beyond which levels would be considered pre-diabetic. Clearly, temporally disordered behavioural rhythms can incur metabolic consequences, likely due, in part, to an uncoupling of centrally and peripherally located clocks.

Further evidence in support of this comes from studies which have explored the effects of irregular meal patterns on metabolism and appetite. The increased availability of inexpensive,

convenient, high-calorie foods within modern society likely facilitates greater variation in daily meal frequency and timing (Gill & Panda, 2015), and observational studies have linked meal irregularity with impaired metabolic health (Pot et al., 2014; Sierra-Johnson et al., 2008). In a series of 14-day intervention studies, lean women (Farshchi et al., 2004*a*; Farshchi et al., 2004*b*), and women with obesity (Farshchi et al., 2005*b*) were asked to eat and drink items from their normal diet, either following a regular meal pattern (6 eating occasions/day), or an irregular meal pattern (daily variation between 3–9 eating occasions/day). In response to a test drink, the increase in energy expenditure attributed to DIT was lower after adhering to the irregular meal pattern (Farshchi et al., 2004*a*; Farshchi et al., 2005*b*), which may possibly have implications for weight gain in the long term. Additionally, the irregular meal pattern increased postprandial insulin concentrations in the absence of changes in glucose concentrations, indicating impaired insulin sensitivity (Farshchi et al., 2004*b*; Farshchi et al., 2005*b*). More recently, these studies were repeated, but with food intake provided by the researchers to prevent differences in self-selected energy intake from influencing the results (Alhussain et al., 2016; Alhussain et al., 2022). Similar to the earlier studies, 14 days of irregular eating reduced DIT (Alhussain et al., 2022), impaired postprandial glucose control, and increased subjective hunger (Alhussain et al., 2016), compared to an isoenergetic regular meal pattern.

2.6.7 Nutrient Timing

Another mechanism which may underlie the adverse metabolic responses to inappropriately timed eating stems from the diurnal variations in postprandial metabolism (Pickel & Sung, 2020). For example, the metabolic responses to a meal are likely to be influenced by the time of day that it is consumed (Garaulet et al., 2013; Jakubowicz et al., 2013; Jakubowicz et al., 2015). Over a 20-week weight loss intervention, subjects with overweight and obesity choosing to consume an early lunch (<15:00) lost ~2.2 kg more weight than those eating a late lunch (>15:00), despite no reported differences in energy expenditure, energy intake, or diet composition between groups (Garaulet et al., 2013). Similarly, greater weight loss was observed after 12 weeks of consuming a hypocaloric diet, in which energy intake distribution was manipulated so that more energy was consumed early in the day (700 kcal breakfast, 500 kcal lunch, and 200 kcal dinner), compared to late in the day (200 kcal breakfast, 500 kcal lunch, and 700 kcal dinner) (Jakubowicz et al., 2013), despite similar self-reported energy intakes between groups. The ‘early’ group also showed greater improvements in fasting and

postprandial glycaemic control, and fasting blood lipid profiles. Superior improvements in glycaemic control have also been shown in individuals living with type 2 diabetes following 7 days of early (700 kcal breakfast, 600 kcal lunch, and 200 kcal dinner) versus late (200 kcal breakfast, 600 kcal lunch, and 700 kcal dinner) energy intake distribution (Jakubowicz et al., 2015).

Although it is possible that these mealtime-induced differences in body weight reduction are, in part, a product of behavioural adaptation (*i.e.,* increased/decreased physical activity in either the early or late feeding conditions) (Ruddick-Collins et al., 2018), there are a number of potential circadian rhythm-related mediators. DIT is shown to be elevated in the morning compared to the evening (Bo et al., 2015; Morris et al., 2015), an effect governed by endogenous circadian influence and not the behavioural cycle. For instance, a simulated work-shift protocol to induce circadian misalignment by inverting behavioural rhythms by 12 h diminished this time-dependent difference in DIT (Morris et al., 2015). Thus, greater elevation of DIT in the morning may encourage a more negative energy balance in response to early versus late calorie distribution. It is possible that this observation is mediated by diurnal variations in rates of gastric emptying, which have been shown to be higher in the morning relative to evening (Goo et al., 1987).

Glucose metabolism also demonstrates a circadian rhythm, with basal glucose concentrations typically elevated first thing in the morning in a response commonly termed ‘the dawn phenomenon’ (Bolli et al., 1984). However, postprandial glucose tolerance also shows a peak in the morning, before gradually reducing to its zenith in the evening (Roberts, 1964; Simon et al., 1994; Van Cauter et al., 1989; Van Cauter et al., 1992; Van Cauter et al., 1997). This phenomenon is thought to be mediated by a combination of reduced glucose utilisation (Lee et al., 1992), decreased peripheral insulin sensitivity (Gibson & Jarrett, 1972; Lee et al., 1992; Saad et al., 2012), and impaired β-cell responsiveness (Melani et al., 1976; Saad et al., 2012) later in the day. Regarding lipid metabolism, circulating concentrations of NEFA, triglyceride, and cholesterol are generally elevated later in the day and into the night (Dallmann et al., 2012; Morgan et al., 1999; Pan & Hussain, 2007; Zimmet et al., 1974). For protein, most amino acids show circadian rhythms, with peak concentrations occurring in the afternoon/evening and a nadir in the early morning (Feigin et al., 1967; Wurtman et al., 1967). The result is that protein synthesis appears to be greater during the day (Kelu et al., 2020).

This demonstrates that human postprandial metabolism has evolved to function optimally during the active/light phase and that eating during the rest/dark phase is likely to confer elevated or extended postprandial excursions. Findings from studies examining human shift workers are in accordance with this. In a sample of 12 healthy, regular night-shift workers, postprandial plasma glucose, insulin, and triglyceride concentrations were elevated, whilst NEFA was supressed, in response to a test meal consumed at 01:30 during the second night of a 7-day night shift protocol (00:00–08:00), compared to 13:30 during a day shift (09:00–17:00) (Lund et al., 2001). Similar findings were observed by Al-Naimi and Colleagues (2004), who exposed 8 lean males to a single simulated day shift (12:00–20:00) and night shift (00:00–08:00) in a randomised, crossover design. During the day shift trial, meals were consumed at 13:00 and 19:00, with a snack at 16:00. In contrast, during the night shift, meals were consumed at 01:00 and 07:00, with the snack at 04:00. In accordance with the study by Lund et al. (2001), the postprandial triglyceride response was elevated during the 8-h measurement period in the night shift trial relative to the day shift. Postprandial glucose and insulin concentrations also exhibited a similar trend, whilst NEFA was unaffected (Al-Naimi et al., 2004).

2.7 Circadian Influence on Responses to Exercise

It is clear that a multitude of physiological and behavioural processes are subject to circadian influence and can, therefore, be entrained by several environmental stimuli such as light/dark cycles, food intake, and physical activity. Disruption of circadian rhythms due to shift work, abnormal eating patterns, exposure to artificial light, and irregular sleeping habits, is implicated in the development of detrimental health outcomes. Whilst exercise is understood to have the capacity to entrain the circadian system and ameliorate the consequences of circadian disruption, this is a two-way relationship, in that the circadian system also has the capacity to influence the responses to exercise (Mansingh & Handschin, 2022). As such, this mutual interaction has led several authors to question whether there exists an optimal time of day to exercise to maximise the metabolic and weight management benefits that are attained (Blankenship et al., 2021; Fillon et al., 2020; Gabriel & Zierath, 2019; Heden & Kanaley, 2019; Janssen et al., 2022; Mancilla et al., 2020; Mansingh & Handschin, 2022; Wolff & Esser, 2019).

2.7.1 Metabolic Responses

Most of the research investigating the potential circadian influence on metabolic responses to exercise has been conducted in rodent models (de Goede et al., 2018; Ezagouri et al., 2019; Sato et al., 2019). Such studies have identified diurnal changes in processes such as substrate utilisation and energy expenditure (Sato et al., 2019), activity of metabolic regulatory proteins including AMPK (Ezagouri et al., 2019), and mitochondrial function (de Goede et al., 2018). In humans, skeletal muscle strength peaks in the late afternoon (~16:00–20:00) (Douglas et al., 2021), and *in vitro* work has showed that mitochondrial oxidative capacity peaks in the late evening (van Moorsel et al., 2016). Accordingly, athletic performance is reported to be superior in the early evening compared to the morning (Atkinson & Reilly, 1996; Kusumoto et al., 2021), and larger gains in muscle mass have been shown in response to evening- compared to morning-based strength and endurance training (Küüsmaa et al., 2016). More recently, studies have begun to explore the potential effects of exercise timing on metabolic health outcomes in humans, although research in this area is sparse.

Savikj et al. (2019) were the first to explore this in a randomised, crossover trial, with a sample of 11 males with type 2 diabetes. In this study, subjects completed 2 weeks of either morning (08:00) or afternoon (16:45) high-intensity interval training (3 sessions per week), before a 2-week washout period and completion of the alternative 2-week training programme. Continuous glucose monitors were used to assess blood glucose concentrations during the first 3 (week 1) and last 3 (week 2) exercise and resting days. Morning exercise increased glucose concentrations on exercise days compared to baseline and to afternoon exercise during both weeks. Morning exercise also increased glucose concentrations on rest days during week 2. Conversely, afternoon exercise reduced glucose concentrations on exercise days during both weeks, suggesting a superiority of afternoon exercise for improving glycaemic profiles in men with type 2 diabetes. However, it is important to note that in this study, Savikj et al. (2019) measured glucose concentrations in the absence of insulin, meaning it cannot be assumed that the reduced blood glucose concentrations were a product of improved insulin sensitivity. For example, training-induced improvements in insulin sensitivity have previously been reported in the absence of any changes in glucose concentrations (Edinburgh et al., 2020).

A subset of 8 subjects from the Savikj et al. (2019) study underwent further measures (blood samples and skeletal muscle and adipose tissue biopies) to explore whole-body and tissue-specific metabolic adaptations (Savikj et al., 2022). Whilst the majority of the metabolic and

proteomic adaptations were similar following 2 weeks of morning and afternoon high-intensity interval training, some distinctive differences in adaptations were identified. Specifically, morning exercise training elevated plasma carbohydrate metabolites to a greater extent than afternoon exercise training, which contrastingly resulted in greater elevations in skeletal muscle lipid and mitochondria content. These data suggest that afternoon exercise had a greater effect on oxidative capacity than morning exercise. When considering the impaired mitochondrial oxidative capacity in individuals with type 2 diabetes, these findings may help to explain the improved glycaemic control observed following afternoon, compared to morning exercise (Savikj et al., 2019).

Mancilla et al. (2021) retrospectively analysed data from a previous study (Brouwers et al., 2018) to assess whether the metabolic adaptations to a 12-week combined aerobic and resistance exercise training regime were influenced by the time of day in which exercise was performed. In this sample of 32 metabolically compromised males, 12 completed all of their sessions (3 sessions per week) between 08:00–10:00, and 20 completed sessions between 15:00–18:00. A 2-step hyperinsulinemic-euglycemic clamp was performed before and after the training period to assess peripheral insulin sensitivity. Importantly, the post-intervention clamp was performed 48–72 h after the final training session to prevent the acute carry-over effect of the last exercise bout. Compared to those who exercised in the morning, subjects who exercised in the evening experienced superior improvements in peripheral insulin sensitivity, fasting plasma glucose concentrations, and exercise performance. These findings may be mediated, in part, by the greater reductions in fat mass observed in the afternoon exercise group.

Further supporting the efficacy of afternoon/evening exercise for improvements in metabolic health is a study by Moholdt et al. (2021). Here, 24 males with overweight or obesity adhered to a prescribed high-fat diet for 11 days (65% fat) and completed daily aerobic exercise for the final 5 days of the diet. Fasting and postprandial blood samples were collected before and after the exercise programme, and continuous glucose monitors were used to assess nocturnal glucose concentrations. Relative to a non-exercising control group, subjects randomised to exercise in the evening (18:30) experienced greater reductions in fasting glucose and low-density lipoprotein (LDL)-cholesterol compared to those exercising in the morning (06:30). Additionally, whilst postprandial insulin was similarly reduced in both exercise groups, postprandial TAG and LDL-cholesterol, and nocturnal glucose concentrations were reduced only with evening exercise. In this study, improvements in metabolic profiles with evening

exercise were unlikely related to changes in body composition or improvements in aerobic capacity, as there were no between-group differences for these outcomes.

In addition to structured exercise, whether the distribution of moderate-to-vigorous physical activity bouts accumulated throughout the day influences health outcomes has been examined in a recent cross-sectional study (Hetherington-Rauth et al., 2022). Results of this study were in agreement with existing experimental data, showing favourable associations between markers of cardiorespiratory fitness, HbA1c, and whole-body fat mass with afternoon moderate-to-vigorous physical activity.

In contrast to the aforementioned studies, Teo et al. (2019) showed that 12 weeks of multimodal exercise training (3 sessions per week) improved fasting and postprandial markers of glycaemic control in a group of 40 males and females classified as overweight, irrespective of whether exercise sessions were conducted in the morning (08:00–10:00) or evening (17:00–19:00). The discrepancy in findings between previous studies may be due to the heterogeneity in applied methodologies, including subject characteristics, type/duration/intensity/modality of exercise, and duration of exposure. Additionally, other than one study (Moholdt et al., 2021), previous studies failed to control the timing and/or composition of meals, which, as outlined in **Chapter 2.4**, can profoundly influence the outcomes of exercise training. Therefore, despite the existing data leaning in the favour of afternoon/evening exercise for enhancing the metabolic responses to exercise, there is clearly a requirement for more research to be conducted in this area.

2.7.2 Appetite and Energy Balance Responses

In the first study to examine the appetite and energy intake responses to morning (08:15) and evening (19:15) exercise (aerobic and muscle conditioning), Maraki et al. (2005) showed that irrespective of time of day, exercise increased subjective appetite, but did not affect self-reported energy intake in a sample of 12 healthy females. Importantly, the energy deficit created by exercise was not compensated for, resulting in a similar reduction in relative energy intake in both exercise trials, compared to a non-exercising control trial. A decade later, similar findings were reported by Alizadeh et al. (2015), who showed that in a group of 50 females classified as overweight, 24-h self-reported energy intake was again not different following morning (08:00–10:00) or afternoon (14:00–16:00) aerobic exercise. Additionally, subjective hunger, fullness, prospective food consumption, and desire for sweet, salty, savoury, and fatty foods did not change significantly in response to either exercise trial. More recently, Larsen et

al. (2019) reported greater post-exercise acylated ghrelin concentrations following 30 min high-intensity interval training when performed in the afternoon (14:00–16:00), compared to the morning (06:00–07:00), and the evening (19:00–20:00), in a sample of 11 men classified as overweight. Despite this, no between-trial differences were shown for post-exercise appetite, or 48-h self-reported energy intake.

In the above studies, the timing and composition of the pre-exercise meal was not standardised between trials, making it difficult to isolate and examine the effects of time of day *per se*. This methodological consideration has since been addressed by McIver et al. (2019*b*). In this randomised, crossover study, 12 healthy males performed 45 min treadmill walking in the morning (~09:00) and afternoon (~16:00), 1 h after consuming a standardised meal. In accordance with previous findings, no time-of-day differences were observed for post-exercise appetite or for 24-h self-reported energy intake. These studies all relied upon self-reported energy intake, which has inherent limitations (Dhurandhar et al., 2015; Rennie et al., 2007), potentially masking any differences in energy intake based on exercise timing, although it should be noted that the absence of any clear time-of-day differences in appetite make this unlikely. One randomised, crossover study objectively assessed energy intake responses over 26 h following 45 min treadmill running conducted in the morning (07:00) and the afternoon (17:00) by measuring *ad-libitum* consumption (O'Donoghue et al., 2010). In the small sample of 9 healthy men, there were no differences in energy or macronutrient intake during the 26-h monitoring period, or at any individual meal, between trials. Collectively, the findings from the above discussion suggest that, at least acutely, there appears to be no clear effect of exercise timing on appetite and energy intake responses.

Studies have also explored whether the timing of exercise affects weight management outcomes of training interventions. Two groups have performed secondary analysis on data from larger studies to explore whether exercise timing influenced weight loss outcomes. In the first of these, Chomistek et al. (2016) performed a cross-sectional analysis among 7157 women who participated in the Women's Health Study, which measured physical activity using accelerometers. The women in the lowest quartile for accelerometer counts before 12:00 had 26% higher odds of being obese, compared to those in the highest quartile. The authors concluded that women less active in the morning hours may be at higher risk of obesity. More recently, Willis et al. (2019) performed secondary analysis on data from a 10-month supervised exercise programme (5 sessions per week) in a sample of 88 physically inactive males and females classified as overweight or obese. Subjects were retrospectively categorised as early

(07:00–11:59) or late (15:00–19:00) exercisers, based on the time-of-day in which they completed >50% of their sessions. For subjects not meeting these criteria, a 'sporadic' exercise group was created, and there also existed a non-exercising control group. At 10 months, weight loss was greater in the early (-7.2%) and sporadic (-5.5%) groups, compared to the control (+0.5%). In line with the findings reported by Chomistek et al. (2016), early exercisers also lost more weight than the late exercisers (-2.1%). Free-living energy intake and energy expenditure were measured using food diaries and doubly-labelled water at the beginning and end of the intervention and revealed greater daily energy intake (80–230 kcal·day^{-1}) and reduced daily energy expenditure (~100 kcal·day^{-1}) in the late, compared to early exercise group, although these findings did not reach statistical significance. Whilst interesting, the ability to draw conclusions from these correlational data is confounded by other factors, such as the possibility of greater commitment or conscientiousness in the early exercisers (Schumacher et al., 2020).

In a follow up to their acute study, Alizadeh et al. (2017) randomised 48 women classified as overweight to a morning (08:00–10:00) or afternoon (14:00–16:00) exercise group. Exercise consisted of 30 min treadmill running and was performed 3 times per week for 6 weeks. Self-reported energy intake declined over the 6-week training period in the morning group only, which manifested as a greater reduction in body weight in this group compared to the evening exercisers. In another study, 20 inactive adults classified as overweight completed a 12-week exercise intervention and were randomised to either a morning exercise (06:00–09:00), evening exercise (16:00–19:00), or a non-exercising control group (Brooker et al., 2019). Subjects were prescribed a minimum of 250 min moderate-to-vigorous exercise per week, with sessions supervised during the initial 4 weeks. Body composition was assessed using dual-energy X-ray absorptiometry at weeks 0, 6 and 12. Given the small sample size, and because the primary aim of this pilot study was to assess acceptability and feasibility of the intervention, between-group data was not analysed statistically, and data are presented descriptively. Change from pre- to post- intervention for body mass index (-1.5 versus -1.1 kg·m^{-2}) and percentage body fat (-1.2 versus -0.6%) appeared to favour morning, compared to evening exercise.

Whilst these initial observations appear to support the efficacy of morning exercise for weight management efforts, findings are inconclusive. For example, Moholdt et al. (2021) showed no time-of-day effect on body mass or composition after 5 days of daily morning (06:30) or evening (18:30) exercise in men with overweight/obesity who were adhering to a high-fat diet. Teo et al. (2021) also failed to observe any differences in body weight loss or improvements in

body composition following 12 weeks of morning (08:00–10:00) versus evening (17:00–19:00) multimodal exercise. In another pilot study designed to assess acceptability and feasibility (Creasy et al., 2022), adults with overweight or obesity prescribed to exercise in the morning (06:00–10:00) or evening (15:00–19:00) lost similar amounts of weight and fat mass over the 15-week intervention period, although due to the preliminary nature of the study, no formal between-group statistical analyses were performed. In contrast to previous studies, a group of 29 postmenopausal women experienced greater reductions in fat mass (-1.71 versus -0.24 kg) after a 3-month walking intervention (50 min walking 4 times per week) when sessions were performed in the evening (18:00–20:00), compared to the morning (07:00–09:00) (De Blasio et al., 2010). Mancilla et al. (2021) also showed greater reductions in fat mass (-1.2 versus 0.2 kg) and percentage body fat (-1.0 versus -0.3%) in a sample of 32 metabolically compromised males after 12 weeks of combined aerobic and resistance exercise training when sessions were conducted between 15:00–18:00, compared to 08:00–10:00. Finally, as highlighted previously in this discussion, evening-based resistance exercise may elicit superior gains in muscle mass (Küüsmaa et al., 2016).

Whilst it is clear that the diurnal timing of exercise leads to different metabolic and weight management outcomes, larger randomised controlled trials are required before conclusions can be drawn regarding an optimal time of day to enhance adaptations (Janssen et al., 2022). In the real world, several other social, environmental, and logistical factors likely dictate the diurnal timing of exercise, and it has recently been concluded that finding an exercise time that fits within an individual's schedule and preference likely supersedes circadian considerations (Janssen et al., 2022; Mansingh & Handschin, 2022). Because the timing of exercise is also likely to influence adherence (Schumacher et al., 2019; Schumacher et al., 2020) – a major determinant of the long-term effectiveness of dietary and exercise interventions (Alhassan et al., 2008; Gibson & Sainsbury, 2017; Stonerock & Blumenthal, 2017) – future exercise interventions should be developed with an appreciation of their timing, thus allowing for their convenient incorporation within the daily lives of the largest proportion of the target population. Information regarding the exercise timing behaviours and preferences of the general population would act as a vital tool in facilitating the development of such interventions, although these data are lacking.

2.8 Thesis Aims

In light of the reviewed literature, this thesis will examine the appetite, energy intake, and metabolic responses to novel exercise and nutrition strategies that are aimed at achieving fasted metabolic conditions. Ensuring that exercise and nutrition interventions can be conveniently incorporated into lifestyles is fundamental in achieving adherence and long-term success. As such, the studies within this thesis will consider exercise timing and the macronutrient manipulation of existing meals as important factors in study design and implementation. The aims of the thesis are as follows:

- To investigate exercise timing behaviours, preferences, and barriers amongst the population to inform the design of two laboratory studies aimed at achieving fasted metabolic conditions via the manipulation of pre-exercise nutrition.
- To explore the appetite, energy intake, metabolic, and performance responses to fasted exercise performed at the most common time of day based on survey data.
- To explore the appetite, energy intake, and metabolic responses to exercise performed after a low-carbohydrate, high-protein meal.
- To explore the utility of a novel, viscous meal, containing virtually no energy in overcoming some of the appetite-related challenges associated with fasting-based interventions.

Chapter 3 – General Methods

This chapter describes the general methodological techniques employed in the laboratory-based experimental studies within this thesis (**Chapters 5, 6 and 7**). The methods employed during the online survey study which comprises **Chapter 4** are described within that chapter to avoid repetition. All studies were conducted according to the guidelines laid down in the 1964 Declaration of Helsinki and its later amendments, and all procedures were approved by the Nottingham Trent Human Invasive Ethical Committee (HIEC) or the Non-Invasive Human Research Ethical Committee (NIHREC); ethics application numbers: **Chapter 4** – 19/20-121; **Chapter 5** – 670; **Chapter 6** – 704; **Chapter 7** – 632. All subjects provided written informed consent prior to their participation in studies. Laboratory studies were registered at ClinicalTrials.gov: **Chapter 5** – NCT04742530; **Chapter 6** – NCT05107583; **Chapter 7** – NCT04735783.

3.1 Subjects

Subjects were recruited from Nottingham Trent University and the surrounding area by word of mouth, poster, email, and social media advertising. Subjects were provided with information sheets detailing the rationale, procedures, and demands of the study. After a verbal explanation and an opportunity to ask any questions about the study, volunteers provided written informed consent (**Appendix B; Appendix C; Appendix D**) and completed a health screen questionnaire (**Appendix E**) at least 24 h prior to participation in experimental trials. Physical activity levels (**Appendix F**) and eating habits (Stunkard & Messick, 1985; **Appendix G**) were also determined. To maximise adherence to standardised diets, subjects' food preferences were also ascertained, and alterations were made to diets, if possible (**Appendix H**). Female subjects completed a menstrual cycle questionnaire (**Appendix I**) so that the timing of experimental trials could be standardised within-subjects to limit the confounding effects of fluctuations in circulating sex-hormones on outcome measures.

The inclusion criteria for participation were:

- Aged 18–40 years
- Non-smoker
- Not currently on any weight management diet
- Weight stable for the last 6 months
- No history of cardiovascular, metabolic, digestive, or renal disease
- No severe dislike or intolerance of any study foods
- Recreationally active (<10 $h \cdot week^{-1}$)
- Not classified as a restrained, disinhibited, or hungry eater
- Female specific: must be using a monophasic, low dose combined OCP (containing less than 50 μg oestradiol and a synthetic progestin) OR females with regular menstrual cycles (**Chapter 5** only; self-reported).

3.2 Preliminary Measures

3.2.1 Body Mass and Composition

Height was measured to the nearest 0.01 m using a stadiometer (Seca, Germany), with the subject wearing no shoes and standing in an upright position. Body mass was measured to the nearest 0.1 kg using a calibrated digital scale (Adam CFW150; Adam Equipment Ltd., UK) with the subject wearing minimal clothing. Body mass index (BMI) was then calculated by dividing the weight in kilograms by the height in meters squared. Subcutaneous body fat was estimated by a Level 1 qualified International Society for the Advancement of Kinanthropometry (ISAK) practitioner. Skinfold callipers (Harpenden, UK) were used at four upper-body sites on the right-hand side of the body (triceps, biceps, subscapular, and iliac crest) with the subject standing in a relaxed position. Duplicate measurements were taken at each skinfold site, and the mean of the two values was used. A third measurement was taken if the difference between the initial two measurements was ≥10%, with the median of the three values being accepted. Body density (Durnin & Womersley, 1974) and percentage body fat (Siri, 1956) were estimated using the sum of all four skinfold sites.

3.2.2 Familiarisation Trials

Subjects completed a preliminary trial prior to experimental trials, in which height, weight and skinfold thickness were assessed as outlined above. They were also familiarised to all procedures to be used in main trials, such as *ad-libitum* meals, subjective appetite assessments, expired gas collection (**Chapters 5 and 6 only**), exercise protocols (**Chapters 5 and 6 only**), and blood sampling procedures (**Chapters 6 and 7 only**). These procedures are described in detail below. In **Chapters 5 and 6**, subjects also completed a maximal exercise test during the preliminary trial for the determination of $\dot{V}O_{2peak}$. A second preliminary trial was also completed in these chapters, involving an additional familiarisation to the exercise protocols.

3.2.3 Pre-Trial Standardisation

Subjects recorded all dietary intake (including caffeine intake) and physical activity in the 24 h before the first experimental visit, replicating these before remaining trials. Strenuous activities and alcohol intake were prohibited during this period, with adherence confirmed verbally upon arrival at the laboratory prior to each trial. On the evening before trial days, subjects ceased food intake at 20:00 in **Chapters 6 and 7** and consumed a standardised dinner at 20:30 in **Chapter 5**. After this, only plain water was permitted, which was standardised between trials, and caffeine intake was not permitted until the end of the trial period. Subjects arrived at the laboratory via motorised transport. Experimental trials were administered in randomised (by drawing trial orders for subjects out of a bag), counterbalanced, cross-over order.

3.3 Standardised Meals

Estimates of subjects' daily energy requirements (EER) were calculated using the Mifflin-St Jeor equation multiplied by a physical activity factor (Mifflin et al., 1990). The Mifflin-St Jeor equation takes into consideration subjects' sex, body mass, height, and age, and reduces the risk of overestimating energy requirements compared to the commonly employed Harris-Benedict equation (Mifflin et al., 1990). The Mifflin-St Jeor equation for males and females is detailed below:

$$\text{RMR (males)} = (10 \text{ x body mass in kg}) + (6.25 \text{ x height in cm}) - (5 \text{ x age in y}) + 5$$

$$\text{RMR (females)} = (10 \text{ x body mass in kg}) + (6.25 \text{ x height in cm}) - (5 \text{ x age in y}) - 161$$

In **Chapters 5 and 6**, a physical activity factor of 1.7 was used to account for the exercise component of the trial (Food and Agricultural Organization (FAO)/WHO, 2004). In **Chapter 7**, RMR was multiplied by a physical activity factor of 1.6, indicating light activity. As different standardised meals were used in each chapter, information relating to specific food items and compositions of meals is provided in the relevant chapter.

3.4 *Ad-Libitum* Energy Intake

3.4.1 Pasta Meal

Energy intake was assessed at distinct timepoints using a laboratory-based *ad-libitum* pasta meal of a fixed macronutrient composition. The meal used to assess energy intake at lunch (**Chapter 7**) and dinner (**Chapters 5 and 6**) consisted of fusilli pasta, Bolognese sauce and extra virgin olive oil. On the day prior to an experimental trial, 400 g of dry pasta (Sainsburys, UK) was cooked in unsalted water in a microwave at 900 W for 9 min, stirred, and then returned to the microwave for a further 9 min. Cooked pasta was then drained and was weighed 1 min after being removed from the microwave. To ensure energy density of each batch of pasta was closely matched, batches were required to weigh between 1070–1090 g, with further cooking periods of 0.5–1 min used to achieve this. Cooked pasta was then mixed with 400 g Bolognese sauce (Dolmio Original Bolognese Sauce, Mars, UK) and covered with a plastic film. The meal was allowed to cool before being refrigerated overnight.

Approximately 60 min before serving, 32 g of extra virgin olive oil (Sainsburys, UK) was mixed into the meal. Immediately before serving, the whole meal was weighed, before being heated for 3 min, stirred thoroughly, and heated for a further 3 min. The pre-meal weight was recorded after 5 min of cooling and was served in its entirety to subjects. The meal provided 1.25 ± 0.01 kcal·g^{-1} (69% carbohydrate, 11% protein, 18% fat, and 2% fibre).

Subjects ate this meal in a personal booth to avoid distractions. Directly outside the booth, a table was set up behind a screen to ensure total privacy. On this table, a serving spoon and a large plastic bowl containing the entire pasta meal were placed and subjects self-served pasta into a smaller bowl before returning to the booth to eat with the cutlery provided. Subjects were able to repeat this process as many times as they desired within the allotted 20 min but were explicitly instructed to eat until they felt ‘comfortably full and satisfied’. *Ad-libitum* water intake was permitted during the 20-min period. Total food and water intake were quantified by

weighing the food and water before and after the eating period. Subjects were required to remain in the booth for the entire 20-min period, even if they had ceased eating, and all subjects reported they had ceased eating within this time in all trials.

3.4.2 Snacking

In **Chapter 6**, evening snack energy intake was measured by providing subjects with a selection of snacks to consume *ad-libitum* outside the laboratory between 20:00–22:00 only. The selection of snacks included 4 small chocolate bars (Mars, UK), 2 cereal bars (Special K, Kellogg's, UK), 2 packets of ready salted crisps (Walkers, UK), 2 apples, and 2 satsumas. Items consumed outside the laboratory were weighed before being provided to subjects and reweighed the following day.

3.5 Subjective Responses

3.5.1 Subjective Appetite Responses

In **Chapter 7**, hunger, fullness, desire to eat (DTE), prospective food consumption (PFC), and nausea were assessed at distinct timepoints using previously validated 100 mm visual analogue scales (VAS; **Appendix J**) (Flint et al., 2000). VAS had written anchors of 'not at all'/'no desire at all'/'none at all' and 'extremely'/'a lot' placed at 0 and 100 mm, respectively. Subjects rated appetite sensations by placing a vertical mark along the 100 mm line corresponding to the degree to which they were experiencing each sensation at that given moment. These responses were then numerically quantified by measuring the distance from the left-hand side of the scale to the point on the line indicated by the subject.

In **Chapters 5 and 6**, VAS were digitised and sent to subjects' mobile telephones at the relevant timepoints. All digital VAS were administered using Surveymonkey.com, and the positioning of the subjects' response on the 0–100 line was recorded numerically on the Surveymonkey.com software and subsequently exported into Microsoft Excel. Subjects' were instructed to complete all digital VAS using the same mobile phone to eliminate the potential effects of differences in screen size on responses.

3.5.2 Pre- and Post-Exercise Subjective Responses

Immediately pre-exercise in **Chapters 5 and 6**, motivation to exercise, readiness to exercise, tiredness, and energy were assessed using 0–100 digital VAS (**Appendix K**). Subjects also completed a paper-based Positive and Negative Affect Schedule (PANAS; **Appendix L**; Watson et al., 1988) pre-exercise.

A paper-based, shortened version of the Physical Activity Enjoyment Scale (PACES-8) was completed immediately post-exercise to measure exercise enjoyment (Kendzierski & DeCarlo, 1991; Raedeke, 2007). PACES-8 uses a series of eight, seven-point bipolar scales which subjects use to rate their agreement with one of the two statements at either end of the scale (*e.g.*, 'I enjoyed it' – 'I hated it') (**Appendix M**).

3.6 Expired Gas Samples

In **Chapters 5 and 6**, resting gas samples were collected after 20 min supine rest. For 10 min, subjects breathed through a silicone mouthpiece, one-way valve, and falconia tube (Hans Rudolf, USA) (Compher, 2006). The first 5 min of each sample was discarded, with the subsequent 5 min being collected into a Douglas bag (HaB International Ltd., UK). During exercise, samples were collected for 4 min, with the latter 2 min being collected into a Douglas bag.

Expired gas samples were assessed for oxygen and carbon dioxide concentrations using a paramagnetic oxygen analyser and an infrared carbon dioxide analyser (MiniHF 5200, Servomex, UK). The analyser was calibrated to certified reference gases prior to sample analysis. Samples were also analysed for volume (Harvard Dry Gas Meter, Harvard Ltd., UK), and temperature. The volume of the sample extracted by the Servomex for assessment of oxygen and carbon dioxide concentrations was recorded, and total sample volume was subsequently corrected. Laboratory oxygen and carbon dioxide concentrations were measured before each sample analysis period to account for variation in ambient air (Betts & Thompson, 2012). Stoichiometric calculations were adjusted for laboratory temperature (608-H2 Hygrometer, Testo Ltd., UK), and barometric pressure (Greisinger G1110 Alti-/Barometer, GHM Group, Germany). Volumes of gas samples were converted to standard temperature and pressure dry (273 K and 760 mmHg; $V_{E(STPD)}$), and the Haldane Transformation was used to

determine the volume of inspired air. Differences between inspired and expired samples was used to determine oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$).

Energy expenditure and substrate oxidation were calculated using modified stoichiometric equations, accounting for increased glycogen contribution to carbohydrate metabolism during moderate-intensity exercise (Jeukendrup & Wallis, 2005). Rates of energy expenditure were calculated based on fat, glucose, and glycogen providing 9.75, 3.74, and 4.15 $kcal \cdot g^{-1}$, respectively. At rest, calculations were based on glucose providing all of the carbohydrate for metabolism, whereas during moderate-intensity exercise, calculations were based on glucose and glycogen contributing 20 and 80%, respectively, to carbohydrate oxidation (Jeukendrup & Wallis, 2005).

3.7 Blood Sampling and Analysis

Venous blood samples (~10mL) were collected from the antecubital vein using either venepuncture (**Chapter 7**) or cannula (**Chapter 6**). To ensure that cannulas were kept patent, they were flushed with ~10 mL non-heparinised saline (0.9% sodium chloride, BD PosiFlush, UK) after every sample and at regular intervals between samples. Prior to each blood sample, subjects rested in a supine position for ≥20 min and remained in this position during the collection, to limit any posture-related changes in plasma volume (Shirreffs & Maughan, 1994).

The first 2 mL of each collection was discarded to ensure samples did not contain saline from the cannula, before 4.9 mL blood was collected into an EDTA monovette (1.6 $mg \cdot mL^{-1}$; Sarstedt AG & Co., Germany). A further 2.7 mL blood was collected into an EDTA monovette (1.6 $mg \cdot mL^{-1}$) containing 27 μL of a solution containing potassium phosphate buffer (PBS; 0.05 M), P-hydroxymercuribenzoic acid (PHMB; 0.05 M), and sodium hydroxide (NaOH; 0.006 M) to prevent the degradation of acylated ghrelin to unacylated ghrelin. Following collection, blood samples were centrifuged (1700 *g*, 15 min, 4 °C), the supernatant (1 mL) of the PHMB/PBS/NaOH treated blood was mixed with 100 μL of hydrochloric acid (HCl; 1 M). Plasma was separated into ~1 mL aliquots and stored at -20 °C until frozen and then transferred to -80 °C until further analysis.

Capillary blood samples were collected in **Chapter 7** by piercing an alcohol-swabbed fingertip (Unistick 3 Extra, Owen Mumford, UK). The first drop of blood was discarded to ensure the sample did not contain any alcohol, and a free-flowing capillary blood sample (20 μL) was

collected into a glass capillary tube which was then added to 1 mL of a haemolysing solution. This solution was thoroughly mixed before being analysed immediately using a desktop blood glucose analyser (Biosen, EKF Diagnostics, UK).

In **Chapters 6 and 7**, plasma concentrations of acylated ghrelin (Bertin Technologies, France) and total PYY (Merck Millipore Ltd., UK) were measured using commercially available ELISA. In **Chapter 6** only, plasma concentrations of total GLP-1 (Merck Millipore Ltd., UK), and insulin (Mercodia, Sweden) were measured using commercially available ELISA, and glucose (Horiba Ltd., UK), NEFA (Randox Laboratories Ltd., UK), and glycerol (Randox Laboratories Ltd., UK) were determined by enzymatic colorimetric assay using a benchtop analyser (glucose and NEFA: Pentra 400; Horiba Ltd., UK; glycerol: Daytona; Randox Laboratories Ltd., UK). To avoid potential differences between plates or reagents, it was ensured that all samples for an individual subject were analysed on the same ELISA plate, or during the same analysis cycle on the Pentra and Daytona. Intra-assay coefficients of variation are presented in **Table 3.1**.

Table 3.1. Intra-assay coefficient of variation for each assay conducted.

Variable	**Chapter 6**	**Chapter 7**
Total PYY	3.1 (1.6–4.0) %	4.5 (4.0–4.9) %
Acylated Ghrelin	4.0 (1.8–6.2) %	6.7 (4.7–8.7) %
Total GLP-1	3.7 (2.2–4.4) %	Not analysed
Glucose (Pentra)	0.3 (0.2–0.4) %	Not analysed
Glucose (Biosen)	Not analysed	3.3 %
NEFA	1.0 %	Not analysed
Glycerol	7.5 %	Not analysed
Insulin	4.0 (2.7–5.8) %	Not analysed

CV data is presented as mean (range)

3.8 Exercise Testing

3.8.1 Maximal Aerobic Capacity

During the first laboratory visit in **Chapters 5 and 6**, cycling $\dot{V}O_{2peak}$ was determined during a discontinuous incremental exercise test on an electronically braked cycle ergometer (Lode Corival, Netherlands). The initial workload was set at either 75 or 100 W, depending on the self-reported existing fitness level of the subject being tested. The test involved 4-min incremental stages separated by ~5 min rest, with workload increasing by 50–100 W during each increment until volitional exhaustion. Verbal encouragement was provided throughout the test. Expired gas samples were collected during the final minute of each stage and the final minute of the test to determine $\dot{V}O_2$, with $\dot{V}O_{2peak}$ being defined as the highest $\dot{V}O_2$ achieved during the test. Heart rate and rating of perceived exertion were also collected during the final minute of each increment.

3.8.2 Heart Rate

During exercise, heart rate was recorded using short-range radio telemetry (Polar, Finland).

3.8.3 Rating of Perceived Exertion (RPE)

The Borg (1982) scale, ranging from 6 (no exertion) to 20 (maximal exertion) was used to measure subjects' levels of perceived exertion during exercise.

3.9 Sample Size Estimation

Sample size estimations were calculated using G*Power software v3.1 (Heinrich Heine University, Germany). In **Chapter 5**, using an α of 0.05, β of 0.8 and data from a previous study (Clayton et al., 2015), it was estimated that 15 subjects would be required to detect a 5% difference in voluntary exercise performance, and 12 subjects to detect a 15% difference in energy intake. To ensure that the study was counterbalanced, 16 subjects were recruited. In **Chapter 6**, using α of 0.05, β of 0.90, fat oxidation data from a previous study (Rothschild et al., 2021) and energy intake data from **Chapter 5**, it was estimated 11 subjects would be required to detect a 15% difference in fat oxidation during exercise, and 8 subjects to detect a 10% difference in post-exercise energy intake. To ensure that the study was counterbalanced,

12 subjects were recruited. For **Chapter 7**, there was a lack of published data to inform a sample size calculation, therefore, the sample size used was similar to previous studies which assessed energy intake at lunch in response to breakfast omission (Clayton et al., 2015; Clayton et al., 2016*a*).

3.10 Statistical Analyses

Data were analysed using SPSS v26.0 (IBM, USA). All data were checked for normality using a Shapiro-Wilk test. Total area under the curve (AUC) values were calculated using the trapezoidal method. AUC were calculated for subjective appetite-related variables, plasma concentrations of substrates/hormones (**Chapters 6 and 7**), energy expenditure (**Chapter 6**), and substrate oxidation (**Chapter 6**), and were averaged over specific time periods (**Chapters 5 and 6**). In chapters comprising >2 trials (*i.e.*, **Chapters 6 and 7**), data containing one factor (*ad-libitum* energy intake at individual meals, exercise performance, exercise subjective responses, and AUC values) were analysed using one-way repeated measures ANOVA. When ANOVA main effects were significant, and in **Chapter 5** due to the inclusion of only 2 trials, data were further analysed using paired samples *t*-tests (normally distributed) or Wilcoxon Signed-Rank tests (not normally distributed), as appropriate. Holm-Bonferroni stepwise adjustments for multiple comparisons were made to reduce type I error rate. Data containing two factors (appetite sensations, plasma substrate/hormone concentrations, and energy expenditure and substrate oxidation rates) were analysed using two-way repeated-measures ANOVA. Significant ANOVA main effects were explored with *post-hoc* paired samples *t*-tests, or Wilcoxon Signed-Rank tests, with Holm-Bonferroni stepwise correction. Assumptions of sphericity of the ANOVA were checked and adjustments for the degrees of freedom were made using the Greenhouse-Geiser ($\varepsilon < 0.75$) or Huynh-Feldt ($\varepsilon > 0.75$) correction, where appropriate. Data sets were considered statistically different when $P < 0.05$. Data are presented as mean ± 1 standard deviation, unless otherwise stated. Where appropriate, effect sizes (Cohen's *dz*) were calculated for within-measures comparisons, with 0.2, 0.5, and 0.8 representing small, medium, and large effect sizes, respectively (Cohen, 1988).

Chapter 4 – Exercise timing behaviours: opportunities, barriers, preferences, proximity to eating, and the impact of COVID-19

4.1. Introduction

Regular exercise is implicated in successful weight management and the prevention of several chronic diseases, such as obesity, cardiovascular disease, and type 2 diabetes (Pedersen & Saltin, 2015; Warburton et al., 2006). A number of endogenous processes, such as macronutrient metabolism, substrate oxidation, and mitochondrial function, undergo circadian (24-h) oscillations (Jiank & Turek, 2017; Rynders et al., 2020; van Moorsel et al., 2016). Therefore, the time of day in which exercise is performed could affect the physiological response (Parr et al., 2020*b*). For example, afternoon/evening exercise has been shown to augment improvements in insulin sensitivity compared to the same exercise performed in the morning (Mancilla et al., 2021; Moholdt et al., 2021; Savikj et al., 2019).

Diurnal variations in eating behaviours may also contribute to these divergent outcomes. Consuming the same meal at different times of the day elicits a different metabolic response (Sopowski et al., 2001; Van Cauter et al., 1992), meaning responses to exercise are also likely to vary based on its timing around meals (Edinburgh et al., 2018; Gonzalez et al., 2013; Hansen et al., 2017). As such, meal-exercise timing could be harnessed to maximise the benefits that are attained from exercise (Heden & Kanaley, 2019; Mancilla et al., 2020). One popular strategy involves exercising after an extended period of fasting (Wallis & Gonzalez, 2019), which acutely increases fat oxidation (Edinburgh et al., 2018; Gonzelez et al., 2013), and may enhance improvements in insulin sensitivity with training (Edinburgh et al., 2020; Van Proeyen et al., 2010).

Despite the aforementioned evidence which alludes to the influence of timing on the responses to exercise, interventions aiming to investigate the effectiveness of exercise training on health outcomes do not typically consider, nor control, the timing of its prescription (Brooker et al., 2019). The consideration of exercise timing is particularly important, not solely due to circadian variations in physiology and eating behaviours, but also because it may have a bearing on adherence (Schumacher et al., 2019; Schumacher et al., 2020) – a major determinant of the long-term effectiveness of dietary and exercise interventions (Alhassan et al., 2008; Gibson & Sainsbury, 2017; Stonerock & Blumenthal, 2017). Compliance with exercise guidelines is generally poor (Guthold et al., 2018), and when considering the busy lifestyles many people lead, it is perhaps no surprise that a ‘lack of time’ is a frequently cited barrier to

performing sufficient exercise (Cerin et al., 2010; Trost et al., 2002). As such, it is increasingly important that exercise interventions are developed and assessed with an appreciation of their timing, thus allowing for their convenient incorporation within the daily lives of the population to aid adherence.

Because the overnight fast offers a practical and convenient opportunity to achieve a fasted state, the application of fasted exercise at other times of day is relatively unknown. The adoption of an overnight fasted exercise regime might not be appropriate or achievable for a large proportion of the general population (*e.g.,* full-time workers and/or parents). Therefore, it should be considered whether the metabolic adaptations to fasted exercise could be attained at an alternative time of day (*i.e.,* afternoon/evening) to increase the application of this intervention. The populations' perceptions of, and willingness to engage with such an intervention should first be explored before efforts are made to empirically examine its effectiveness.

The COVID-19 pandemic in 2020 forced governments of counties worldwide to enforce states of lockdown and implement social distancing measures as a means of decelerating infection rates within the population. Data collection for this survey took place during this lockdown period, therefore, questions relating to 'typical' exercise timing behaviours were asked to subjects retrospectively. These same questions were also asked to subjects in the context of their 'current' behaviours during lockdown restrictions, which allowed for the examination of exercise timing behaviours outside of typical routine.

This survey aimed to explore and understand (1) exercise timing behaviours and preferences; (2) perceived barriers to exercise at specific times of the day; and (3) experiences and perceptions of fasted exercise. The findings from this survey would inform the design of subsequent studies within this thesis.

4.2. Methods

Subjects

A cross-sectional online survey was completed by 516 adults. All subjects provided informed consent prior to completing the survey (**Appendix A**). Subjects had to be ≥18 years and engaging in some form of planned and/or structured physical exercise at least once per week for at least 6 months prior to the implementation of COVID-19 lockdown restrictions and/or at least once per week since the implementation of COVID-19 lockdown restrictions (self-reported). Due to not satisfying the inclusion criteria, responses from 4 subjects were excluded, and analysis was performed on the remaining 512 responses. When subject characteristics (*i.e.*, body mass index (BMI) and age) were included as co-factors within the analysis, subjects were grouped into categories defined by specific criteria. For BMI, the categories were: underweight (<18.5 $kg \cdot m^{-2}$), normal weight (18.5–24.9 $kg \cdot m^{-2}$), overweight (25–29.9 $kg \cdot m^{-2}$), and obese (≥30 $kg \cdot m^{-2}$) (Centers for Disease Control and Prevention, 2022). For age, these were: 18–24, 25–34, 35–44, 45–54, 55–64, and ≥65 years.

Questionnaire

An internet-based survey (Jisc Online Surveys: www.onlinesurveys.ac.uk) was opened on 13 May 2020 for a period of 5 weeks and subsequently closed on 17 June 2020. Subjects were recruited via several avenues, including social media platforms (Twitter and Facebook), text messaging, email to Nottingham Trent University staff and students, and word-of-mouth. Additionally, a link to the survey in English was included within a news article published online (The Conversation, 2020). The survey was purposely designed to achieve the objectives of the study with the support of a qualitative research expert at Nottingham Trent University with considerable experience of conducting survey-based research studies. A pilot version of the survey was completed by 16 individuals on 8 May 2020 to obtain information on questionnaire completion times, acceptability, and clarity of the questions and answer options. The pilot version was subsequently revised based on feedback, and the final survey comprised questions that were divided into five sections, obtaining information about (1) demographic characteristics; (2) exercise behaviours before lockdown restrictions were implemented; (3) exercise behaviours since the implementation of lockdown restrictions; (4) exercise timing opportunities, preferences, and barriers; and (5) perceptions and experiences of fasted exercise.

For the questions in Sections 4 and 5, respondents were asked to answer based on the absence of any lockdown restrictions. Questions included in each section are detailed below:

Section 1: Demographic characteristics

Subjects reported their biological sex, age, height, weight, employment status prior to lockdown restrictions, the impact of lockdown restrictions on their working status, and their number of dependent children under 18 years of age.

Section 2: Exercise behaviours before lockdown restrictions were implemented

Exercise behaviours in the month prior to the implementation of lockdown restrictions were reported by subjects. Questions were designed to assess the typical timing of exercise with regards to the time of day, as well as the proximity to food intake. Additional questions were also included to assess the types of exercise that subjects were engaging in, and the locations in which exercise took place. For time of day, respondents selected the single time window within which they most often began exercising from a selection of five windows ('earlier than 08:00', '08:00–11:59', '12:00–15.59', '16:00–19:59', and '20:00 or later'). Separate responses were obtained for a typical weekday (Monday–Friday), and for a typical weekend day (Saturday–Sunday). Subjects could also state that they did not typically exercise during the week or weekend. Next, subjects reported when they typically had their last meal, snack, or calorie containing drink before exercising at the time selected in the previous question. Again, separate weekday and weekend responses were obtained. Options were: 'less than 1 h before', '1–2 h before', '2–3 h before', '3–4 h before', '4–5 h before', 'more than 5 h before', and 'no typical time (different every time)'. Subjects then selected all types of exercise they engaged in (from: 'cardiovascular/aerobic', 'resistance training', 'body-weight exercises', 'yoga/pilates', 'competitive sport', and 'other') and the locations in which their exercise took place (from: 'gym/leisure centre', 'home', 'public outdoor spaces', 'sport-specific facilities', and 'other').

Section 3: Exercise behaviours since the implementation of lockdown restrictions

Subjects repeated the questions stated in section 2, this time reporting their exercise behaviours since the implementation of lockdown restrictions.

Section 4: Exercise timing opportunities, preferences, and barriers

Subjects reported the times of day that they had *opportunity* to exercise on a typical weekday from the following options: 'earlier than 08:00', '08:00–11:59', '12:00–15:59', '16:00–19:59', and '20:00 or later'. Subjects were asked to consider any work, family, or social commitments in their answer to this question and could select multiple options. From the same selection, subjects reported a single window as their *preferred* time of day for exercise, if they had the full 24 h available to them, in the absence of any work, family, or social commitments. Subjects then reported the specific barriers/commitments preventing them from exercising at their preferred time, selecting all applicable options from a selection, including 'job/work commitments', 'spending time with family', 'providing care for children', and 'time spent on other hobbies/pastimes' (full list provided in **Table 4.2**). 'Not applicable. I am able to exercise at my preferred time' was also included as an option to be selected, if appropriate.

Section 5: Perceptions and experiences of fasted exercise

Subjects reported whether they would be willing to fast for an extended period of time (6–8 h, not including the overnight fast) prior to exercise if it was shown to provide additional benefits for their health (options: 'yes', 'no', or 'unsure'). Those selecting 'no' and 'unsure' were then asked to report how long they would consider fasting for before exercise from choices ranging from less than 1 h to 5–6 h. Finally, subjects were asked to report whether they had previously experienced fasting for 6–8 h before exercise (not including exercising after the overnight fast). Those with experience were asked to report how this had made them feel during exercise, whereas those without experience were asked to postulate how they thought exercise would feel under these conditions. In both cases, subjects selected all applicable options from a selection of seven positive and eight negative feelings (full list provided in **Table 4.3**).

Data Analysis

Data were analysed using Microsoft Excel and SPSS v26.0 (IBM, USA). To prevent duplicate data, the database was searched for non-unique subject-generated 6-digit codes (generated based on answers to a series of security questions). In cases of identical 6-digit codes, data were visually checked to confirm that the responses were different. Because not all questions were relevant to all subjects, details on sample size are provided where relevant, and all analyses were performed on the full data set for each question. Pearson's chi-squared analyses were used to examine the relationships between categorical variables. Fisher's exact tests were used for 2x2 contingency tables where >20% of expected cell counts were <5 (Quinn & Keogh, 2002). For contingency tables larger than 2x2, the Monte Carlo method was applied in instances where >20% of expected cell counts were <5 (Mehta & Patel, 2011). Exercise timing windows have been categorised into times of the day for the purpose of aiding the discussion, *i.e.*, early morning (earlier than 08:00), morning (08:00–11:59), afternoon (12:00–15:59), early evening (16:00–19:59), and late evening (20:00 or later). It was agreed that the period of time preceding the implementation of lockdown restrictions was more representative of subjects 'normal' behaviours. Therefore, results regarding exercise timing behaviours are initially presented exclusively for the pre-lockdown period. Comparisons of exercise behaviours before and during lockdown restrictions are made later in the section. Data are presented as mean ± 1 standard deviation (SD), frequencies (*n*), and distributions (%), and differences were considered statistically significant when $P < 0.05$.

4.3. Results

Sample Description

The sample consisted of 512 respondents (71.9% female; age 39 ± 13 years; BMI 25.7 ± 4.7 $kg \cdot m^{-2}$). Demographic information including work status prior to the implementation of lockdown restrictions, the impact of lockdown restrictions on work status, and the number of dependent children is summarised in **Table 4.1**.

Table 4.1. Demographic information of subjects (*n*=512).

Variables		
Sex	**Frequency (*n*=512)**	**(%)**
Male	144	28.1
Female	368	71.9
Age (years)	**Frequency (*n*=512)**	**(%)**
18 – 24	81	15.8
25 – 34	162	31.6
35 – 44	96	18.8
45 – 54	94	18.4
55 – 64	67	13.1
≥65	12	2.3
BMI classification	**Frequency (*n*=506*)**	**(%)**
Underweight (<18.5 $kg.m^{-2}$)	4	0.8
Normal Weight (18.5 – 24.9 $kg.m^{-2}$)	255	50.4
Overweight (25 – 29.9 $kg.m^{-2}$)	167	33.0
Obese (≥30 $kg.m^{-2}$)	80	15.8
Work status before social distancing	**Frequency (*n*=512)**	**(%)**
Employed Full-Time	278	54.3
Employed Part-Time	76	14.8
Student	62	12.1
Retired	21	4.1
Self-Employed	43	8.4
Unemployed	11	2.1
Paid or unpaid leave of absence	2	0.4
Other	19	3.7
Impact of social distancing on working status	**Frequency (*n*=512)**	**(%)**
Not changed	64	12.5
Changed, but still able to work from usual location	49	9.6
Changed, now working remotely	283	55.3
Changed, not working due to being furloughed/reduced workload	90	17.6
Other	26	5.1
Number of dependent children	**Frequency (*n*=512)**	**(%)**
0	392	76.6
1	51	10.0
2	51	10.0
3	13	2.5
4	5	1.0

* Due to obvious errors in inputting of height and/or weight information, BMI data from 6 subjects has been omitted.

Exercise Timing During the Week and Weekend

Of the 512 subjects included, 99.0% (*n*=507) reported that they engaged in exercise at least once during the week, and 87.9% (*n*=450) reported exercising at least once during the weekend. One subject reported that they did not exercise at least once during either the week or the weekend. Significant differences were observed for exercise timing during the week compared to the weekend ($P<0.001$). The most common time to begin exercise during the week was the early evening (16:00–19:59) (40.4%), whereas during the weekend it was the morning (08:00–11:59) (57.6%). In contrast, only 10.4% selected the early evening during the weekend and only 22.1% selected the morning during the week. The late evening (20:00 or later) was identified by the smallest proportion of respondents during the week (8.1%) and weekend (1.8%), respectively (**Figure 4.1**).

A significant association between employment status and weekday exercise timing was identified ($P < 0.001$; **Figure 4.2**). A greater proportion of subjects in full-time employment engaged in exercise in the early evening (49.6%) compared to those not working (11.8%), in self-employment (18.6%), and in part-time employment (32.9%). A smaller proportion of those in full-time employment exercised in the morning (11.6%) compared to those not working (52.9%), in self-employment (37.2%), and part-time employment (34.2%). Age was also associated with weekday exercise timing ($P < 0.001$; **Figure 4.3**). A greater proportion of subjects aged ≥45 years engaged in exercise in the morning (35.1%) compared to those aged <45 years (15.5%). Also, a smaller proportion of subjects aged ≥45 years engaged in exercise in the early evening (26.9%) compared to those aged <45 years (47.3%). Exercise timing behaviours were not associated with sex ($P = 0.118$), BMI ($P = 0.729$), or number of dependent children ($P = 0.600$; **Figures 4.4–4.6**). Weekend exercise timing was not associated with employment status ($P = 0.314$), age ($P = 0.127$), sex ($P = 0.103$), BMI ($P = 0.567$), or number of dependent children ($P = 0.413$).

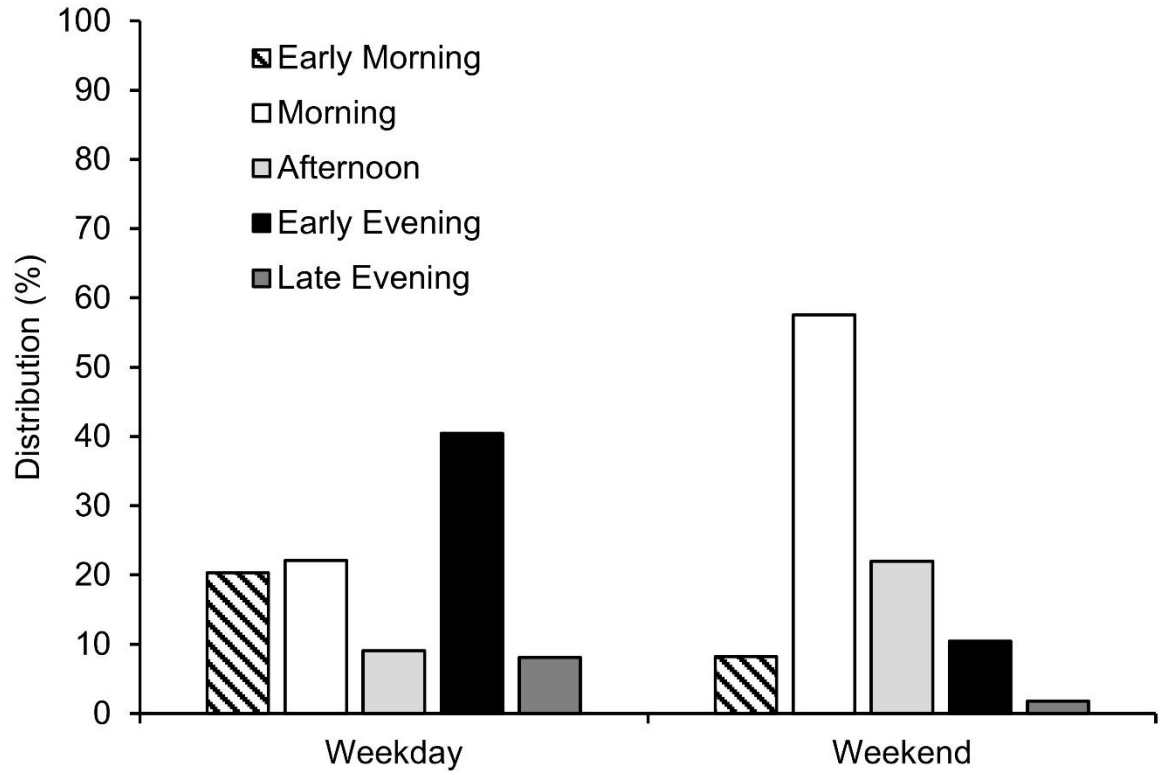

Figure 4.1. Distribution of responses (%) for the timing of exercise during the week (*n*=507) and weekend (*n*=450).

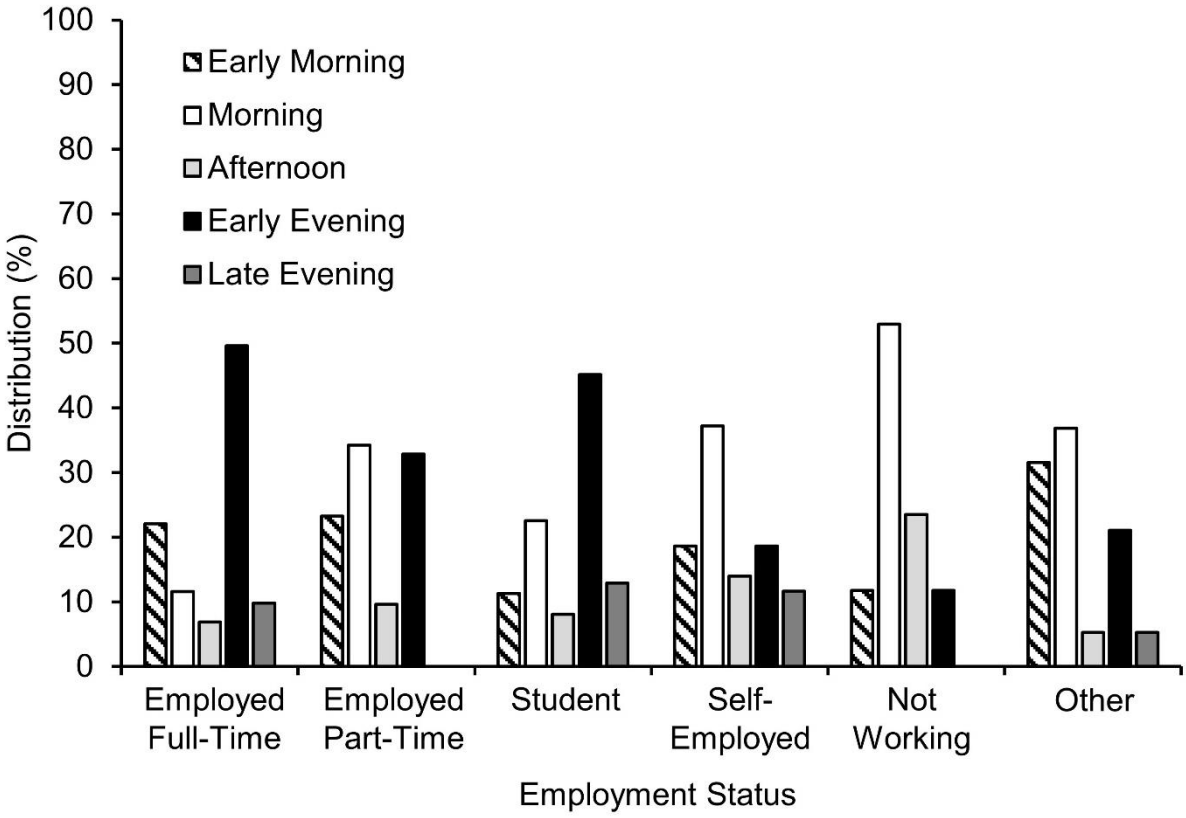

Figure 4.2. Distribution of responses (%) for the timing of exercise during the week by subject employment status (*n*=507). Unemployed, retired, and paid/unpaid leave of absence (e.g., maternity leave) have been grouped under "Not Working".

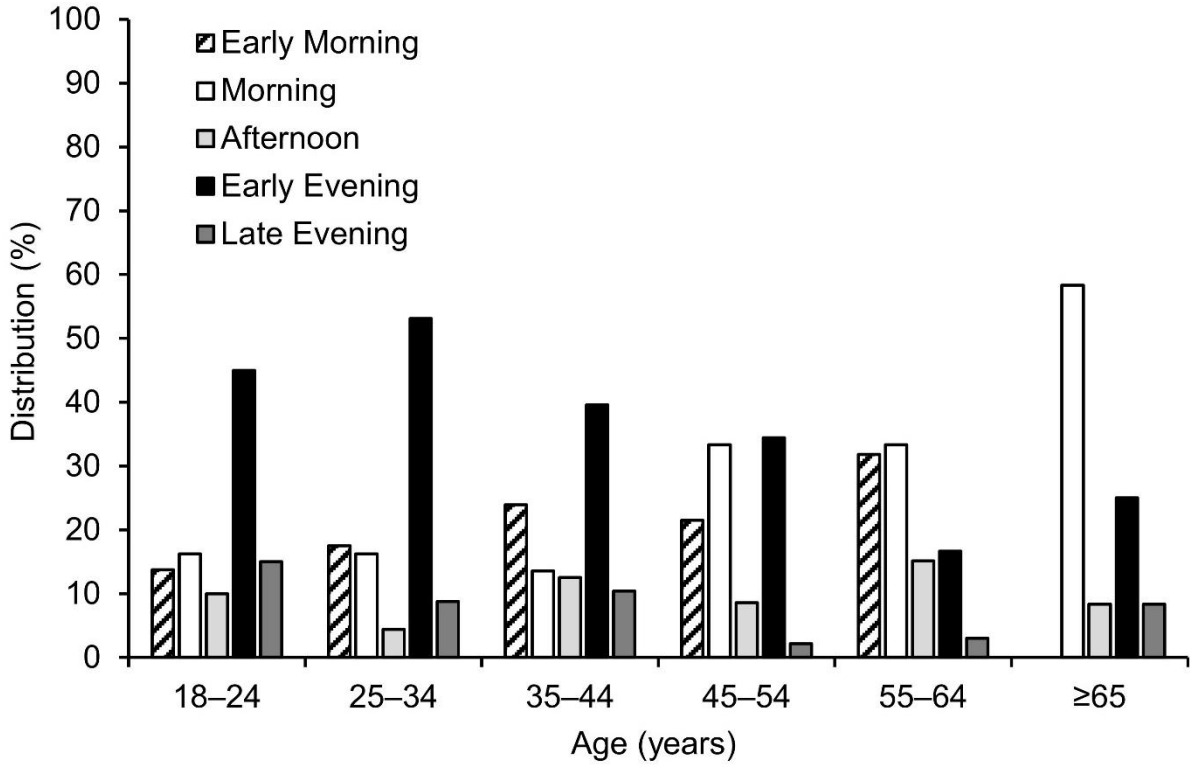

Figure 4.3. Distribution of responses (%) for the timing of exercise during the week by subject age category (*n*=507).

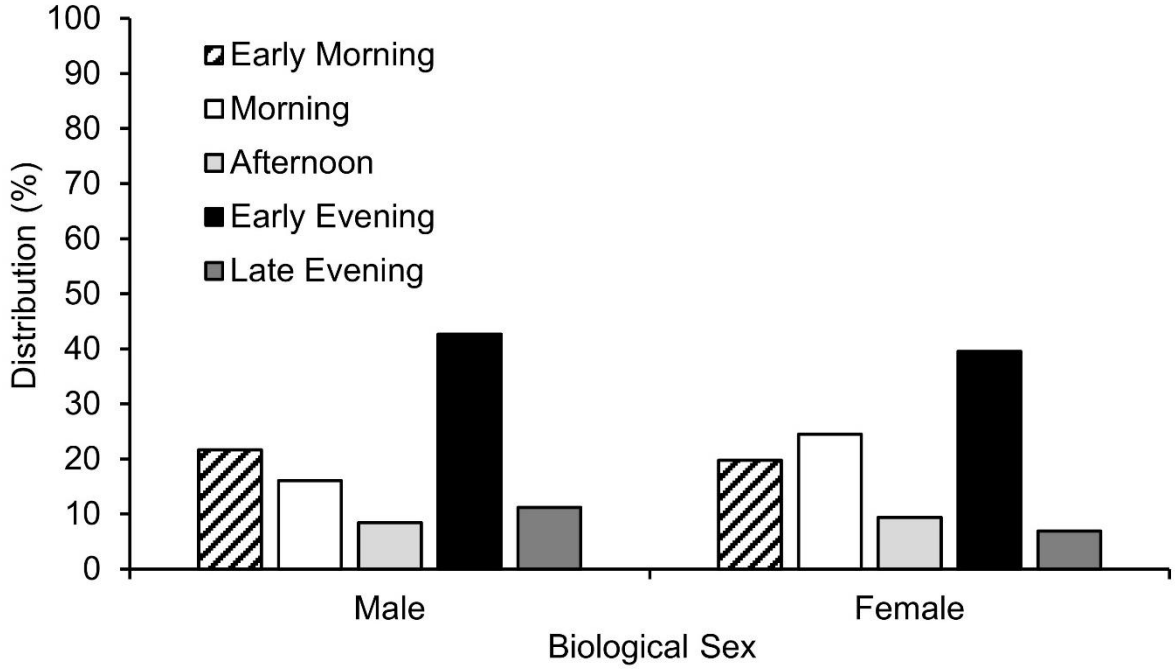

Figure 4.4. Distribution of responses (%) for the timing of exercise during the week by subject biological sex (*n*=507).

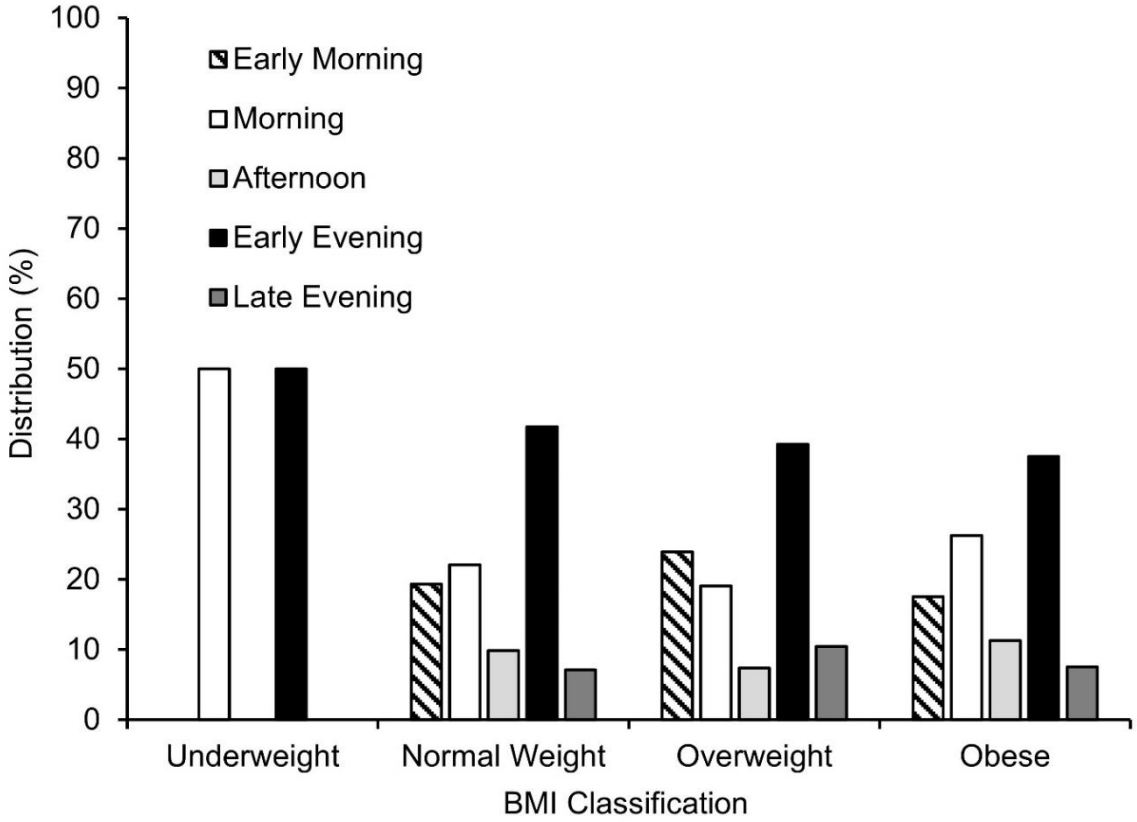

Figure 4.5. Distribution of responses (%) for the timing of exercise during the week by subject BMI classification (*n*=501).

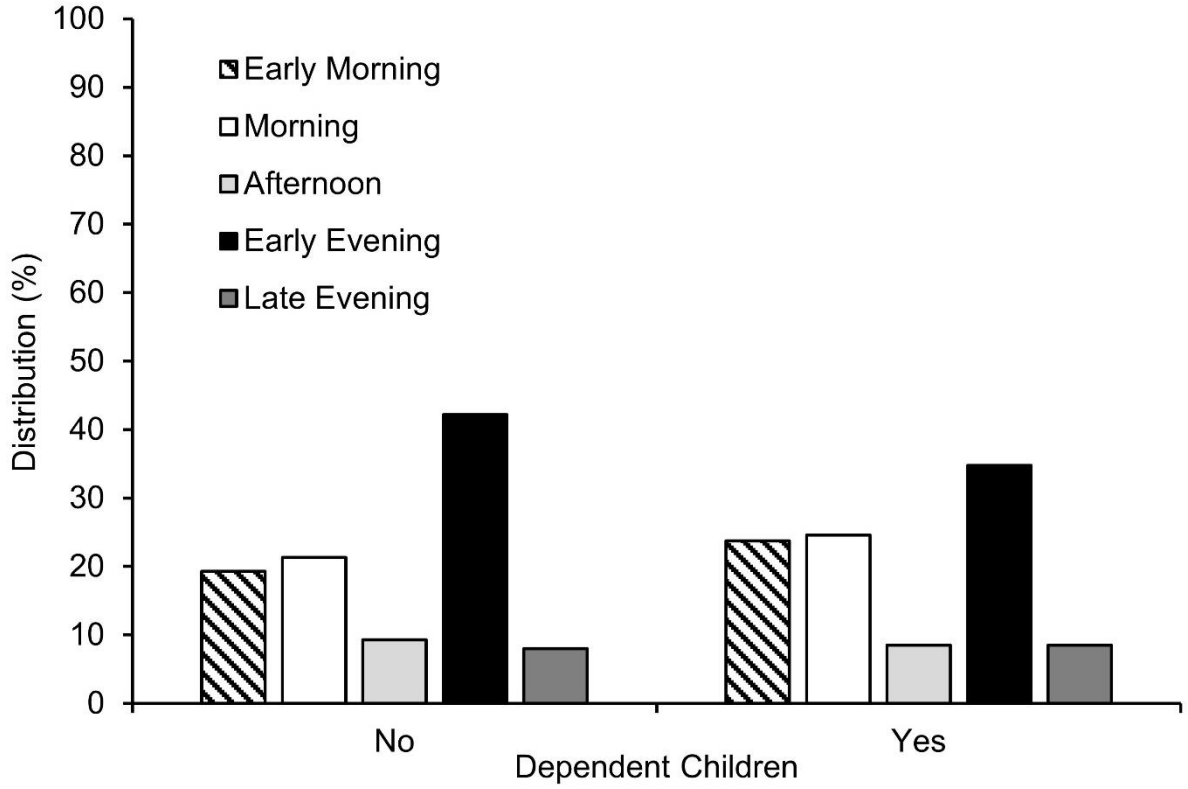

Figure 4.6. Distribution of responses (%) for the timing of exercise during the week by whether the subject has any dependent children (*n*=507). Subjects reporting 1, 2, 3, and 4 dependent children have been grouped under "Yes".

Meal-Exercise Timing

During the week and the weekend, the timing of the last meal or snack prior to beginning exercise was associated with the time of day that exercise took place (both $P < 0.001$). When exercising in the early morning, the largest proportion of subjects reported consuming their last meal or snack 'more than 5 h before' exercise during the week (59.2%) and the weekend (56.8%). This option was selected less frequently when exercise occurred later in the day (≤27.0%), with '1–2 h before' representing the most common option at all other times of the day during both the week and weekend (32.1–55.3%; **Figure 4.7**).

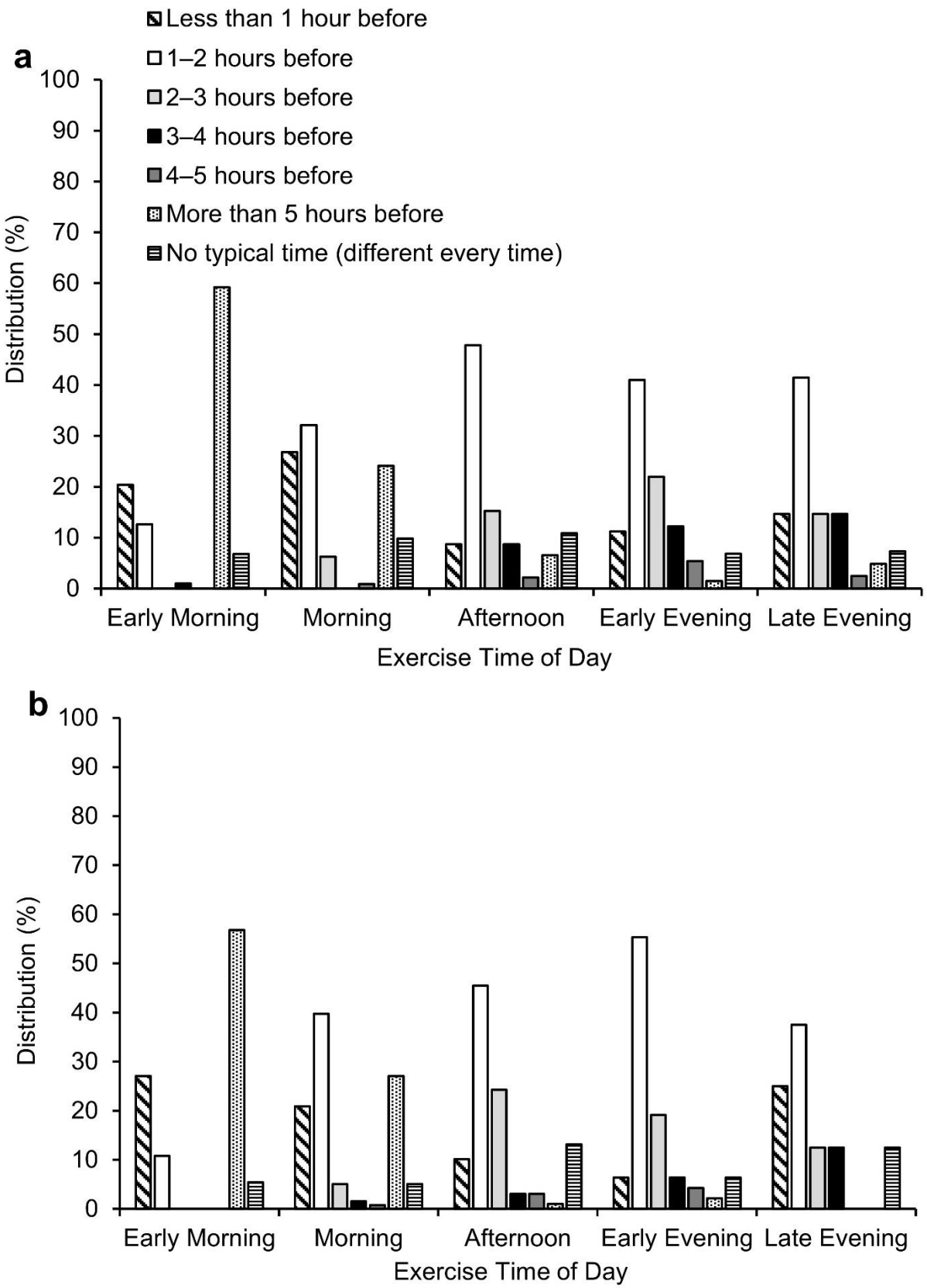

Figure 4.7. Typical proximity of exercise to the prior meal/snack based on the time of day of exercise **(a)** during the week (*n*=507) and **(b)** weekend (*n*=450).

Exercise Timing Opportunities, Preferences, and Barriers

On a typical weekday, the most commonly reported *opportunity* to exercise was in the early evening (59.0%), followed by the early morning (47.7%), late evening (42.2%), morning (30.9%), and afternoon (22.9%). The most commonly selected *preferred* time to exercise was the morning (52.4%), followed by the early morning (20.1%), afternoon (15.0%), early evening (10.0%), and late evening (2.5%).

A modest proportion of subjects (30.7%) reported that they had opportunity to exercise at their preferred time and 'job/work commitments' was the most commonly cited barrier preventing exercising at preferred times (**Table 4.2**).

Table 4.2. Barriers preventing exercise at preferred times (*n*=512).

Barriers	Frequency (*n*=512)	%
Job/work commitments	327	63.9
Not applicable. I am able to exercise at my preferred time	157	30.7
Spending time with family	84	16.4
Providing care for children	73	14.3
Time spent on other hobbies/pastimes	43	8.4
Other	26	5.1
Spending time with friends	23	4.5
Providing care for a dependent friend or family member	13	2.5

Experiences and Perceptions of Fasting Before Exercise

In total, 36.5% (*n*=187) respondents had previously experienced exercise following an extended period of fasting (6–8 h, not including exercising after the overnight fast). Positive feelings towards fasted exercise were reported by a greater proportion of subjects with experience (67.4%), compared to those without experience of fasted exercise (23.1%; $P < 0.001$). Also, a smaller proportion of subjects with experience reported negative feelings (50.8%), compared to those without experience (89.5%; $P < 0.001$) (**Table 4.3**).

Table 4.3. Prevalence of reported positive and negative feelings associated with previous experience (*n*=187) and expected experience (*n*=325) of 6–8 h fasted exercise (not including exercising after the overnight fast).

	With experience		**Without experience**	
	Frequency (*n*=187)	**%**	**Frequency (*n*=325)**	**%**
Positive Feelings				
No different to when I ate/eat closer to exercise	42	22.5	15	4.6
Light/weightless	40	21.4	44	13.5
Motivated	38	20.3	11	3.4
Energetic	37	19.8	10	3.1
Comfortable	32	17.1	5	1.5
Focused	30	16.0	11	3.4
Like my performance was/is improved/enhanced	27	14.4	7	2.2
Selected positive feeling	**126**	**67.4**	**75**	**23.1***
Did not select positive feeling	**61**	**32.6**	**250**	**76.9***
Negative Feelings				
Tired	46	24.6	217	66.8
Hungry	44	23.5	213	65.5
Like my performance was/is reduced/impaired	41	21.9	145	44.6
Lightheaded	35	18.7	169	52.0
Nauseous/sick	17	9.1	97	29.8
Uncomfortable	17	9.1	70	21.5
Heavy	8	4.3	26	8.0
Demotivated	5	2.7	74	22.8
Selected negative feeling	**95**	**50.8**	**291**	**89.5***
Did not select negative feeling	**92**	**49.2**	**34**	**10.5***

* Indicates a significant effect of experience ($P < 0.05$).

Willingness to Engage in Fasted Exercise

When asked: ‘not including the overnight fast (whilst sleeping), would you consider not eating for 6–8 h before exercise, if it was shown to provide additional benefits for your health?’, 49.6% (*n*=254) of respondents answered ‘yes’, 30.9% (*n*=158) answered ‘no’, and 19.5% (*n*=100) answered ‘unsure’. Significantly more of those who reported a previous experience of exercise following an extended period of daytime fasting answered ‘yes’ (83.4%), compared

to those reporting no experience (30.2%; $P < 0.001$). Of the subjects with experience, only 8.6% selected ‘no’ and 8.0% selected ‘unsure’, compared to 43.7% and 26.1%, respectively, for those with no experience.

From the of respondents who selected ‘no’ (n=158) and ‘unsure’ (n=100), the most frequently selected maximum pre-exercise fasting duration that individuals would consider was ‘3–4 hours’ (31.4%), followed by ‘2–3 hours’ (29.8%), ‘4–5 hours’ (15.5%), ‘1–2 hours’ (13.2%), ‘5–6 hours’ (5.4%) and ‘less than 1 hour’ (4.7%).

Effect of Lockdown Restrictions on Exercise Behaviours

Whilst lockdown restrictions were in place, 98.2% (n=503) and 90.0% (n=461) respondents reported engaging in exercise at least once during the week and the weekend, respectively. Three subjects reported that they did not exercise at least once during either the week or the weekend. Significant differences in exercise timing were observed compared to before lockdown restrictions were implemented for weekday ($P < 0.001$), but not weekend ($P = 0.117$) exercise. During the week, the morning (32.0%) and early evening (31.2%) were the most commonly selected time windows for exercise to commence during lockdown restriction, and the morning was the most frequently selected option during the weekend (49.2%). Exercise commencing in the late evening represented the minority of responses during both the week (4.6%) and the weekend (2.0%) during lockdown restriction (**Figure 4.8**).

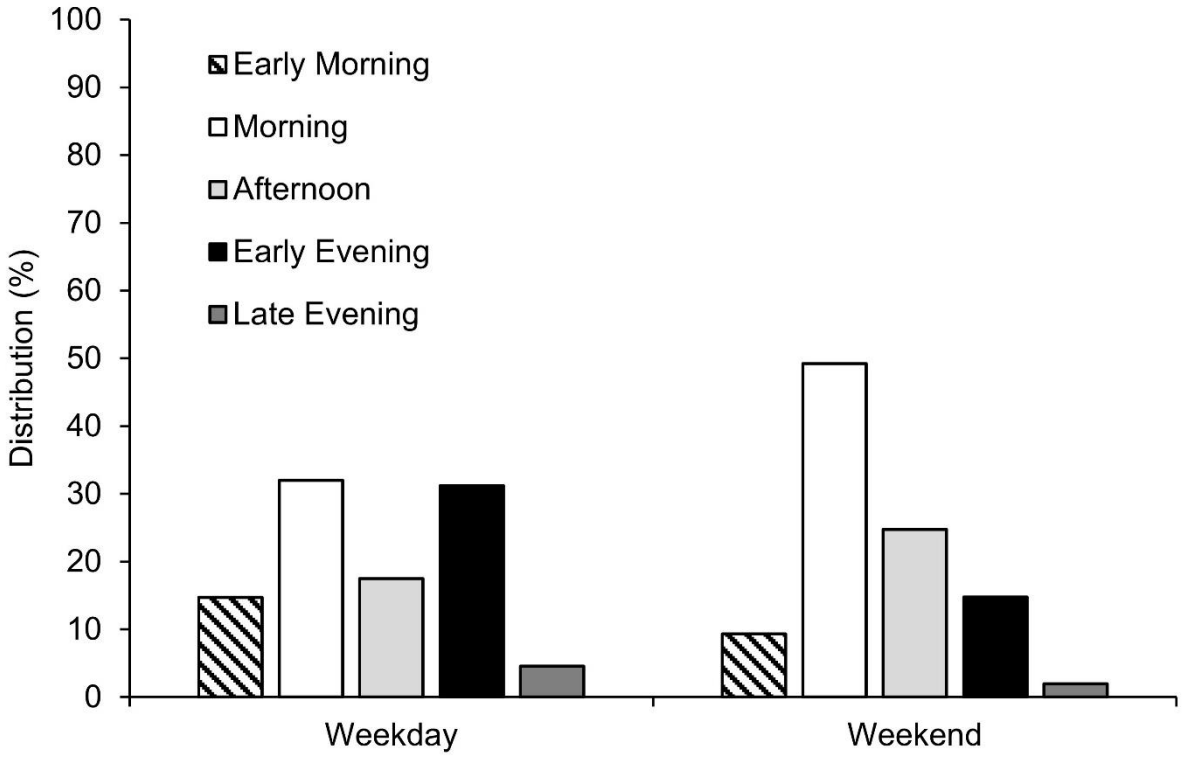

Figure 4.8. Distribution of responses (%) for the timing of exercise during the week (*n*=503) and weekend (*n*=461) since the implementation of lockdown restrictions.

The timing of the last meal or snack prior to beginning exercise was also associated with the time of day that exercise took place during lockdown restriction ($P < 0.001$). Similar to the responses observed prior to the implementation of lockdown restrictions, when exercising in the early morning, the largest proportion of subjects reported consuming their last meal or snack 'more than 5 h before' exercise during the week (62.2%) and the weekend (53.5%). 'More than 5 h before' was also selected by the largest proportion of respondents exercising in the morning during the week (32.3%). This option was selected less frequently at all other exercise times during the week and weekend (≤26.0%), with '1–2 h before' representing the most common option (29.4–55.6%).

Since the implementation of lockdown restrictions, there was an increase in the proportion of respondents who reported engaging in aerobic/cardiovascular exercise ($P < 0.05$) and body weight exercise ($P < 0.01$) in comparison to before lockdown restriction. In contrast, the proportion who reported engaging in resistance training and competitive sport decreased after the implementation of lockdown restrictions ($P < 0.001$). More people reported exercising at home ($P < 0.001$) and in public outdoor spaces ($P < 0.001$) since lockdown restriction, and less

reported exercising within a gym/leisure centre ($P < 0.001$) and within sport-specific facilities ($P < 0.001$) (**Table 4.4**).

Table 4.4. Type and location of exercise before (n=511) and since (n=509) the implementation of COVID-19 lockdown restrictions.

Question	**Before COVID-19 Lockdown**		**Since COVID-19 Lockdown**		***P* value**
Type	**Frequency (n=511)**	**(%)**	**Frequency (n=509)**	**(%)**	
Cardiovascular/aerobic	443	86.7	462	90.8	0.040*
Resistance training	296	57.9	163	32.0	0.001*
Body-weight exercises	235	46.0	279	54.8	0.005*
Yoga/Pilates	145	28.4	173	34.0	0.053
Competitive sport	98	19.2	8	1.6	0.001*
Other	10	2.0	19	3.7	0.088
Location	**Frequency (n=511)**	**(%)**	**Frequency (n=509)**	**(%)**	
Gym/leisure centre	335	65.6	8	1.6	0.001*
Home	154	30.1	410	80.6	0.001*
Outdoor spaces	287	56.2	374	73.5	0.001*
Sport-specific facilities	152	29.7	5	1.0	0.001*
Other	18	3.5	9	1.8	0.081

* Indicates significant difference between before and since COVID-19 lockdown ($P < 0.05$).

4.4. Discussion

In this sample of 512 adults, the early evening (16:00–19:59) was the most frequently reported time for exercise to commence on a typical weekday (Monday–Friday), compared to the morning (08:00–11:59) during the weekend (Saturday–Sunday). These data suggest that the early evening should be considered a primary target for the implementation of exercise interventions to improve public health.

Weekdays account for the greatest proportion of working hours amongst society (Bryson & Forth, 2007) and as such, a large proportion of the population are likely constrained in when they are able to exercise due to work commitments. This is supported by our observations that, as well as the early evening, exercise in the early morning was also more prevalent during the week. However, early evening was still the most common exercise time, suggesting that the post-work period is the most feasible time for weekday exercise for many. In support of this, subjects in full-time employment exhibited this weekday distribution pattern most clearly,

whereas unemployed, retired, and self-employed subjects displayed a distribution of exercise across the day that closely mirrored that of the overall weekend distribution, with exercise typically occurring during the morning. A similar exercise timing distribution has been reported previously, with more early-morning and evening exercisers being in employment compared with late-morning exercisers (Schumacher et al., 2019). The tendency towards choosing to exercise earlier in the day in the absence of work commitments is supported by our data showing that the largest proportion (52.4%) of the sample selected the morning as their preferred time of day for exercise. These findings concur with a study in older adults, in which '09:00–12:00' was the preferred exercise time for the majority of subjects (Cohen-Mansfield et al., 2004). However, in the present study, just 30.9% of subjects had the opportunity to exercise in the morning, compared to 59.0% who had opportunity to exercise in the early evening. Amongst other commitments such as 'spending time with family' and 'providing care for children', 'job/work commitments' was the most commonly cited barrier preventing exercise at preferred times.

There is clearly discordance between when people prefer to exercise and when they have opportunity to exercise, and our data suggest that opportunity is the primary determinant of exercise timing behaviours. These findings are particularly relevant to employers and policy makers, who are increasingly encouraged to support employees to be more physically active. As well as improving the health status of the employees, workplace initiatives can also increase work productivity and reduce sickness absence rates (Mills et al., 2007). The National Institute for Health and Care Excellence (2008) recommend that organisations develop a plan that includes measures to maximise the opportunity for all employees to participate in physical activity and suggest that flexible working policies may facilitate this. This recommendation is supported by data from the present study, and employers may wish to consider adopting flexible working policies, where feasible, as a means of increasing opportunity for employees to engage in regular exercise.

Whilst strategies that provide individuals with the opportunity to exercise at their preferred time of day may improve adherence, there is some evidence to suggest that this may not be absolutely necessary. Brooker and Colleagues (2019) conducted a study to examine the feasibility and acceptability of exercise performed in the morning and the evening over a 12-week period, and despite random trial allocation resulting in some subjects being assigned to an exercise condition which did not align with their preferred time to exercise, adherence rates were high, and 'unfavourable group allocation' was not reported by any subjects as a reason

for study withdrawal. These findings were attributed to 'temporal consistency', which is the idea that performing a behaviour, *i.e.*, exercise, at a specific time regularly, creates a protected time for exercise habits and increases both engagement and adherence (Kaushal & Rhodes, 2015; Schumacher et al., 2019; Schumacher et al., 2020), regardless of the exercise timing preference (Brooker et al., 2019). Adherence to physical activity guidelines is poor (Guthold et al., 2018), and findings from the present study indicate that the early evening is the time of day with most opportunity for consistent exercise for a large proportion of the population. Future studies should, therefore, aim to evaluate the effects of exercise sessions specifically conducted in the early evening.

Whilst adherence is likely to have the greatest effect on the success or failure of an exercise intervention, there is also evidence to suggest that the physiological responses to exercise at different times of day could differ, mediated in part, by circadian rhythms. The circadian system is comprised of a master pacemaker (suprachiasmatic nucleus) located in the hypothalamus, which synchronises peripheral clocks located in nearly every cell of the body, to daily light and dark cycles (Mohawk et al., 2012). Specifically, peripheral clocks located within tissues such as the liver, pancreas, adipose tissue, stomach, and skeletal muscle are collectively involved in a plethora of key metabolic processes (Dibner et al., 2010). There is evidence that performance in sports requiring physical effort is superior later in the day (Atkinson & Reilly, 1996; Kusumoto et al., 2021), and whilst the optimal timing of exercise for improvements in metabolic health and weight management remains to be elucidated (Gabriel & Zierath, 2019; Janssen et al., 2022; Mansingh & Handschin, 2022; Parr et al., 2020*b*), it should not be assumed that the responses to a tested intervention will be reproducible when performed at different times of the day.

For instance, it is well established that the same meal consumed at different times of the day can result in distinct metabolic responses (Sopowski et al., 2001; Van Cauter et al., 1992). Given that meal consumption rapidly alters the profile of circulating metabolites, ultimately impacting on substrate oxidation during exercise and subsequent energy storage (Hawley et al., 2011), it is likely that interactions between nutrition status and exercise may be profoundly influenced by the time of day. Furthermore, our data demonstrate that exercise occurring at different times of the day is likely to be performed in different nutritional states due to diurnal alterations in the timing of nutrient intake prior to exercise. Early morning exercise is more likely (53.5–62.2% of subjects) to take place following a period fasting (>5 h), possibly due to exercising after an overnight fast, whereas exercise after midday typically occurs 1–2 h after

consuming a meal (29.4–55.6% of subjects). Acute exercise performed in either the fed or fasted state leads to distinct responses for substrate metabolism and subsequent glycaemic control (Edinburgh et al., 2018; Gonzalez et al., 2013), further highlighting the potential impact of nutrition-exercise interactions, in addition to circadian rhythms, on the outcomes of exercise performed at different times of the day.

Interestingly, evidence exists to show that chronic exercise performed in a fasted state may enhance adaptations which lead to improved markers of metabolic health (Edinburgh et al., 2020; Robinson et al., 2015*a*; Van Proeyen et al., 2010). However, almost all research on fasted exercise has been undertaken in the morning. There is evidence to suggest that the metabolic responses to exercise may vary when performed at different times of the day, independent of the timing of the prior meal (Ezagouri et al., 2019). Therefore, despite it being common practice for laboratory trials to take place in the morning due to its practicality regarding diet and physical activity control, diurnal variations in physiology and nutrient metabolism indicate that findings derived from overnight-fasted exercise cannot necessarily be applied to situations where exercise is performed at an alternative time of the day. In order to maximise ecological validity and utility, future studies should be designed so that exercise timing coincides with when the majority of people have the opportunity to exercise, which our findings indicate is the early evening.

In the present study, just 36.5% of the sampled population had experience of exercising after a 6–8 h period of fasting (not including exercising after the overnight fast). Interestingly, 83.4% of those with prior experience reported willingness to engage in this practise if it were shown to benefit health, compared to only 30.2% of those without experience. Subjects with experience of exercising after a period of daytime fasting associated this type of exercise with feeling light/weightless, having greater motivation, or simply reported feeling no different to when they exercise after eating. In contrast, subjects without experience expressed concerns that it would make them feel tired, hungry, lightheaded, and impair their performance, which presumably contributed to their reluctance to engage in such interventions. A recent survey study in endurance athletes also noted similar reasons for avoiding exercise following an overnight fast (Rothschild et al., 2020), suggesting a lack of experience of performing fasted exercise may be conducive to negative preconceptions, but these appear to be replaced with positive feelings in those with experience. This is supported by data from a 12-week fasted exercise intervention in individuals with type 2 diabetes, which showed excellent tolerance and adherence rates (91%) (Verboven et al., 2020). This data is valuable as an aid to encourage

participation in this type of exercise intervention and explore its usage as a clinical tool to improve or maintain health.

A secondary aim of this study was to explore the effect of lockdown restrictions that were introduced during the COVID-19 pandemic on exercise behaviours within this population. The weekday-specific bias towards exercising later in the day was replaced during the lockdown period by a pattern that more closely reflected that of the weekend exercise pattern before lockdown restrictions (*i.e.,* morning was the most common time of day for exercise to occur). Following the implementation of lockdown restrictions, nearly three quarters of our sample reported they were either working from home, no longer working (furloughed or redundancy), or experiencing a reduced workload due to self-employment. These findings provide further support for our observation that work commitments have a strong influence on exercise behaviours, and when these commitments are lessened, there is a natural shift towards exercising in the morning, thus aligning with exercise timing preferences. It should be noted that countries internationally took measures that were different to one another, and in response to the severity of COVID-19 outbreak in that specific country. Therefore, the degree to which lockdown restrictions affected physical activity and exercise behaviours may have differed between countries (Pépin et al., 2020).

Data from the present survey also highlight that the implementation of lockdown restrictions had an important bearing on both the types of exercise that individuals were engaging in, and the locations in which that exercise took place. Specifically, there was a reduction in the number of respondents engaging in resistance exercise and competitive sports since the implementation of lockdown restrictions, whereas the number engaging in cardiovascular/aerobic exercise and bodyweight exercise increased. Perhaps unsurprisingly, this was mirrored by a reduction in the number of people exercising within gyms and sport-specific facilities, and an increase in those exercising at home and in public outdoor spaces. Alongside reduced work commitments, these shifts in exercise type and setting may have influenced the pattern of exercise timing behaviours during lockdown restriction. For example, commercial gyms in the UK are generally at their busiest between 17:00–19:00 (PureGym UK, 2022), and it is commonplace for sport-specific training sessions to take place in the evening. On the contrary, it may be more common for outdoor exercises, such as running, to take place in the morning due reduced daylight hours in the post-work period for many months of the year. Therefore, it is plausible that the movement in exercise away from gyms and sports

facilities, towards outdoor spaces and the home environment, facilitated the shift towards exercise earlier in the day during lockdown restriction.

This apparent influence of shifts in exercise type on the times of day that individuals engage in exercise may have been facilitated by when the survey was released. Specifically, the survey was open during the early UK summer months of May and June 2020. Therefore, when participants recalled their typical exercise behaviours from before the implementation of lockdown restrictions, this would likely have reflected exercise habits during the winter months. As alluded to earlier, the reduced daylight hours and less pleasant weather conditions during the winter may have made indoor, gym-based exercise, and thus exercise later in the day, more common. On the contrary, the increase in outdoor-based cardiovascular/aerobic exercise reported since the implementation of lockdown restrictions may have been due partly to the seasonal shift during the summer months. Therefore, the findings from the present study should be viewed within the context of when the survey was released.

This cross-sectional survey study provides important data relating to exercise timing with potential implications for the design of future research studies and the implementation of exercise interventions, although it does come with its limitations. Modern lifestyles are often characterised by hectic schedules underpinned by late-night exposure to artificial light, long working hours, extended commute times, and increased leisure time activities, which may promote considerable variability in daily behaviour patterns. For example, eating patterns are highly variable from day to day (Gill & Panda, 2015) and irregular sleep schedules are common (Taylor et al., 2016). It is probable then, that exercise timing also undergoes daily variation, which would not have been captured by the present survey due to the requirement for subjects to select a single time window within which they most often engaged in exercise. Although this study attempted to account for this variation by collecting distinct responses for weekday and weekend exercise timing, it would be prudent to explore how exercise timing behaviours vary on a day-to-day basis.

Second, the demographic variation amongst survey respondents was low, with the majority of subjects being young females (aged <45 years) without children. Therefore, the interpretation of the data may be largely constrained to this cohort, and larger, comparative studies involving a more even distribution of subjects across factors including sex, age, ethnicity, and socioeconomic status, would be insightful. Although a common challenge within research, engaging a more diverse sample could be facilitated by several strategies, including actively

engaging with diverse groups of individuals through targeted recruitment strategies within their typical settings (*i.e.*, advertising within online/offline groups dedicated to male- or female-specific topics), encouraging subjects from underrepresented groups to share details of the study amongst their peers, or by using a wider range of recruitment strategies, including those which do not rely on access to technology which may not be accessible across all cultural and socioeconomic groups (*i.e.*, social media and email). However, the latter approach would have been practically challenging during the COVID-19 lockdown when the present survey was conducted.

Finally, it should be highlighted that, due to the survey being conducted during the COVID-19 pandemic, data relating to typical exercise timing behaviours relied upon subjects accurately recalling exercise habits from before the COVID-19 pandemic. As such, there may be some discrepancy between actual behaviours and what was reported in the survey.

Conclusion

The current study showed that the largest proportion of people perform exercise sessions in the early evening during the week. However, this trend is inverted towards morning exercise during the weekend, in individuals who are unemployed/retired/self-employed, and in response to home-working as a result of COVID-19 lockdown restrictions, more closely aligning with exercise timing preferences. This indicates that weekday temporal restrictions resulting from full-time employment is a primary factor governing exercise timing behaviours, with the post-work period clearly identified as the most feasible time in which to exercise. This data should be considered in the future implementation of exercise interventions for health as a means of increasing adherence, which may partially be mediated through temporal consistency. Furthermore, the potential impact of the circadian system on exercise outcomes highlights the importance of the consideration, standardisation, and reporting of the time of day that exercise is conducted in future laboratory studies.

Chapter 5 – Fasting before evening exercise reduces net energy intake and increases fat oxidation, but impairs performance in healthy males and females

5.1. Introduction

A change in weight occurs due to an energy imbalance, but the counter-regulatory changes to energy balance systems appear more profound for an energy deficit than an energy surplus (Hill et al., 2012). Therefore, early intervention in lean individuals to prevent weight gain might be a more efficacious approach than attempting to reduce obesity once established (Monnier et al., 2021). Physical activity/exercise causes an acute increase in energy expenditure, but long-term exercise interventions for weight management are often less effective than predicted (Martin et al., 2019). This is likely explained by compensatory reductions in energy expenditure (Thompson et al., 2014), and/or increases in energy intake (King et al., 2008).

Exercise after a prolonged fast (>12 h) may aid in regulating energy balance by facilitating a lower 24 h energy intake (Bachman et al., 2016; Edinburgh et al., 2019). Additionally, fasted morning exercise increases fat oxidation (Edinburgh et al., 2019; Gonzelez et al., 2013), which may drive adaptations leading to improved markers of metabolic health (Robinson et al., 2015*a*). Almost all research on fasted exercise has been undertaken in the morning because the overnight fast offers a practical and convenient opportunity to achieve a fasted state without the need to skip meals. The response to fasted exercise at other times of day is not well researched, and it was shown in the previous chapter (**Chapter 4**) that a large proportion of the population perform weekday exercise in the early evening, primarily due to job/work commitments. There is also evidence that evening exercise is perceived as requiring less effort (Maraki et al., 2005), and may improve glycaemic control more than morning exercise (Moholdt et al., 2021). These diurnal differences may be explained by the circadian system, which regulates several endogenous processes, including macronutrient metabolism, appetite, and components of energy balance, in 24 h oscillations (Smith & Betts, 2022). Therefore, findings from overnight-fasted exercise might not translate to exercise performed later in the day.

Only one previous study has examined the energy intake and metabolic responses to fasted exercise performed at a time of day other than the morning. McIver et al. (2019*b*) showed similar 24 h energy intakes following fed and fasted exercise commencing in the morning or

early-evening, indicating fasted exercise may reduce daily energy intake, irrespective of the time of day. Furthermore, fat oxidation was similarly elevated during both fasted morning and fasted early-evening exercise. The amount of exercise performed and motivation to exercise are, however, important to maximise both the energy deficit achieved and the positive health outcomes from exercise training (Foulds et al., 2014). Skipping breakfast has been shown to reduce voluntary exercise performance (Clayton & James, 2016), but the effect of afternoon fasting on evening exercise performance is unknown.

Whilst providing novel insight into the responses to fasted exercise performed later in the day, the study by McIver et al. (2019*b*) examined a 9-h period of fasting between breakfast at 07:00 and exercise at 16:00, which may be challenging to adhere to in the real world. Previous work has shown that substrate oxidation patterns during exercise are comparable when exercise is performed either 6, 8, or 12 h after a meal, whereas carbohydrate oxidation rates are elevated when exercise is performed 2 and 4 h post-meal (Montain et al. 1991). Therefore, a pre-exercise fasting duration of ≥6 h is likely required to achieve a shift in substrate oxidation akin to overnight fasted exercise. Considering this, the present study will adopt a more feasible fasting duration, which may allow individuals to make small alterations to their existing meal patterns (*i.e.*, eat a slightly earlier lunch) before commencing exercise in the popular post-work period identified in **Chapter 4**.

The aim of this study was to examine the effects of fasting for 7 h before evening cycling exercise on post-exercise *ad-libitum* energy intake, appetite, voluntary exercise performance, and substrate oxidation in healthy, recreationally active males and females.

5.2. Methods

Subjects

Sixteen healthy, recreationally active (<10 $h \cdot wk^{-1}$) males and females (n=8 each) completed the study (**Table 5.1**). Female subjects were regular monophasic combined oral contraceptive users (≥6 months use before commencing the study; n=3) or eumenorrheic (self-reported; n=5), and not using a hormonal contraceptive.

Table 5.1. Subject baseline characteristics.

Characteristic	Overall (n=16)	Males (n=8)	Females (n=8)
Age (y)	25 ± 3	25 ± 2	24 ± 4
Weight (kg)	70.9 ± 12.1	80.6 ± 8.3	61.2 ± 4.9
Height (m)	1.74 ± 0.11	1.83 ± 0.06	1.65 ± 0.05
BMI ($kg \cdot m^{-2}$)	23.3 ± 1.9	24.1 ± 2.0	22.6 ± 1.6
Body fat (%)	20 ± 7	14 ± 3	26 ± 3
$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	39 ± 6	43 ± 6	36 ± 5
Dietary restraint[a]	8 ± 3	6 ± 3	9 ± 3
Dietary disinhibition[a]	5 ± 3	5 ± 3	5 ± 3
Hunger[a]	5 ± 3	5 ± 3	4 ± 2
Estimated resting metabolic rate ($kcal \cdot day^{-1}$)[b]	1557 ± 265	1754 ± 237	1395 ± 77

Values are means ± SD
[a] Three-factor eating questionnaire (Stunkard & Messick, 1985)
[b] Estimated via predictive equation (Mifflin et al., 1990)

Study Design

Subjects completed two preliminary trials, followed by two experimental trials that were separated by ≥4 days. To control for fluctuations in appetite associated with sex hormone concentrations (Buffenstein et al., 1995), eumenorrheic women completed experimental trials in the follicular phase (3–14 days after the onset of menstruation – self-reported) and oral contraceptive users completed all trials between days 4–17 of the pill-taking phase. This was individually standardised within a 4-day period for each female subject. Experimental trials involved consuming a 24 h standardised diet before an exercise session at 18:30. Exercise consisted of 30-min steady-state cycling and a 15-min all-out performance test, which required subjects to complete as much work as possible within the allotted time. In FAST, subjects ceased food intake at 11:30 and commenced exercise after a 7 h fast. In FED, subjects consumed a pre-exercise meal at 16:30 and commenced exercise after a 2 h fast.

Preliminary Trials

During the first preliminary trial, subjects' body mass and height were measured, before body fat percentage was estimated by measuring skinfold thickness. Cycling $\dot{V}O_{2peak}$ was determined during a discontinuous incremental exercise test on an electronically braked cycle (**Chapter 3**). After ~30 min rest, subjects then completed the 15-min performance test. During the second preliminary trial, subjects were familiarised with the exercise protocol and the *ad-libitum* meal.

Protocol

Subjects consumed a standardised dinner at 20:30 the evening before trial days, after which food and caffeine intake were not permitted. Subjects then consumed a standardised breakfast at 08:30, and a lunch at 11:30. In FED, subjects consumed a standardised pre-exercise meal at 16:30, which was replaced with a prescribed volume of water in FAST. Subjects arrived at the laboratory at 18:00 and measures of subjective appetite, mood, and exercise readiness were completed. After 20 min supine rest, a 5-min expired gas sample was collected. Exercise commenced at 18:30, with 30-min steady-state cycling (60% $\dot{V}O_{2peak}$). During exercise, heart rate and RPE were measured every 5 min, with 2-min expired gas samples collected every 10 min. After 3-min rest, subjects commenced a 15-min all-out performance test (described below). An *ad-libitum* pasta meal was served (1.25 ± 0.01 kcal·g^{-1}) 15 min after the cessation

of exercise, and subjects were permitted 20 min to eat. Subjects then left the laboratory and were instructed to consume nothing other than the prescribed water and to refrain from engaging in exercise and caffeine intake until after completing the final subjective appetite questionnaire at 08:30 the following day. Adherence to this was confirmed via text messaging.

Exercise Performance Test

The ergometer was set in linear mode, with the linear factor (L) calculated using the formula: $L = W/(rpm)^2$ to elicit a workload (W) of 85% $\dot{V}O_{2peak}$ at the subjects' preferred cadence identified during the $\dot{V}O_{2peak}$ test. Power output could be increased and decreased with an increase or decrease in cadence. Subjects were instructed to complete as much work as possible within 15 min and were blinded to all outcome measures, except time remaining. No encouragement was provided, and standardised instructions were provided before each trial. Work completed (kJ) and heart rate were recorded every minute, and RPE was recorded every 2 min from the first minute.

Standardised Meals

Subjects were provided with weighed meals and water to be consumed at home, with clear, written guidelines on timing of intake and instruction to consume nothing else. Subjects were regularly contacted via text messaging to encourage adherence with these instructions. Meals were designed to provide a percentage of estimated energy requirements (EER; resting metabolic rate [Mifflin et al., 1990] multiplied by a physical activity level of 1.7).

Standardised dinner and lunch meals were identical (814 ± 129 kcal), consisting of tuna/chicken sandwiches prepared by the researchers (white bread (Hovis, UK), tuna chunks in brine (Princes, UK)/chicken breast chunks (Bernard Matthews, UK), and full-fat mayonnaise (Hellmann's, UK)), ready salted crisps (Walkers, UK), and chocolate (Cadbury, UK). Standardised breakfast and pre-exercise meals were also identical (543 ± 86 kcal), consisting of instant porridge oats (Oatso Simple Golden Syrup, Quaker, UK), cereal bars (Strawberry Nutri-Grain, Kellogg's, UK), and yoghurt (Ski Strawberry, Nestlé, UK) (**Table 5.2**). Water intake was provided at 30 $mL \cdot kg^{-1}$ body mass during trials (2126 ± 363 mL), distributed into 5 equal volumes consumed: 1) between waking and lunch (<11:30); 2) during lunch (11:30–

12:00); 3) early afternoon (12:00–17:30); 4) 1 h before exercise (17:30); and 5) between the *ad-libitum* meal and sleep (>20:00).

Expired Gas Samples

A 5-min expired gas sample was collected into a Douglas bag immediately pre-exercise following 20 min of supine rest. During steady-state cycling, 2-min expired gas samples were collected between 8–10, 18–20, and 28–30 min. Expired gas samples were collected and analysed as described in **Chapter 3**.

Subjective Responses

Subjects rated feelings of hunger, fullness, desire to eat (DTE), prospective food consumption (PFC), and nausea on digital visual analogue scales (VAS) that were sent to their personal mobile telephone at each timepoint (0, 2, 3, 3.5, 5, 7, 8, 10, 11, 11.5, 13.5, and 24 h). Additional subjective feelings of motivation to exercise, readiness to exercise, tiredness, and energy were added to the pre-exercise questionnaire (10 h). Subjects also completed a paper-based Positive and Negative Effect Schedule (PANAS; Watson et al., 1988) pre-exercise. A paper-based, shortened version of the Physical Activity Enjoyment Scale (PACES-8) was completed immediately post-exercise to measure enjoyment of exercise sessions (Raedeke, 2007).

After the second and final experimental trial, subjects completed an exit survey designed to gather further subjective and perceptual responses to fasted evening exercise. Firstly, subjects were asked to rate how easy/difficult it was to fast throughout the afternoon. Subjects could select one option from the following choices: ‘very easy’, ‘easy’, ‘neither easy nor difficult’, ‘difficult’, and ‘very difficult’. Secondly, subjects were asked to report how often they would be willing to engage in fasted evening exercise if it was shown to provide additional benefits for their health. Choices were: ‘≥3 times/week’, ‘2 times/week’, ‘1 time/week’, ‘1 time/month’, ‘<1 time/month’, and ‘never’. Thirdly, subjects were asked to rate how fasted evening exercise compared to exercise after an overnight fast. Choices were: ‘N/A, I never exercise after an overnight fast’, ‘a lot easier’, ‘slightly easier’, ‘no different’, ‘slightly harder’, and ‘a lot

harder'. Finally, subjects reported which trial they felt that they had performed better in by selecting either 'fed evening exercise' or 'fasted evening exercise'.

Statistical Analyses

For subjective appetite-related variables, area under the curve (AUC) values were calculated and averaged over time in response to breakfast (0–3 h), lunch (3–7 h), pre-exercise meal (7–11 h), and *ad-libitum* meal (11–24 h). Because fluctuations in circulating sex hormone concentrations can influence appetite and energy intake in females, sex was entered as a between-subjects factor in repeated-measures ANOVA to test for sex-by-trial-by-time interactions and/or sex-by-trial interactions. Data were analysed using the methods described in **Chapter 3**. Due to equipment issues, heart rate data is missing for one subject.

5.3. Results

Energy Intake

Ad-libitum energy intake post-exercise was 99 ± 162 kcal greater during FAST ($dz = 0.61$; $P < 0.05$), but cumulative energy intake across the day was 443 ± 128 kcal lower during FAST than FED ($dz = 3.42$; $P < 0.001$; **Table 5.2**).

There was a sex-by-trial interaction effect for *ad-libitum* energy intake ($P < 0.001$), with greater energy intake during FAST than FED in males (+203 ± 122 kcal; $dz = 1.67$; $P < 0.01$), but not females (-5 ± 129 kcal; $dz = 0.04$; $P = 0.919$; **Figure 5.1**).

Table 5.2. Macronutrient composition of each meal.

	Carbohydrate (g)	Protein (g)	Fat (g)	Fibre (g)	Energy (kcal)
Standardised Breakfast					
FAST FED	93.2 ± 15.7	14.5 ± 1.0	11.2 ± 1.9	5.5 ± 0.9	543 ± 86
Standardised Lunch					
FAST FED	72.5 ± 11.1	36.8 ± 6.9	41.0 ± 6.2	4.1 ± 0.6	814 ± 129
Standardised Pre-Exercise Meal					
FAST	0	0	0	0	0
FED	93.2 ± 15.7	14.5 ± 1.0	11.2 ± 1.9	5.5 ± 0.9	543 ± 86
***Ad-Libitum* Post-Exercise Meal**					
FAST	152.1 ± 60.3	23.9 ± 9.5	17.9 ± 7.2	8.4 ± 3.3	882 ± 350*
FED	135.0 ± 48.4	21.2 ± 7.6	15.9 ± 5.7	7.4 ± 2.7	783 ± 281
Total					
FAST	317.7 ± 82.4	75.3 ± 16.3	70.1 ± 14.4	18.0 ± 4.6	2239 ± 533*
FED	393.8 ± 80.9	87.1 ± 14.6	79.3 ± 14.3	22.5 ± 4.5	2682 ± 519

Data are mean ± SD

* Values are significantly different from FED ($P < 0.05$).

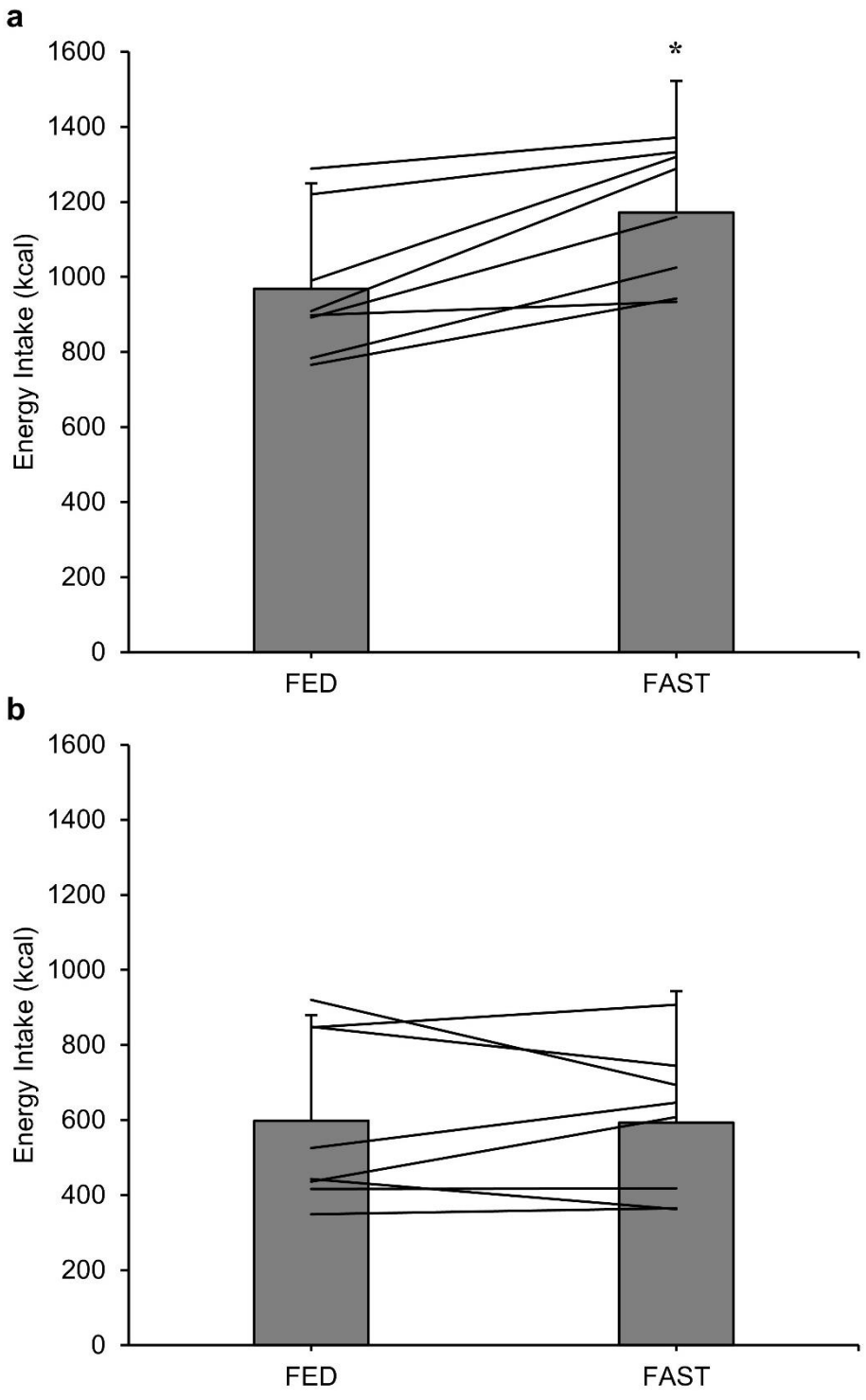

Figure 5.1. Energy intake (kcal) at the *ad-libitum* meal for **(a)** males (*n*=8), and **(b)** females (*n*=8). The bars display mean values, with vertical error bars representing SD. The lines display individual subjects' *ad-libitum* energy intake for each experimental trial. * FED vs. FAST ($P < 0.05$).

Subjective Appetite Responses

There were trial ($P < 0.01$) and time ($P < 0.01$) main effects and a trial-by-time interaction ($P < 0.001$) effect for hunger, fullness, DTE, and PFC. Subjects reported increased hunger, DTE, and PFC, and reduced fullness, in the period following the pre-exercise meal until immediately before the post-exercise *ad-libitum* meal (16:30–19:30) during FAST ($P < 0.05$). Nausea showed a main effect of time ($P < 0.01$), and a trial-by-time interaction effect ($P < 0.05$), but no main effect of trial ($P = 0.149$). Nausea tended to be greater immediately pre-exercise in FAST ($P = 0.06$; **Figure 5.2**).

AUC for hunger, DTE, PFC, and nausea were all greater, and fullness was lower, between the pre-exercise meal and the *ad-libitum* meal in FAST ($P < 0.01$). No further AUC differences were shown between trials in response to breakfast ($P \geq 0.398$), lunch ($P \geq 0.458$) or *ad-libitum* meal ($P \geq 0.464$; **Figure 5.3**).

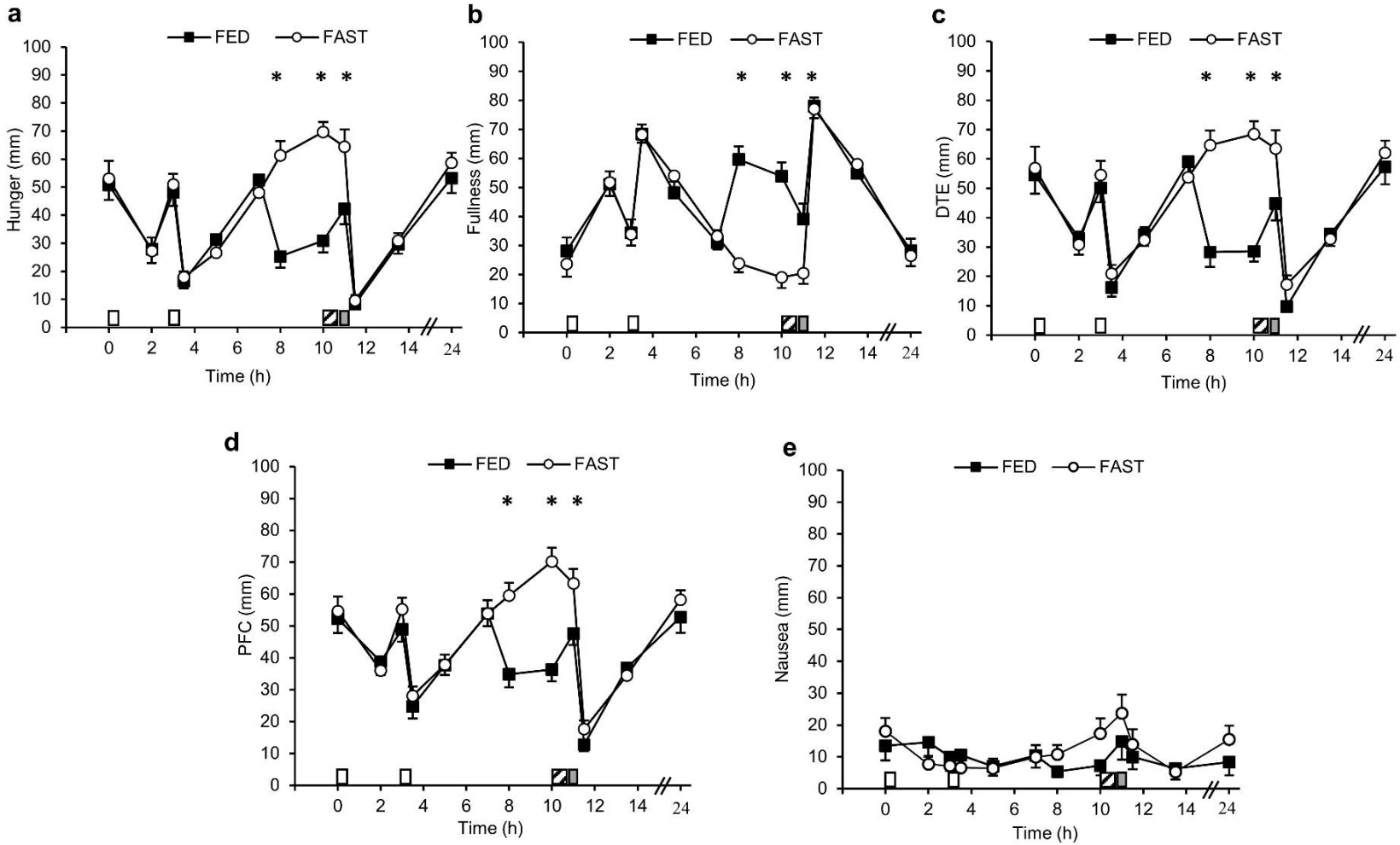

Figure 5.2. (a) Hunger, **(b)** fullness, **(c)** desire to eat (DTE), **(d)** prospective food consumption (PFC), and **(e)** nausea in FED and FAST. Data are mean ± SEM. White rectangles represent standardised meals; grey rectangle represents *ad-libitum* meal; diagonal striped rectangle represents exercise. * FED vs. FAST ($P < 0.05$).

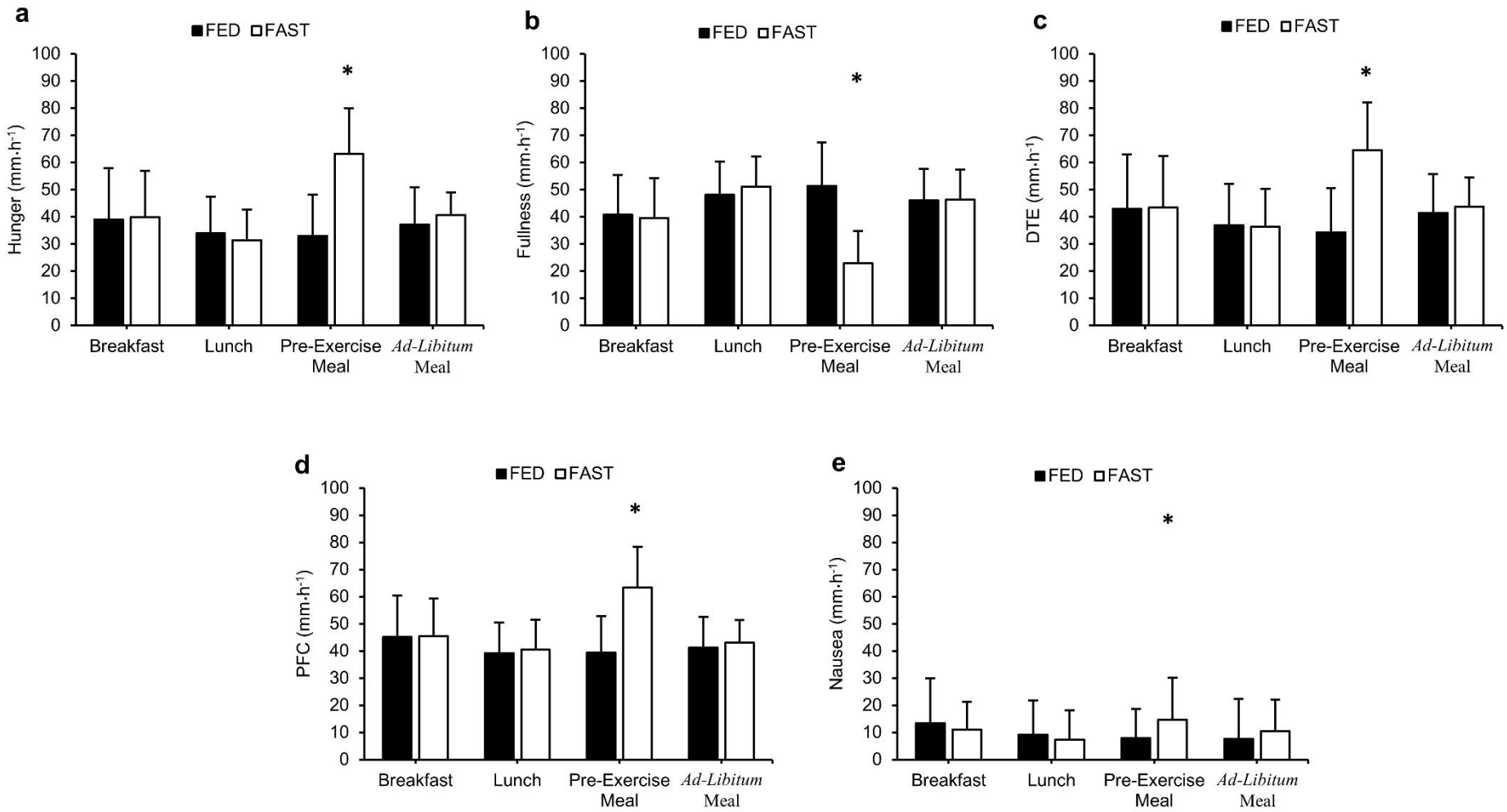

Figure 5.3. (a) Hunger, **(b)** fullness, **(c)** desire to eat (DTE), **(d)** prospective food consumption (PFC), and **(e)** nausea time-averaged area under the curve (AUC) in FED and FAST. Data are mean ± SEM. * FED vs. FAST ($P < 0.05$).

Energy Expenditure and Substrate Oxidation

At rest, carbohydrate oxidation was lower (0.04 ± 0.03 vs 0.13 ± 0.06 g·min^{-1}; *dz* = 1.25; *P* < 0.001) and fat oxidation was higher (0.11 ± 0.02 vs 0.09 ± 0.03 g·min^{-1}; *dz* = 0.67; *P* < 0.01) in FAST. Energy expenditure at rest was lower in FAST (1.3 ± 0.2 kcal·min^{-1} vs 1.2 ± 0.2 kcal·min^{-1}; *dz* = 0.67; *P* < 0.001). There was a sex-by-trial interaction effect for resting energy expenditure (*P* < 0.05), which was lower in FAST than FED in males (1.5 ± 0.2 kcal·min^{-1} vs 1.3 ± 0.2 kcal·min^{-1}; *dz* = 1.12; *P* < 0.05) but was not different between trials in females (1.2 ± 0.1 kcal·min^{-1} vs 1.1 ± 0.1 kcal·min^{-1}; *dz* = 0.14; *P* = 0.602).

During steady-state exercise, total fat oxidation was greater (+3.25 ± 1.99 g; *dz* = 1.64; *P* < 0.001), and total carbohydrate oxidation was lower (-9.16 ± 5.80 g; *dz* = 1.58; *P* < 0.001) in FAST (**Figure 5.4**). Total energy expenditure in the steady-state exercise was lower in FAST (-6 ± 8 kcal; *dz* = 0.59; *P* < 0.05).

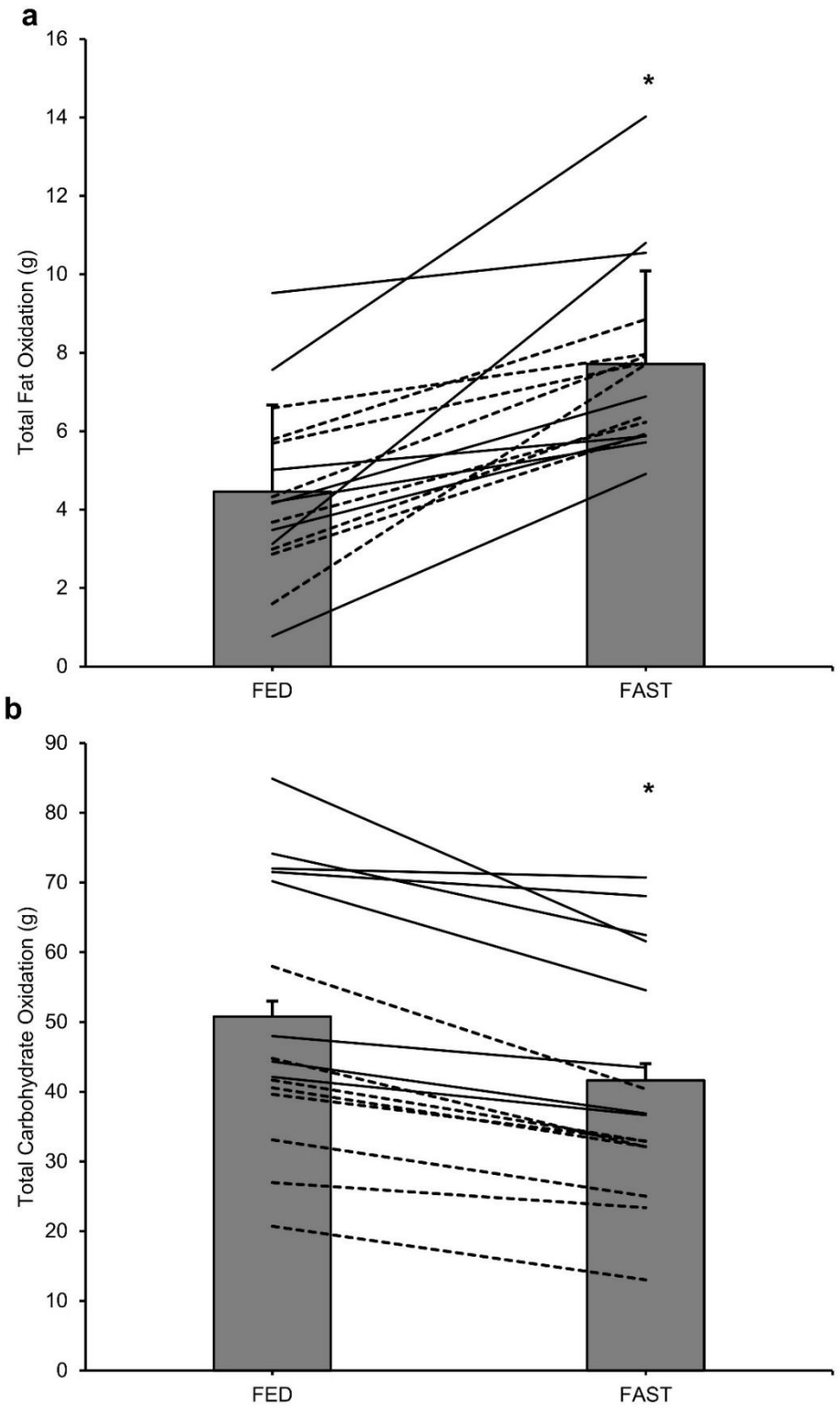

Figure 5.4. (a) Total fat oxidation (g), and **(b)** total carbohydrate oxidation (g) during the 30-min steady-state bout of cycling in FED and FAST. Data are mean ± SD. The lines display individual subjects' substrate oxidation during each experimental trial (dotted line: females; block line: males). * FED vs. FAST ($P < 0.05$).

Exercise Performance and Responses

Work completed during the 15-min performance test was 5 ± 8 kJ lower during FAST (*dz* = 0.62; $P < 0.05$; **Figure 5.5**).

Mean $\dot{V}O_2$ achieved during steady-state exercise was lower in FAST (57.9 ± 5.6% $\dot{V}O_{2peak}$ vs. 59.0 ± 6.1% $\dot{V}O_{2peak}$; $P < 0.01$). Mean heart rate ($P = 0.079$) and RPE ($P = 0.806$) were not different between trials during the 30-min steady-state bout. Mean heart rate during the performance test was lower in FAST ($P < 0.05$), but RPE was not different between trials ($P = 0.739$).

Laboratory temperature ($P = 0.212$), humidity ($P = 0.702$), and pressure ($P = 0.442$) were not different between trials.

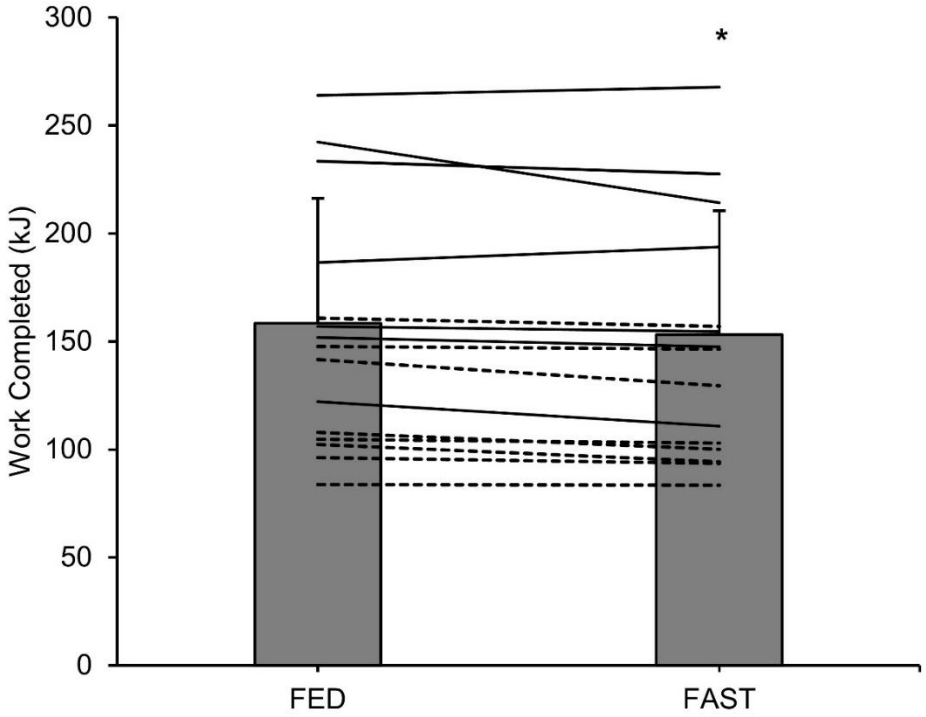

Figure 5.5. Total work completed (kJ) during the 15-min exercise performance test in FED and FAST. Data are mean ± SD. The lines display individual subjects' completed work during each experimental trial (dotted line: females; block line: males). * FED vs. FAST ($P < 0.05$).

Exercise Subjective Responses

Subjects reported lower pre-exercise motivation, energy, and readiness to exercise in FAST ($P < 0.001$), although tiredness was not different between trials ($P = 0.270$). The PANAS questionnaire revealed lower positive affect pre-exercise in FAST ($P < 0.05$), but negative affect was not different between trials ($P = 0.238$). Mean score on the PACES-8 questionnaire was lower in FAST ($P < 0.01$), suggesting that the exercise session was enjoyed less in FAST (**Table 3**).

Table 5.3. Pre- and post-exercise subjective responses.

	FAST	FED
PANAS Positive Affect[a]	22 ± 6*	26 ± 6
PANAS Negative Affect[a]	13 ± 3	12 ± 3
PACES-8 Score[b] (%)	49 ± 12*	57 ± 13

Values are means ± SD
* Values are significantly different from FED ($P < 0.05$)
[a] PANAS questionnaire (Watson et al., 1988)
[b] PACES-8 questionnaire (Raedeke, 2007)

Exit Survey

Out of the 16 subjects who completed the study 10 subjects reported fasting throughout the afternoon to be either 'difficult' or 'very difficult'. 'Neither easy nor difficult' and 'easy' were each reported by 3 subjects. If it was shown to provide additional benefits for their health, 8 subjects reported that they would be willing to engage in fasted evening exercise '2 times/week', 3 subjects selected '1 time/week', and 2 subjects selected 'never'. '≥ Three times/week', '1 time/month' and '<1 time/month' were each selected by 1 subject. When asked to report how fasted evening exercise compared to overnight-fasted exercise, 12 subjects selected either 'slightly harder' or 'a lot harder', and 2 subjects reported that it felt 'no different to overnight-fasted exercise'. 'Slightly easier' and 'N/A, I never exercise after an overnight fast' were each selected by 1 subject. Finally, when asked which trial they felt they had performed better in, 15 of the 16 subjects selected 'fed evening exercise'.

5.4. Discussion

This study showed that fasting for 7 h before evening exercise increased *ad-libitum* energy intake by ~100 kcal compared to exercise performed 2 h after eating, but this did not compensate for the omission of a pre-exercise meal. Accordingly, net energy intake was lower when evening exercise was performed following a 7 h fast. However, fasting before evening exercise reduced performance by ~3.8%, and was associated with reduced motivation and exercise enjoyment. Further study is required to determine whether fasting before evening exercise can be used chronically to assist in weight and health management, or whether it's associated negative perceptions impede long-term success.

Most studies explore fasted exercise in the morning due to the convenience of extending the overnight fast. However, as shown in the previous chapter (**Chapter 4**) morning exercise is not always convenient or possible, with the early evening representing the most common time of day for exercise to occur during the week. Therefore, this study assessed the metabolic and behavioural responses to fasted exercise in the evening. Previously, McIver et al. (2019*b*) showed that fasting for 9 h before exercising at 16:00 increased appetite pre-exercise, but there were no differences in post-exercise appetite regardless of whether exercise was performed 1 h (fed) or 9 h (fasted) after a meal. This aligns with some (Gonzalez et al., 2013; McIver et al., 2019*a*), but not all (Bachman et al., 2016; Griffiths et al., 2020) morning fasted exercise studies. Findings from the present study are in-line with the latter, demonstrating elevated appetite extending into the post-exercise period following a bout of evening exercise performed after a 7 h fast. Interestingly, post-exercise energy intake was ~100 kcal (~13%) greater, which contrasts with a number of studies comparing fed and fasted exercise performed in the morning (Bachman et al., 2016; Gonzalez et al., 2013; Griffiths et al., 2020). As such, the present study provides novel data suggesting a potential disparity in post-exercise energy intake responses between fasted exercise that is performed in the morning and in the evening, with fasted evening exercise appearing to provoke compensatory eating which is not typically observed with fasted morning exercise. However, further studies directly comparing the responses to fasted morning and evening exercise are still needed.

Interestingly, this increase in energy intake was driven predominantly by males, with seemingly no such compensation occurring in females. Appetite and energy intake responses to acute exercise are generally similar between males and females (Dorling et al., 2018), although only a small number of studies have directly compared males and females. Moreover,

nutrient-exercise interactions have not been considered (Frampton et al., 2022), so the sex-specific responses to fasted exercise are not well understood. Findings from this study suggest that fasted evening exercise may not provoke a compensatory energy intake response in females, potentially making it a more effective weight management strategy for females than males. Sex hormones may influence appetite and energy intake (Buffenstein et al., 1995). To control for this, female subjects conducted trials in the same phase of the menstrual or pill-taking cycle. However, it was not possible to standardise this to the exact day within the phase, and hormones were not measured directly, both of which can be considered limitations of the present study. Sex hormone concentrations may still fluctuate within the same cycle phase (Buffenstein et al., 1995), meaning larger sample size studies of both males and females, with measurement of ovarian hormone concentrations in female subjects, are required to further explore these preliminary findings.

Aside from the influence of circulating sex hormones, the absence of energy intake compensation following fasted evening exercise in females may be partly explained by sex-related differences in psychological aspects of eating behaviour. For example, restrained eating is more common amongst females than males (Conner et al., 2004), and previous research has shown that females typically display much smaller compensatory energy intake responses under acute test meal conditions, even in response to test meals with considerably different energy/macronutrient compositions (Astbury et al., 2010; Davy et al., 2007). In agreement with this concept, females scored higher for dietary restraint than males in the present study, although this difference was not statistically significant (P = 0.08). Therefore, it is plausible that the single laboratory-based eating occasion used in the present study was not suitable for assessing compensatory energy intake responses in females. Future studies should explore whether females would exhibit different compensatory energy intake responses to fasted evening exercise when measured in a free-living environment over several eating occasions.

Despite post-exercise energy intake being greater following fasted evening exercise, this increase only compensated for ~18% of the pre-exercise meal in FED. Therefore, energy intake over the course of the entire day was ~443 kcal lower in the fasted trial. Energy intake was only measured at a single post-exercise meal, so it is possible that further compensation occurred later in the evening or during the subsequent day. Consistent with other studies (Bachman et al., 2016; McIver et al., 2019*a*; McIver et al., 2019*b*; Griffiths et al., 2020), differences in appetite were abolished after the post-exercise meal, implying that future eating behaviour may not differ between trials. Indeed, studies tracking energy intake for up to 24 h

post-exercise demonstrate that the reduction in energy intake caused by fasting (meal skipping) is not compensated for in this time period (McIver et al., 2019*b*; Edinburgh et al., 2019; Bachman et al., 2016). Additionally, recent work suggests energy intake increases in anticipation of energy restriction (James et al., 2020) and/or exercise (Barutcu et al., 2021), but this could not be assessed in the present study as food intake was controlled to ensure similar metabolic conditions at the start of trials.

In the present study, prior fasting for 7 h increased fat oxidation by 3.25 g during 30 min evening exercise. Exercising after a 10–14 h overnight fast increases fat oxidation (Edinburgh et al., 2019; Gonzalez et al., 2013), which if performed regularly, may drive adaptations leading to improved markers of metabolic health (Robinson et al., 2015*a*). Despite circadian variations in several metabolic processes (Smith & Betts, 2022), the present study, and previous work (McIver et al., 2019*b*), show that a shorter, 7–9 h fasting period during the afternoon also increases fat oxidation during evening exercise. However, it must be noted that longer fasting durations that include the overnight fast, and shorter fasting durations such as that used in the present study, likely elicit differences in metabolism beyond changes in substrate oxidation. For example, plasma glycerol concentrations (a marker of lipolysis) increase in direct proportion to the duration of the fast (Montain et al., 1991), meaning the metabolic effects of a shorter period of afternoon fasting may not necessarily mimic those of an overnight fast. Future studies should seek to explore whether elevated fat oxidation during fasted evening exercise improves markers of metabolic health.

The main benefits from exercise are likely to be driven by the volume and intensity of exercise performed (Foulds et al., 2014). This is especially important when time for exercise is often curtailed by other commitments (Cerin et al., 2010). Findings from this study showed that fasting before evening exercise reduced subjective ratings of motivation, readiness, and energy immediately prior to exercise, indicating a suboptimal psychological state for maximising the volume or intensity of voluntary exercise. Accordingly, the amount of work completed during the 15-min performance test was reduced by 3.8% with fasting. Eating, particularly carbohydrate, appears to enhance aerobic performance >60 min due partially to increased endogenous carbohydrate stores (Aird et al., 2018), but effects on aerobic exercise <60 min are less conclusive (Mears et al., 2018; Galloway et al., 2014). Recent evidence suggests that the perception of consuming nutrients prior to exercise using an energy-free "placebo" meal (Mears et al., 2018; Naharudin et al., 2020) or the suppression of hunger (Naharudin et al., 2021), might improve performance. Therefore, the awareness of consuming nutrients and/or

subjective responses during the fed-state exercise trial may have increased self-selected intensity during the performance test.

The absolute difference between trials for work completed was very small (~6 kcal), possibly due to the short duration (15 min) and high intensity (85% $\dot{V}O_{2peak}$) of the selected test. This reduction in performance is unlikely to manifest in a meaningful change to energy balance. However, if motivation to exercise and self-selected duration and/or intensity of exercise are curtailed, as this reduction in performance might imply, this could dramatically impact the success of exercise training programmes. Additionally, given that exercise enjoyment may be an important predictor of long-term adherence to exercise interventions (Raedeke, 2007), the finding of reduced exercise enjoyment in the present study provides further insight into possible challenges with incorporating fasted evening exercise into a weight management programme.

An interesting outcome not measured in this study is the potential effects of fasted evening exercise on subsequent sleep duration and quality. This is a particularly important consideration given the evidence linking impaired sleep with poorer food choices and increased energy intake (Brondel et al., 2010; Shi et al., 2008). The effects of evening exercise on sleep are equivocal, with studies reporting improved sleep (Brand et al., 2014), worsened sleep (Oda & Shirakawa, 2014), or no effect on sleep (Buman et al., 2014). One study has even shown that increased evening exertion can improve sleep quality and reduce hunger the next morning (Brand et al., 2014). Heterogeneous findings may be due to differences in exercise duration/intensity, or the interval between exercise and habitual bedtime. For example, a meta-analysis of 23 studies revealed that performing light to moderate exercise within 4 h of bedtime does not negatively impact sleep, whereas performing vigorous exercise within 1 h of bedtime may impair sleep (Stutz et al., 2019). In-line with these findings, a more recent study showed that sleep quality was improved only when performed 4 h (~18:30), but not 2 h (~20:30), before bedtime, although energy intake at dinner and breakfast on the subsequent day was unaffected by exercise timing (Saidi et al., 2020). It is important to note that previous studies rarely considered the chronotype of subjects, and some evidence suggests that evening exercise may impair sleep only in morning-type, but not evening-type subjects (Vitale et al., 2017). Based on the available evidence, it is unlikely that adopting a fasted evening exercise regime would negatively impact sleep, and may in fact have favourable effects if performed ~4 h before bedtime. However, it is possible that such an intervention may have variable effects based on an individual's habitual sleeping pattern, meaning future studies examining exercise timing and sleep should consider chronotype as an important factor which may mediate the response.

The present study provides novel insight into the effects of fasting before evening exercise, but it is not without limitations. Firstly, caffeine intake was not permitted in the present study due to its potential confounding effects on substrate oxidation (Collado-Mateo et al., 2020), exercise performance (Graham, 2001), and appetite (Schubert et al., 2017). Outside the confines of the laboratory, individuals may utilise caffeinated beverages (*i.e.*, tea and coffee) to offset some of the appetite-related challenges associated with daytime fasting, which may have improved some of the negative perceptual responses to fasted evening exercise. Secondly, subjects were required to consume standardised meals and undergo instructed fasting periods in the absence of experimenter supervision. Although regular contact was made via text messaging to increase compliance, full adherence with these instructions cannot be assumed. Thirdly, the study was conducted in lean and healthy subjects, meaning the results cannot be directly extrapolated to other population groups, particularly individuals with overweight or obesity, who may respond differently to fasting-based interventions (Gonzalez et al., 2018). Finally, this study investigated a single exposure, and compensatory energy intake was only assessed at a single timepoint. As such, it is not known whether these acute findings would persist after multiple exposures within a free-living setting, with greater opportunity for compensatory energy balance behaviours to occur.

Conclusion

This study showed that the energy omitted by skipping a pre-exercise meal before evening exercise was not fully compensated for at dinner. Therefore, fasting for 7 h prior to evening exercise reduced net energy intake between breakfast and dinner, whilst also increasing fat oxidation. The chronic success of this intervention may, however, be compromised by elevations in appetite and reductions in voluntary performance, as well as reductions in the motivation to exercise and the enjoyment of exercise sessions. Future studies are required to explore whether regular fasted evening exercise can be used by lean and healthy individuals as a method of managing body weight and/or composition in the long-term. Additionally, exploring the effects of this intervention on indices of energy balance and metabolic health within overweight/obese populations represents an important avenue for future research.

Chapter 6 – A low-carbohydrate, high-protein lunch increases fat oxidation during evening exercise, whilst suppressing subsequent appetite and energy intake

6.1. Introduction

Pre-exercise nutritional state can mediate the benefits of exercise. For example, exercise performed after a prolonged fast (>12 h) increases fat oxidation (Edinburgh et al., 2019; Gonzelez et al., 2013), and if performed regularly, can increase fat oxidative capacity (De Bock et al., 2008; Van Proeyen et al., 2010). This is associated with improved markers of metabolic health (Robinson et al., 2015*a*), and accordingly, overnight-fasted exercise training may augment improvements in insulin sensitivity (Edinburgh et al., 2020; Van Proeyen et al., 2010). Overnight-fasted exercise also induces a more negative energy balance than fed exercise (Bachman et al., 2016; Edinburgh et al., 2019; Gonzalez et al., 2013; Griffiths et al., 2020), therefore potentially benefiting weight management.

Most fasted exercise studies have been conducted in the morning, but morning exercise may not be possible for many (see **Chapter 4**). Macronutrient metabolism and appetite demonstrate circadian variation (Smith & Betts, 2022), so findings from fasted morning exercise may not translate to other times of day. One study showed that fasted evening exercise upregulates fat oxidation (McIver et al., 2019*b*), but prolonged afternoon fasting also elevates appetite, increases energy intake, and ultimately reduces motivation to exercise, exercise enjoyment, and exercise performance (**Chapter 5**). Perceived effort and exercise enjoyment are not affected during fasted morning exercise (Frampton et al., 2022), therefore, these findings highlight potential difficulties specifically with adopting a fasted exercise regime in the evening. For example, elevated appetite may reduce physical performance (Naharudin et al., 2021), or lead to poorer food choices (Read & van Leeuwen, 1998).

The metabolic benefits of fasted exercise may be driven by carbohydrate restriction, rather than fasting *per se*. There is evidence that consuming a low-carbohydrate, high-protein breakfast before exercise does not blunt fat oxidation compared to fasted exercise (Impey et al., 2015; Rothschild et al., 2021; Taylor et al., 2013). Moreover, markers of training adaptation with implications for improved insulin sensitivity, such as AMPK signalling, and CD36 and PGC-1α mRNA expression are upregulated following protein-only feeding (Aird et al., 2021; Larsen et al., 2020; Taylor et al., 2013). Additionally, a high-protein meal reduces appetite and energy

intake to a greater extent than high-carbohydrate or high-fat meals (Rolls et al., 1988), which may aid weight management efforts. This provides a practical rationale for overcoming some of the difficulties associated with conducting fasted exercise later in the day, but the appetite and metabolic effects of a low-carbohydrate, high-protein pre-exercise meal, relative to a more typical high-carbohydrate pre-exercise meal and fasting are not well understood.

The aim of this study was to examine the effects of consuming a low-carbohydrate, high-protein lunch prior to evening cycling exercise on substrate oxidation, exercise metabolism, appetite, and subsequent energy intake, compared to a high-carbohydrate, lower-protein lunch, and fasting.

6.2. Methods

Subjects

Twelve healthy, recreationally active (<10 $h \cdot wk^{-1}$) males completed the study (**Table 6.1**).

Table 6.1. Subject baseline characteristics (*n*=12).

Characteristic	Mean	SD
Age (y)	25	5
Weight (kg)	81.4	10.2
Height (m)	1.81	0.08
BMI ($kg \cdot m^{-2}$)	24.7	2.1
Body fat (%)	17	6
$\dot{V}O_{2peak}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	45	7
Dietary restraint[a]	7	3
Dietary disinhibition[a]	6	2
Hunger[a]	7	3
Estimated resting metabolic rate ($kcal \cdot day^{-1}$)[b]	1827	152

Values are means ± SD
[a] Three-factor eating questionnaire (Stunkard & Messick, 1985)
[b] Estimated via predictive equation (Mifflin et al., 1990)

Study Design

Subjects completed two preliminary trials and three experimental trials that were separated by ≥7 days. Experimental trials involved consuming a standardised breakfast at home (08:15), before either a low-carbohydrate (LO-CARB), high-carbohydrate (HI-CARB), or no (FAST; ~13:15) lunch in the laboratory. Three hours later (~16:15), subjects completed 60 min steady-state cycling, before *ad-libitum* energy intake was assessed at dinner and from a selection of snacks provided after subjects left the laboratory. Subjects were blinded to the compositional differences between LO-CARB and HI-CARB meals until completion of the study. Laboratory temperature (P = 0.872), humidity (P = 0.115), and pressure (P = 0.640) were not different between trials.

Preliminary Trials

The first preliminary trial involved measuring subjects' body mass, height, and skinfold thickness, before $\dot{V}O_{2peak}$ was determined on an electronically braked cycle ergometer (**Chapter 3**). During the second preliminary trial, subjects were familiarised with the steady-state cycling and *ad-libitum* eating procedures.

Protocol

At 08:15, subjects completed baseline measures of subjective appetite and consumed a standardised breakfast, before arriving at the laboratory between 12:15–12:45. An indwelling cannula was inserted into an antecubital vein and after 30 min supine rest, a baseline blood sample, expired gas sample, and subjective appetite measures were collected. At ~13:15 (0 h), subjects consumed a standardised lunch (LO-CARB and HI-CARB), or volume of water (FAST). After lunch (0.5 h), subjects rested in the laboratory, with blood and gas samples collected at 1, 1.75, and 2.75 h. At 3 h (~16:15), subjective measures of appetite, mood, exercise readiness, and a pre-exercise blood sample were collected before subjects completed 60 min steady-state cycling at ~60% $\dot{V}O_{2peak}$. A 3-h interval between lunch and exercise was selected to ensure sufficient time for the meal to be digested and the contained nutrients to be released into the circulation. This meal-exercise interval is frequently used in studies which examine the effects of a mixed-macronutrient meal on metabolism during exercise (Gonzalez & Stevenson, 2014; Stevenson et al., 2006; Stevenson et al., 2007; Wu et al., 2003). During

exercise, expired gas samples were collected between 28–30 and 58–60 min, venous blood samples collected at 30 and 60 min, and heart rate and RPE recorded every 15 min, with subjective appetite measured immediately post-exercise. Final expired gas and venous blood samples were collected 1 h post-exercise. One hour and fifteen minutes after exercise, an *ad-libitum* meal was served in the laboratory (~18:30), with subjects permitted 20 min to eat. Subjects then left the laboratory, taking a selection of snacks to consume *ad-libitum* between 20:00–22:00 only (**Chapter 3)**. Subjects were instructed to consume only foods provided, until after the final subjective appetite questionnaire was completed at 08:15 the following day, but *ad-libitum* water was permitted (volume recorded). Adherence to these instructions was confirmed via text messaging.

Standardised Breakfast Meal

Meals were provided to subjects as a percentage of their estimated energy requirement (EER; resting metabolic rate [Mifflin et al., 1990] multiplied by a physical activity factor of 1.7). A standardised breakfast (779 ± 66 kcal) consisting of porridge (Oatso Simple Golden Syrup, Quaker, UK), cereal bars (Belvita, Mondelez, UK), yoghurt (Ski Strawberry, Nestlé, UK), and strawberry milkshake (Yazoo, Campina Ltd., UK) was provided in all experimental trials.

Test Lunch Meals

In HI-CARB and LO-CARB, subjects consumed a lunch (1186 ± 140 kcal) consisting of tuna and mayonnaise sandwiches, crisps, and a blended drink. Meals provided either 2 g·kg body mass^{-1} (HI-CARB) or 0.2 g·kg body mass^{-1} (LO-CARB) carbohydrate (**Table 6.2**). Meals were isocaloric via the manipulation of carbohydrate and protein content, with fat content closely matched to limit effects of dietary fat on substrate oxidation. The HI-CARB blended drink comprised of water, maltodextrin (MyProtein, UK), full-fat milk, chocolate milkshake powder (Nesquik, Nestlé, UK), sucralose sweetener, and thickening agent xanthan gum (Doves Farm, UK). The LO-CARB drink consisted of water, chocolate-flavoured soy protein isolate (MyProtein, UK), double cream (ASDA, UK), and sucralose sweetener. Also in LO-CARB, low-carbohydrate, high-protein bread (LivLife, UK) and crisps (MyProtein, UK) replaced the regular bread (Hovis, UK) and crisps (Walkers, UK) provided in HI-CARB. In FAST, subjects consumed water equal to the water content of LO-CARB and HI-CARB meals. Water intake

was provided at 30 mL·kg body mass^{-1} (2441 ± 307 mL) distributed into 7 equal volumes consumed: 08:15–10.30; 10:30–12:30; 14:15–15:15; 15:15–16:15; first half of exercise (16:15–16:45); second half of exercise (16:45–17:15); 17:15–18:15.

Expired Gas Samples

At rest, 5-min expired gas samples were collected into a Douglas bag following 20 min supine rest. During exercise, 2-min expired gas samples were collected after 1 min of breathing through the tubing. Expired gas samples were collected and analysed as described in **Chapter 3**.

Subjective Responses

Subjects rated hunger, fullness, desire to eat (DTE), prospective food consumption (PFC), and nausea on digital visual analogue scales (VAS) sent to their mobile telephone at -5, 0, 0.5, 1, 1.75, 3, 4, 5.25, 5.75, 6.75, 8.75, and 19 h. Additionally, motivation to exercise, readiness to exercise, tiredness, and energy, were rated pre-exercise (3 h). Subjects also completed a paper-based Positive and Negative Affect Schedule (PANAS; Watson et al., 1988) pre-exercise. Enjoyment of exercise was assessed immediately post-exercise using a paper-based, shortened version of the Physical Activity Enjoyment Scale (PACES-8; Raedeke, 2007).

Additional VAS related to perceptions of the overall meal (how pleasant), the sandwich (how pleasant, dry, moist, chewy), and the drink (how pleasant, bitter, sweet, creamy, thick, sticky, salty) were completed by participants immediately after lunch in LO-CARB and HI-CARB.

Blood Sampling and Analysis

Venous blood samples (~10 mL per sample) were collected after 20 min supine rest and were treated and analysed for determination of acylated ghrelin, PYY, GLP-1, insulin, glucose, NEFA, and glycerol concentrations, as described in **Chapter 3**.

Statistical Analyses

For appetite-related variables, AUC values were determined in response to breakfast (08:15–13:15), lunch (13:15–16:15), exercise (16:15–18:30), and dinner/overnight (18:30–08:15). Data were analysed using the methods described in **Chapter 3**.

6.3. Results

Energy Intake

Ad-libitum dinner energy intake in LO-CARB was 262 ± 174 kcal lower than FAST (*dz* = 1.52; $P < 0.001$) and 215 ± 135 kcal lower than HI-CARB (*dz* = 1.58; $P < 0.001$) but was not different between FAST and HI-CARB (*dz* = 0.41; $P = 0.194$). Snack energy intake (LO-CARB: 575 ± 272 kcal, FAST: 696 ± 246 kcal, HI-CARB: 673 ± 245 kcal; $P = 0.274$) and macronutrient intake ($P \geq 0.055$) were not different between trials. Cumulative energy intake across the day was greater in LO-CARB (+803 ± 279 kcal; *dz* = 2.86; $P < 0.001$) and HI-CARB (+1116 ± 315 kcal; *dz* = 3.56; $P < 0.001$) versus FAST but was also lower during LO-CARB than HI-CARB (-313 ± 284 kcal; *dz* = 1.10; $P < 0.01$; **Table 6.2**).

Table 6.2. Macronutrient composition of each meal.

	Carbohydrate (g)	Protein (g)	Fat (g)	Fibre (g)	Energy (kcal)
Standardised Breakfast					
LO-CARB	121.2 ± 9.2	24.1 ± 1.9	20.0 ± 1.7	9.4 ± 0.7	779 ± 66
HI-CARB	121.2 ± 9.2	24.1 ± 1.9	20.0 ± 1.7	9.4 ± 0.7	779 ± 66
FAST	121.2 ± 9.2	24.1 ± 1.9	20.0 ± 1.7	9.4 ± 0.7	779 ± 66
Standardised Lunch					
LO-CARB	18.4 ± 2.5	157.7 ± 18.8	50.1 ± 5.9	15.3 ± 1.4	1186 ± 140†
HI-CARB	163.2 ± 19.3	30.6 ± 3.9	44.3 ± 5.3	6.0 ± 0.5	1186 ± 140#
FAST	0	0	0	0	0
***Ad-Libitum* Dinner**					
LO-CARB	149.8 ± 38.0	23.6 ± 6.0	17.6 ± 4.5	8.2 ± 2.1	869 ± 220*†
HI-CARB	186.9 ± 43.1	29.4 ± 6.8	22.0 ± 5.1	10.3 ± 2.4	1084 ± 250*
FAST	195.0 ± 50.3	30.7 ± 7.9	22.9 ± 5.9	10.7 ± 2.8	1131 ± 292†
***Ad-Libitum* Snack**					
LO-CARB	83.1 ± 39.3	7.2 ± 3.5	22.9 ± 11.4	4.0 ± 2.9	575 ± 272
HI-CARB	98.8 ± 40.2	9.0 ± 4.4	25.5 ± 7.7	6.4 ± 3.9	673 ± 245
FAST	101.7 ± 37.7	9.6 ± 3.1	26.5 ± 9.6	6.2 ± 3.1	696 ± 246
Total					
LO-CARB	372.5 ± 60.4	212.6 ± 24.8	110.6 ± 16.0	36.8 ± 5.2	3409 ± 466*†
HI-CARB	570.1 ± 78.8	93.1 ± 11.4	111.7 ± 13.0	32.0 ± 5.6	3722 ± 478*#
FAST	417.8 ± 67.8	64.4 ± 9.8	69.4 ± 11.1	26.3 ± 4.9	2606 ± 403†#

Data are mean ± SD
* LO-CARB vs. HI-CARB ($P < 0.05$)
† LO-CARB vs. FAST ($P < 0.05$)
# HI-CARB vs. FAST($P < 0.05$)

Subjective Appetite Responses

There were trial-by-time interaction effects for hunger, fullness, DTE, and PFC ($P < 0.001$), but not nausea ($P = 0.367$). Following lunch, hunger, DTE, and PFC were lower, and fullness was higher until 3 h in LO-CARB and HI-CARB versus FAST ($P < 0.001$), and these differences in appetite were still apparent at 4 and 5 h between LO-CARB and FAST ($P < 0.001$). Between LO-CARB and HI-CARB, hunger and DTE were lower in LO-CARB at 4 and 5 h, and with fullness also higher at 5 h ($P < 0.05$).

AUC for hunger, DTE, and PFC were all greater, and fullness lower in response to lunch and exercise in LO-CARB ($P < 0.001$) and HI-CARB ($P < 0.01$) versus FAST, and in LO-CARB versus HI-CARB ($P < 0.01$; **Figure 6.1**).

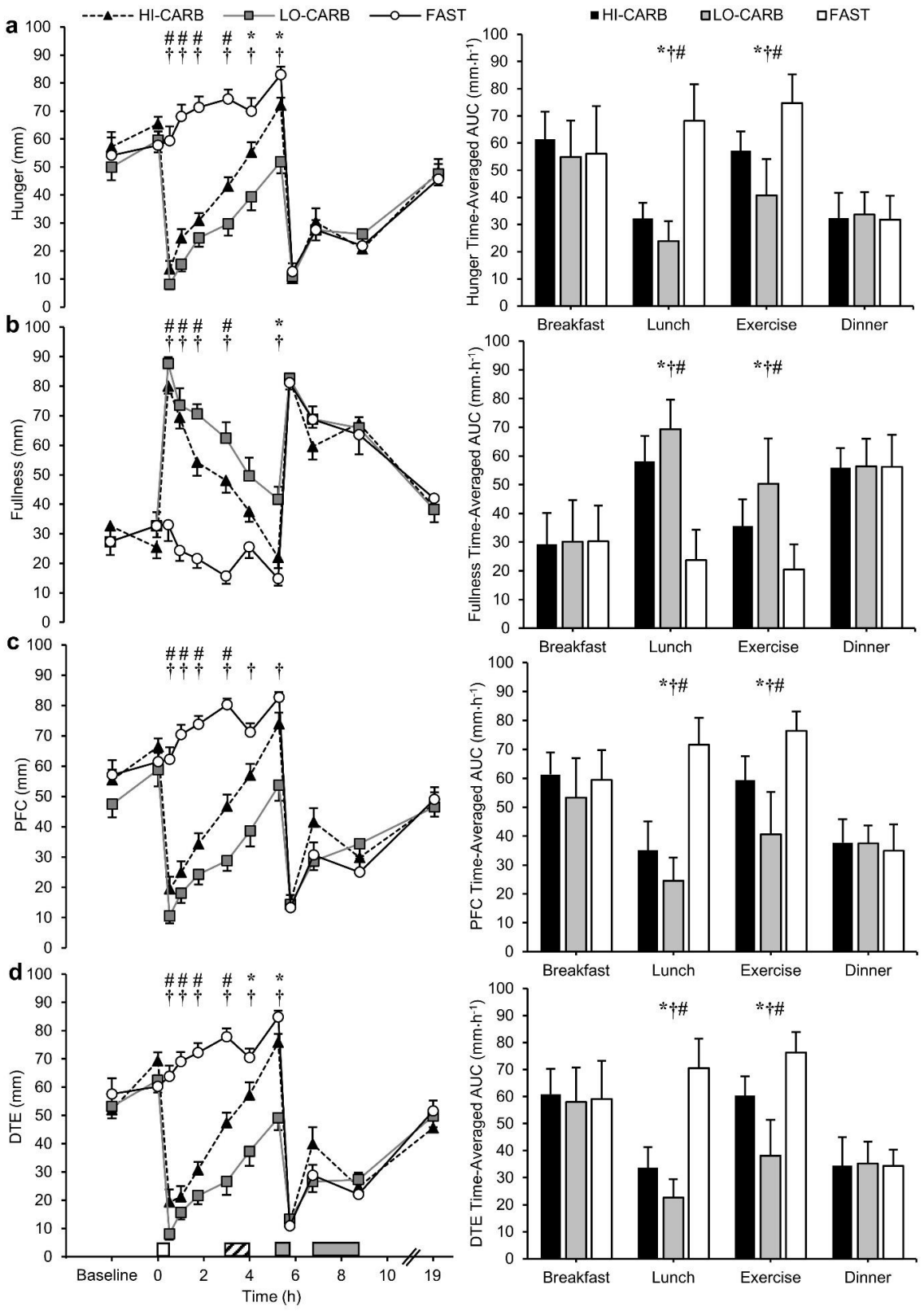

Figure 6.1. (a) Hunger, **(b)** fullness, **(c)** prospective food consumption (PFC), and **(d)** desire to eat (DTE) during HI-CARB, LO-CARB and FAST. Data are presented at each time point (left) and as total area under the curve (AUC) for each trial (right). Data are mean ± SEM. White rectangle represents standardised lunch; grey rectangle represents *ad-libitum* dinner and snacking; diagonal striped rectangle represents exercise. * LO-CARB vs. HI-CARB ($P < 0.05$); † LO-CARB vs. FAST ($P < 0.05$); # HI-CARB vs. FAST ($P < 0.05$).

Energy Expenditure and Substrate Oxidation

There were trial-by-time interaction effects for fat and carbohydrate oxidation rates ($P < 0.001$). Resting fat oxidation was greater in LO-CARB at 1, 1.75, and 2.75 h, and in FAST at 1.75 and 2.75 h, versus HI-CARB ($P < 0.001$). Resting carbohydrate oxidation was lower in LO-CARB and FAST versus HI-CARB at 1, 1.75, and 2.75 h ($P < 0.05$) and was also lower in FAST at 1.75 and 2.75 h versus LO-CARB ($P < 0.001$). At 5 h, resting fat oxidation was greater in LO-CARB versus HI-CARB ($P < 0.01$). Total fat oxidation was higher and carbohydrate oxidation was lower in LO-CARB and FAST versus HI-CARB, as well as in FAST versus LO-CARB ($P < 0.01$). There was a main effect of trial ($P < 0.001$), but no trial-by-time interaction effect for energy expenditure ($P = 0.119$). Total energy expenditure was greater in LO-CARB and HI-CARB versus FAST, and in LO-CARB versus HI-CARB ($P < 0.05$; **Figure 6.2**).

During exercise, total fat oxidation was greater (+8.00 ± 3.83 g; $dz = 2.10$; $P < 0.001$) and total carbohydrate oxidation was lower (-17.21 ± 10.16 g; $dz = 1.15$; $P < 0.01$) in LO-CARB versus HI-CARB. Also, total fat oxidation was greater (+11.66 ± 6.63 g; $dz = 1.75$; $P < 0.001$) and total carbohydrate oxidation was lower (-30.25 ± 17.39 g; $dz = 1.24$; $P < 0.001$) in FAST versus HI-CARB. Total fat oxidation was greater (+3.66 ± 5.07 g; $dz = 0.72$; $P < 0.05$) and carbohydrate oxidation was lower (-13.04 ± 13.55 g; $dz = 0.98$; $P < 0.01$) in FAST versus LO-CARB. Total energy expenditure during exercise was greater in LO-CARB versus FAST (+17 ± 16 kcal, $dz = 1.12$; $P < 0.01$; **Figure 6.3**).

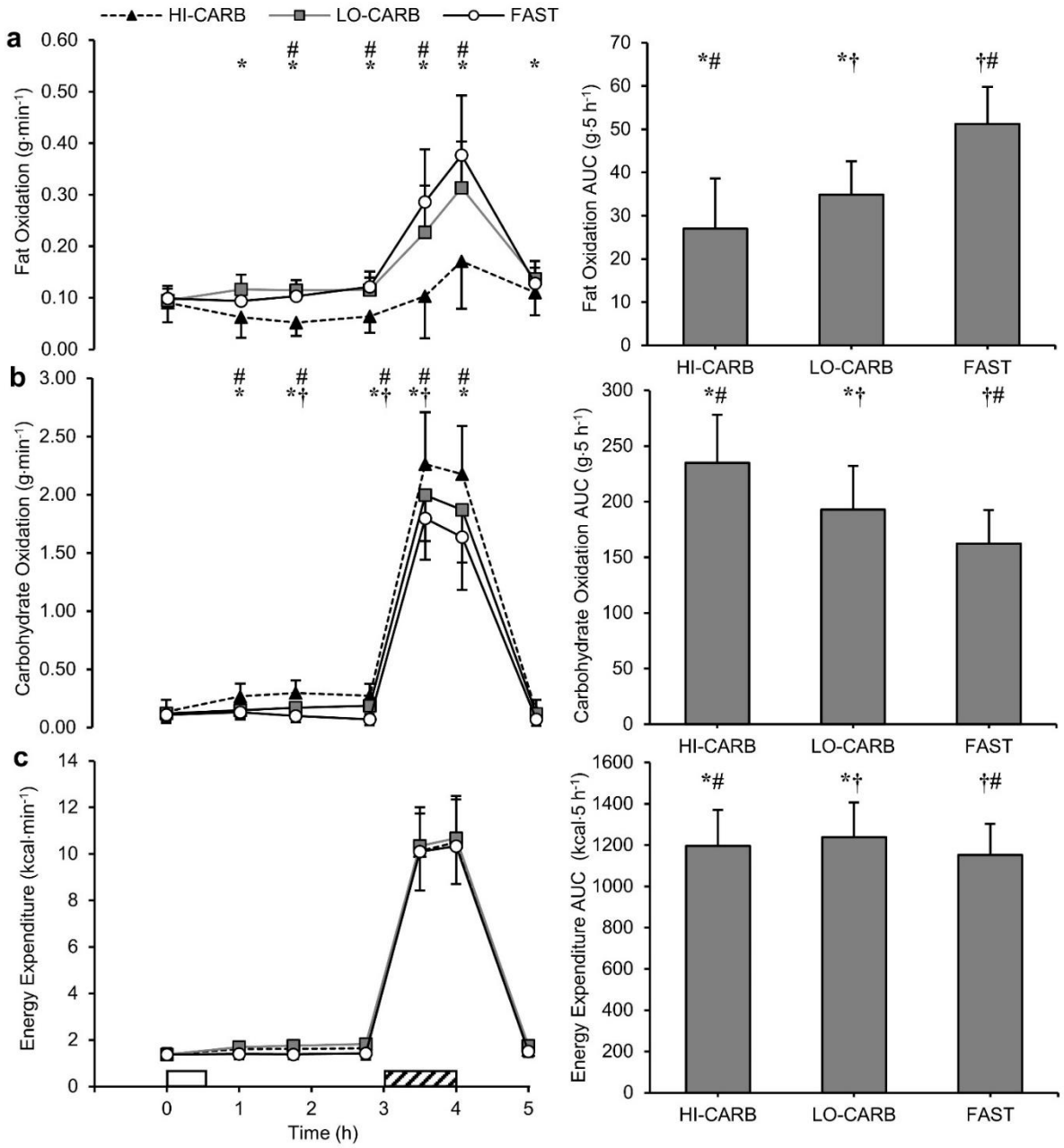

Figure 6.2. (a) Fat oxidation, **(b)** carbohydrate oxidation, and **(c)** energy expenditure during HI-CARB, LO-CARB and FAST. Data are presented at each time point (left) and as total area under the curve (AUC) for each trial (right). Data are mean ± SD. White rectangle represents standardised lunch; diagonal striped rectangle represents exercise. * LO-CARB vs. HI-CARB ($P < 0.05$); † LO-CARB vs. FAST ($P < 0.05$); # HI-CARB vs. FAST ($P < 0.05$).

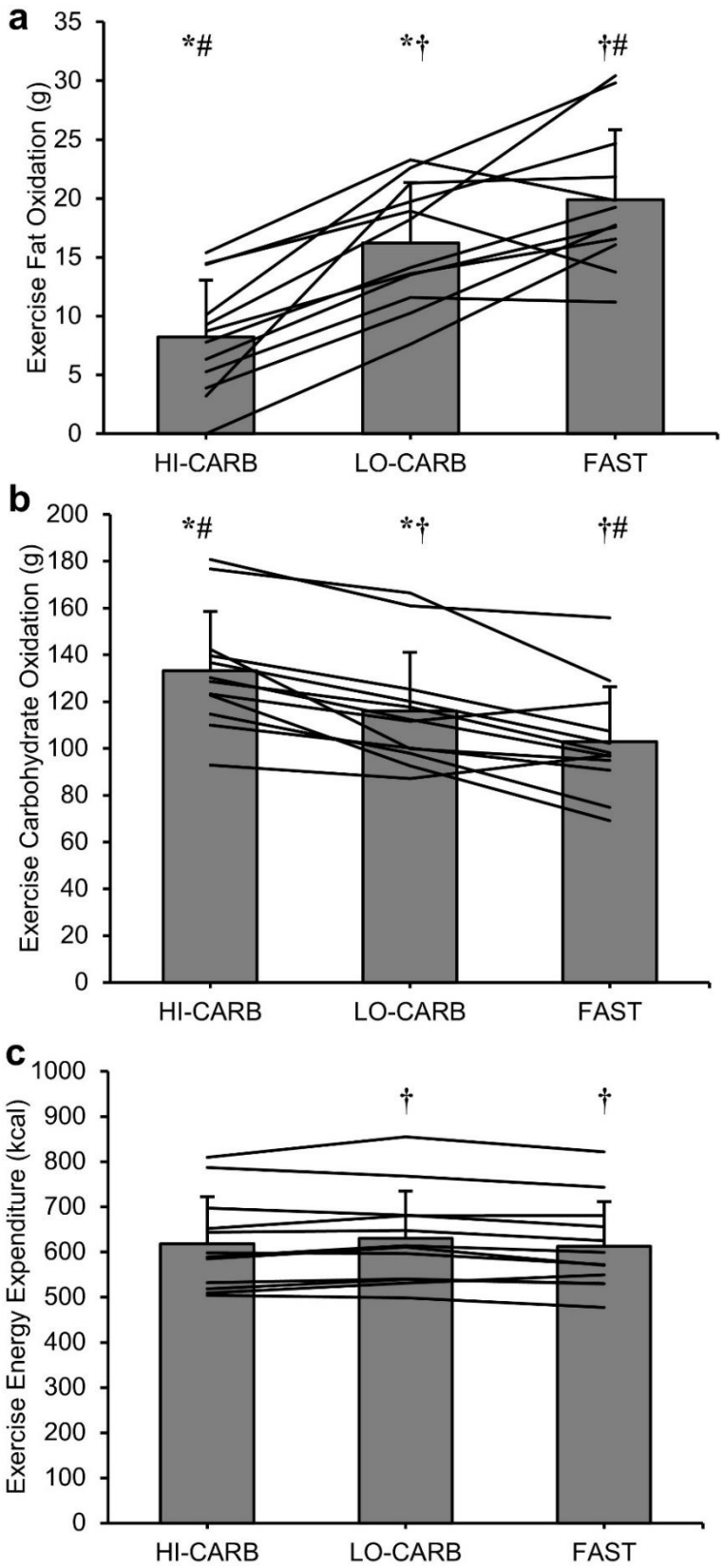

Figure 6.3. (a) Total fat oxidation, **(b)** total carbohydrate oxidation, and **(c)** total energy expenditure during the 60 min cycling exercise in HI-CARB, LO-CARB and FAST. The bars display mean values, with vertical error bars representing SD. The lines display individual subjects' substrate oxidation and energy expenditure for each experimental trial. * LO-CARB vs. HI-CARB ($P < 0.05$); † LO-CARB vs. FAST ($P < 0.05$); # HI-CARB vs. FAST ($P < 0.05$).

Exercise Subjective Responses

Subjects reported lower pre-exercise energy in FAST versus LO-CARB and HI-CARB ($P < 0.05$), although motivation, tiredness, and readiness to exercise were not different between trials ($P \geq 0.121$). Positive affect ($P = 0.103$) and negative affect ($P = 0.137$) immediately pre-exercise (PANAS questionnaire) were not different between trials. Enjoyment of exercise sessions (PACES-8 questionnaire) was not different between trials ($P = 0.186$).

Meal Perceptions

Overall pleasantness of the lunch meal was lower in LO-CARB ($P < 0.05$). The drink was rated as both creamier and thicker in LO-CARB ($P < 0.05$), with no further differences in perceptions ($P \geq 0.058$). The sandwich was rated as less pleasant and chewier in LO-CARB ($P < 0.05$), with no further perceptual differences ($P \geq 0.143$).

Blood Analyses

There were trial-by-time interaction effects for plasma PYY and GLP-1 concentrations ($P < 0.001$). For plasma acylated ghrelin concentrations, there was a main effect of trial ($P < 0.001$), but no trial-by-time interaction effect ($P = 0.067$). Plasma PYY was greater at all timepoints following lunch in LO-CARB ($P < 0.001$) and HI-CARB ($P < 0.05$) versus FAST, and in LO-CARB versus HI-CARB ($P < 0.05$). Plasma GLP-1 was greater at all timepoints after lunch in LO-CARB versus FAST and HI-CARB ($P < 0.001$). Plasma GLP-1 was also greater in HI-CARB at 1, 4, and 5 h versus FAST ($P < 0.01$). AUC for plasma PYY and GLP-1 was greater in LO-CARB ($P < 0.001$) and HI-CARB ($P < 0.01$) versus FAST and in LO-CARB versus HI-CARB ($P < 0.001$). AUC for plasma acylated ghrelin was lower in LO-CARB and HI-CARB versus FAST ($P < 0.01$; **Figure 6.4**).

There were trial-by-time interaction effects for plasma insulin, glucose, NEFA, and glycerol concentrations ($P < 0.001$). Plasma insulin was lower at 1–3.5 h in LO-CARB and FAST versus HI-CARB ($P < 0.05$), and at 1–3 h, and 5 h in FAST versus LO-CARB ($P < 0.05$). AUC for insulin was lower in LO-CARB and FAST versus HI-CARB and in FAST versus LO-CARB ($P < 0.01$). Plasma glucose was lower at 1 h ($P < 0.05$), but higher at 3 h ($P < 0.01$), in LO-CARB and FAST versus HI-CARB. AUC for plasma glucose was lower in LO-CARB versus

HI-CARB ($P < 0.05$). Plasma NEFA was greater at 1–5 h in FAST versus HI-CARB ($P < 0.05$), and also versus LO-CARB ($P < 0.05$), except for 4 h ($P = 0.067$). Plasma NEFA was also greater at 1–3.5 h in LO-CARB versus HI-CARB ($P < 0.05$). AUC for plasma NEFA was greater in LO-CARB ($P < 0.01$) and FAST ($P < 0.001$) versus HI-CARB, and in FAST versus LO-CARB ($P < 0.01$). Plasma glycerol was greater in FAST at 1–3 h, and 4 h versus LO-CARB, and at 1, 1.75, 3, and 4 h versus HI-CARB ($P < 0.05$). AUC for plasma glycerol was greater in FAST versus LO-CARB and HI-CARB ($P < 0.001$; **Figure 6.5**).

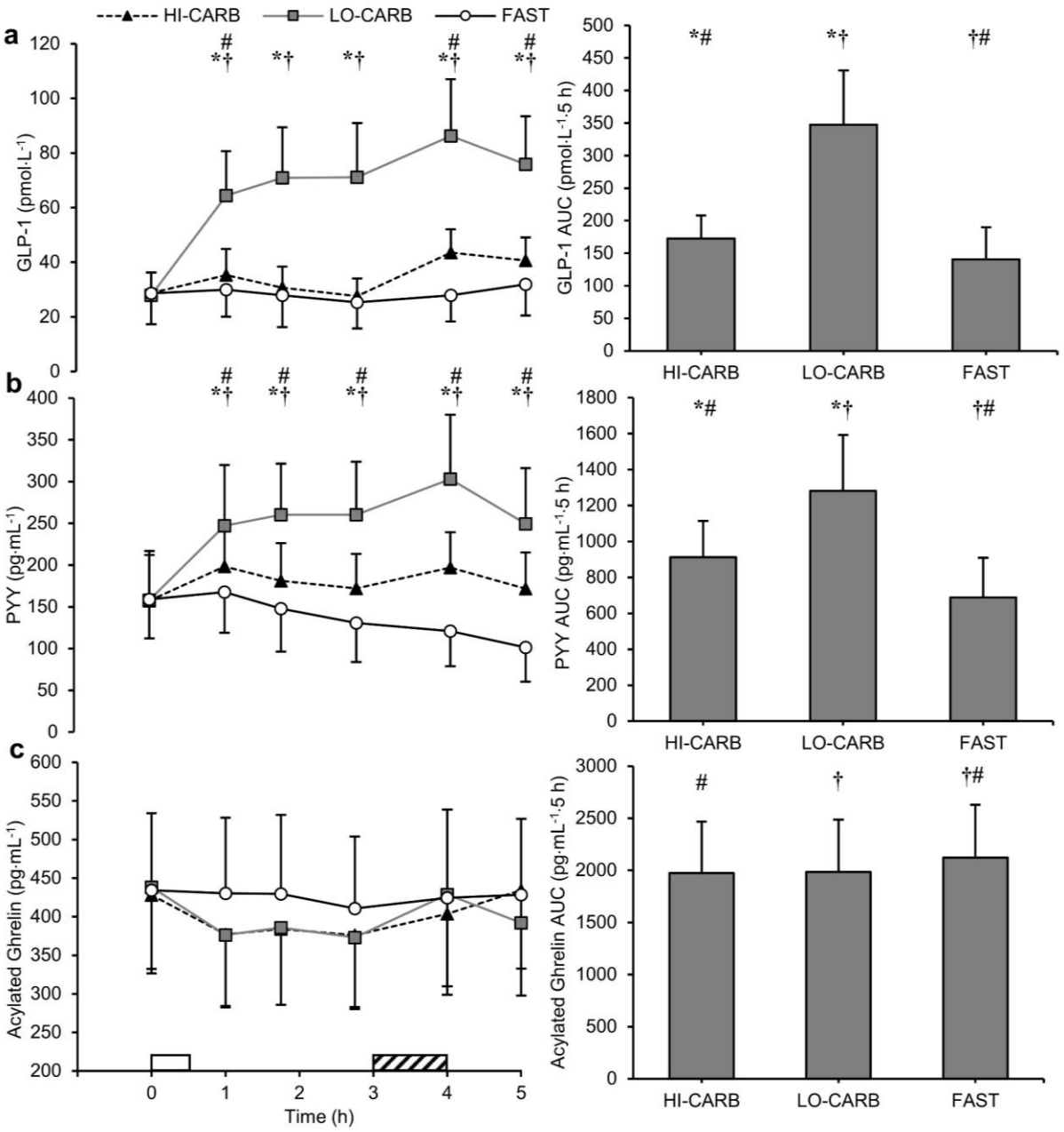

Figure 6.4. Plasma concentrations of **(a)** GLP-1, **(b)** PYY, and **(c)** acylated ghrelin during HI-CARB, LO-CARB and FAST. Data are presented at each time point (left) and as total area under the curve (AUC) for each trial (right). Data are mean ± SD (GLP-1 and PYY) or mean ± SEM (acylated ghrelin). White rectangle represents standardised lunch; diagonal striped rectangle represents exercise. * LO-CARB vs. HI-CARB ($P < 0.05$); † LO-CARB vs. FAST ($P < 0.05$); # HI-CARB vs. FAST ($P < 0.05$).

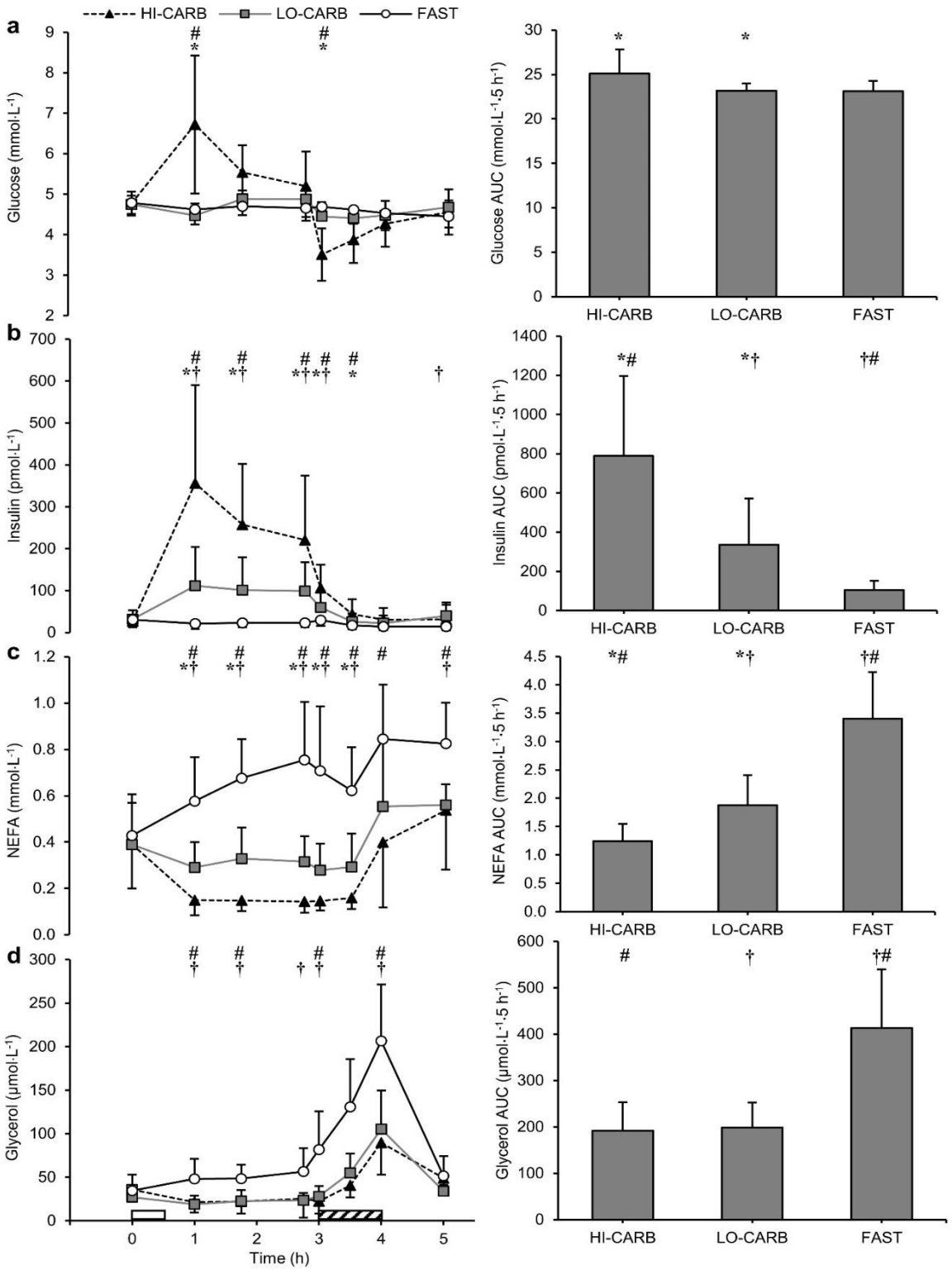

Figure 6.5. Plasma concentrations of **(a)** glucose, **(b)** insulin, **(c)** non-esterified fatty acids (NEFA), and **(d)** glycerol during HI-CARB, LO-CARB and FAST. Data are presented at each time point (left) and as total area under the curve (AUC) for each trial (right). Data are mean ± SD. White rectangle represents standardised lunch; diagonal striped rectangle represents exercise. * LO-CARB vs. HI-CARB ($P < 0.05$); † LO-CARB vs. FAST ($P < 0.05$); # HI-CARB vs. FAST ($P < 0.05$).

6.4. Discussion

This study showed that a low-carbohydrate, high-protein lunch increased fat oxidation during evening exercise compared to a high-carbohydrate, lower-protein lunch, although this increase was less (-3.66 g) than that following an 8 h fast. The LO-CARB lunch also suppressed appetite and reduced *ad-libitum* energy intake in the evening by ~313 kcal compared to HI-CARB, and ~383 kcal compared to fasting. These findings suggest that a LO-CARB lunch could be used to achieve some of the beneficial metabolic responses to fasted exercise, whilst also mitigating the challenges to appetite associated with fasted evening exercise. Future studies are required to assess whether acutely reducing carbohydrate intake and increasing protein intake prior to exercise on a regular basis can be used to assist in weight and health management.

Fasting before exercise increases fat oxidation compared to carbohydrate-fed conditions (Vieira et al., 2016), which, when incorporated into a training programme, may improve insulin sensitivity (Edinburgh et al., 2020; Van Proeyen et al., 2010). These improvements may be partly due to an increased capacity to oxidise fat (Robinson et al., 2015*a*). The inhibition of fat oxidation during fed-state exercise is likely governed primarily by the insulinemic response to carbohydrate ingestion (Vieira et al., 2016). Prior studies have reported that similar fat oxidation can be achieved during morning exercise after a low-carbohydrate, high-protein meal, and complete fasting (Impey et al., 2015; Rothschild et al., 2021; Taylor et al., 2013). The present study extends these findings by showing that a low-carbohydrate (18 ± 2 g carbohydrate), high-protein lunch increased fat oxidation by 8.00 g during 60 min evening exercise compared to an isocaloric high-carbohydrate (163 ± 19 g carbohydrate), lower-protein lunch, albeit to a lesser extent than after an 8 h fast (+11.66 g). These findings suggest that pre-exercise carbohydrate restriction may offer an alternative method of achieving some of the metabolic benefits associated with increased fat oxidation, without enduring extended fasting during the day.

The stepwise increase in fat oxidation between trials was mirrored by a stepwise reduction in insulin concentrations. Consuming carbohydrate increases plasma glucose and insulin concentrations (Coyle et al., 1997), inhibiting hormone-sensitive lipase activity and lipolysis (Saltiel & Kahn, 2001), and stimulating fatty acid re-esterification in adipose tissue (Enevoldsen et al., 2004; Frayn et al., 1995). This ultimately reduces fatty acid availability for oxidation during exercise after carbohydrate feeding (Coyle et al., 1997; Vieira et al., 2016). Accordingly, plasma NEFA concentrations showed stepwise increases between trials in-line

with differences in fat oxidation. The fat content of the pre-exercise meals was closely matched (difference of ~6 g, or ~4% energy from fat), so it is unlikely that the differences in NEFA concentrations following the LO-CARB and HI-CARB meals are a product of dietary fat appearance, and therefore, likely indicate increased endogenous fat oxidation in LO-CARB.

Plasma glycerol concentrations, however, which are often used as a surrogate marker of adipose tissue lipolysis (Robinson et al., 2016), were only elevated during fasted exercise, suggesting different mechanisms may explain the increased fat oxidation in LO-CARB and fasting trials, likely increased intramuscular triglyceride utilisation (Coyle et al., 1997; van Loon et al., 2003). Although, from the static measurement of plasma glycerol in this study, it cannot be determined whether glycerol flux was altered in the LO-CARB trial (Robinson et al., 2016; van Hall et al., 2002). However, studies report elevated NEFA and glycerol concentrations during exercise after smaller doses of protein (Erdmann et al., 2010; Impey et al., 2015; Oliveira et al., 2021), suggesting the high protein dose and the resultant insulin concentrations in the present study might have reduced lipolysis and fat oxidation compared to fasting. This is supported by observations that even small increases in plasma insulin concentrations can suppress lipolysis (Bonadonna et al., 1990).

The high protein content of the LO-CARB lunch profoundly suppressed appetite, and reduced evening energy intake by 22% and 27% compared to HI-CARB and fasting. Oliveira et al. (2021) similarly reported lower post-exercise hunger when performed after a high-protein breakfast. Evidence supports the effects of protein intake on suppressing subjective appetite and increasing post-prandial concentrations of anorexigenic hormones GLP-1 and PYY dose-dependently (Belza et al., 2013; Leidy et al., 2015; Rolls et al., 1988). Consistent with this, GLP-1 and PYY concentrations were also greater in LO-CARB. GLP-1 is also an incretin, enhancing glucose-dependent insulin secretion (Watkins et al., 2021), so the increased GLP-1 in LO-CARB might also benefit postprandial glucose control. The orexigenic hormone acylated ghrelin was suppressed by both lunch meals, suggesting the increase in protein intake did not alter responses. This is consistent with some (Belza et al., 2013; Erdmann et al., 2004), but not all (Blom et al., 2006) studies. However, it should be noted that acylated ghrelin concentrations typically fall rapidly after the onset of food intake, and often reach nadir values within less than 1 h (Callahan et al., 2004). Therefore, although findings from the present study indicate the appetite-suppressing effects of the LO-CARB meal were likely mediated via GLP-1 and PYY, it is possible that by delaying the first blood sample until 1 h after the initiation of

lunch, potential differences in acylated ghrelin concentrations between LO-CARB and HI-CARB may have been missed.

Although every effort was made to ensure that the LO-CARB and HI-CARB meals were matched with regards to their sensory properties, the considerable differences in their macronutrient compositions made this practically challenging, and some perceptual differences were identified. Specifically, the LO-CARB lunch was rated as less pleasant, possibly due to the increased chewiness of the sandwich, and increased thickness and creaminess of the drink. Such perceptual differences have the potential to influence subjective appetite and subsequent energy intake. For example, it is well established that increasing the pleasantness of a meal increases appetite and energy intake within-meal, but may also increase appetite in the hours proceeding the meal (Hill et al., 1984). Additionally, increasing the viscosity of a liquid is known to suppress subjective appetite (Marciani et al., 2001; Solah et al., 2010) and food intake (Ho et al., 2015; Vuksan et al., 2009).Therefore, whilst the protein content of LO-CARB and its subsequent effects on GLP-1 and PYY concentrations likely mediated the appetite suppressing effects of this meal, the apparent perceptual differences likely accentuated this effect.

One important factor to note is that the reduction in *ad-libitum* evening energy intake after the LO-CARB meal only compensated for ~32% of the lunch meal, so energy intake over the course of the day was still ~803 kcal lower in the fasted trial. This is consistent with previous studies comparing fed and fasted exercise performed in the morning (Bachman et al., 2016; Edinburgh et al., 2019; Gonzalez et al., 2013; Griffiths et al., 2020), but it is important to acknowledge the additional challenges associated with fasted evening exercise. In **Chapter 5**, fasting from 11:30 until evening exercise at 18:30 reduced motivation to exercise, exercise enjoyment, and performance during exercise. Similarly, the present study showed that fasting before evening exercise reduced pre-exercise energy levels, although other subjective markers including motivation, tiredness, and readiness to exercise were unaffected by prior fasting. In any case, a low-carbohydrate, high-protein lunch may help achieve a better psychological state for engaging in regular exercise.

Energy expenditure during the trial period was greater after both the LO-CARB and HI-CARB lunch meals compared to fasting. Other than the prescribed exercise, which was standardised between trials, physical activity was minimised within the laboratory. Therefore, the increase in energy expenditure in these trials was likely due to increased dietary induced thermogenesis

(DIT), which refers to the energy expended in the process digesting and assimilating nutrients (Westerterp, 2004). Interestingly, energy expenditure was greater in the LO-CARB trial compared to the HI-CARB trial. DIT is greater for protein (20–30% of energy intake) than it is for carbohydrate (5–10% of energy intake) or fat (0–3% of energy intake) (Westerterp, 2004), suggesting the increased energy expenditure in LO-CARB was likely due to the protein content of the meal. Although energy expenditure was only measured for 1 h post-exercise in the present study, it has previously been shown that a high-protein pre-exercise meal can increase energy expenditure for up to 24 h post-exercise compared to a high-carbohydrate pre-exercise meal (Hackney et al., 2010). As such, increasing energy expenditure is an additional pathway through which a low-carbohydrate, high-protein meal may help create a more negative energy balance with exercise.

Although the present study demonstrates encouraging findings with regards to the acute metabolic and energy balance responses to low-carbohydrate, protein-fed exercise, the longer-term implications of regularly adhering to this intervention in the real world are unknown. The previous findings of Edinburgh et al. (2020) showing that fat oxidation is persistently elevated during overnight-fasted exercise performed regularly (3 times per week) over a 6-week period lend promise to the idea that the acutely elevated fat oxidation during LO-CARB might also persist during repeated exposures. This concept is supported by Aird et al. (2021), who reported that muscle CD36 mRNA expression was similarly upregulated following 3 weeks of sprint interval training performed 3 times per week after either protein-only feeding or after an overnight fast. Regarding weight management, there are several external factors which influence energy balance behaviours in the real world which are controlled for in laboratory-based studies like the present study. This may partly explain the largely null weight management findings when fasted exercise studies are translated into free-living environments (Brinkmann et al., 2019; Edinburgh et al., 2020; Schoenfeld et al., 2014; Verboven et al., 2020). However, due to the superior appetite suppressing effects of the LO-CARB meal compared to fasting, it is possible that regularly consuming a low-carbohydrate, high-protein meal before exercise may mitigate against some of the compensatory energy balance behaviours which occur in response to fasting-based interventions (Betts et al., 2014; Chowdhury et al., 2016*b*), although this requires further study.

Conclusion

This study showed that the acute consumption of a low-carbohydrate, high-protein lunch before evening exercise increased fat oxidation compared to a high-carbohydrate, lower-protein lunch, although the increase was less than that following an 8 h fast. The low-carbohydrate, high-protein lunch also reduced appetite and subsequent energy intake, meaning consuming such a meal might offer some of the metabolic benefits associated with fasted exercise without the need to endure daytime fasting. Future long-term studies are required to explore whether acute exercise after a low-carbohydrate, high-protein meal can be implemented on a regular basis as a method of managing body weight/composition and maintaining metabolic health.

Chapter 7 – Effect of a very low-energy, 'placebo' meal on subsequent appetite and energy intake in healthy males

7.1. Introduction

Obesity is caused by a sustained positive energy imbalance, in which energy intake exceeds energy expenditure (Hall et al., 2012; Hill et al., 2012) and is a risk factor for several chronic diseases (Bray, 2004). Action taken to prevent weight gain may yield greater success than attempts to treat obesity, due to the energy balance system showing a stronger opposition to weight loss than weight gain (Hill et al., 2012). Reducing daily energy intake is a seemingly simple solution to this, although numerous factors often impede the long-term success of such interventions, including compensatory alterations in appetite which can stimulate an increase in energy intake (Polidori et al., 2016).

Extending the overnight fast, thereby restricting the time available for food intake, is a simple and effective dietary strategy for reducing daily energy intake (Betts et al., 2016; Clayton & James, 2016; Clayton et al., 2020). Acutely, morning fasting typically elevates subjective appetite (Chowdhury et al., 2015; Chowdhury et al., 2016*a*; Clayton et al., 2015; Clayton et al., 2016*a*), and concentrations of the appetite-stimulating hormone ghrelin (Cummings et al., 2001), and reduces appetite-suppressing hormones such as peptide tyrosine-tyrosine (PYY) (Chowdhury et al., 2015; Chowdhury et al., 2016*a*; Batterham et al., 2002) during the fasting period. Morning fasting also often leads to increased energy intake at lunch (Astbury et al., 2011; Chowdhury et al., 2015; Clayton et al., 2015; Levitsky & Pacanowski, 2013), although this rarely fully compensates for energy omitted by fasting during the morning (*i.e.*, skipping breakfast). However, longer-term studies have observed increased energy intake during repeated periods of morning fasting. Farshchi et al. (2005*a*) showed that two weeks of delaying the first meal, from 08:00 until 11:00, increased self-reported daily energy intake in lean females, and another study showed that the increased energy intake over the day fully compensated for the energy omitted via six weeks of daily fasting until 11:00 (Chowdhury et al., 2016*b*). These studies highlight the challenge of compensatory eating associated with repeated morning fasting.

The inability to blind subjects to fasting causes a problem for the interpretation of data from these studies (Sievert et al., 2019). Placebo-controlled study designs are used in research to dissociate the physiological and psychological effects of an intervention and have been recently employed in the context of morning fasting. Consuming a virtually energy-free 'placebo' meal

can suppress subjective appetite by a similar extent to an energy-containing (~496 kcal) meal (Naharudin et al., 2020), despite only the high energy-containing meal suppressing total plasma ghrelin concentrations, compared to the placebo meal having no effect on plasma ghrelin concentrations. This disparity between the subjective and physiological markers of appetite suggests the response may, at least partly, be due to psychological factors associated with the act of consuming a meal, rather than physiological effects related to nutrient consumption *per se*. Importantly, eating behaviour does not universally correspond to changes in subjective appetite or ghrelin and PYY concentrations (Chowdhury et al., 2016*a*; Clayton et al., 2014; James et al., 2015), therefore, whether the suppression of subjective appetite caused by a placebo meal manifests as a reduction in energy intake at a subsequent eating occasion is not known.

The aims of this study were to examine the effects of a very low-energy, viscous placebo meal on subjective appetite, appetite-regulatory hormone concentrations, and subsequent energy intake at an *ad-libitum* lunch, compared to a typical whole-food meal and a water-only control.

7.2. Methods

Subjects

Fourteen healthy, recreationally active (<10 $h \cdot wk^{-1}$) males who regularly consumed breakfast (self-reported) completed the study (**Table 7.1**).

Table 7.1. Subject baseline characteristics (*n*=14).

Characteristic	Mean	SD
Age (y)	24	2
Weight (kg)	77.1	6.8
Height (m)	1.81	0.07
BMI ($kg \cdot m^{-2}$)	23.5	2.3
Body fat (%)	13.2	3.4
Dietary restraint[a]	6	2
Dietary disinhibition[a]	5	2
Hunger[a]	6	3
Estimated resting metabolic rate ($kcal \cdot day^{-1}$)[b]	1792	93

[a] Three-factor eating questionnaire (Stunkard & Messick, 1985)
[b] Estimated via predictive equation (Mifflin et al., 1990)

Study Design

Subjects completed a preliminary trial, followed by three experimental trials that were separated by ≥4 days. Each experimental trial involved the consumption of a different breakfast meal, before energy intake was assessed at an *ad-libitum* lunch meal 3.25 h later. The meals investigated were a very low-energy, viscous placebo meal (PLA), a typical whole-food meal (FOOD), and a water-only control (WAT). Subjects were informed that the purpose of the study was to compare subjective and physiological responses to a 'novel meal' and were unaware that the PLA meal contained almost no energy. Following completion of the final experimental trial, the contents of the PLA meal were revealed to the subjects, and they were informed of the true aims of the study.

Preliminary Trial

Subjects' body mass and height were measured, before body fat percentage was estimated by measuring skinfold thickness. Subjects were also familiarised to the *ad-libitum* lunch meal procedures used in experimental trials.

Protocol

Subjects arrived at the laboratory at 08:30 following a ≥11 h overnight fast (water was permitted overnight, but volume was recorded after the first trial and standardised for subsequent trials) and rested in a supine position for 20 min before baseline venous and capillary blood samples were collected. Baseline measures of subjective appetite were obtained immediately before subjects were provided with their allocated breakfast meal (0 h), which was consumed in its entirety within 10 min. A second subjective appetite measurement was obtained immediately after meal consumption. Subjects then rested quietly in the laboratory, with subjective appetite measurements collected at 0.5, 1, 2, and 3.25 h; capillary blood samples were collected at 0.5, 1, 1.5, 2, and 3 h; and venous blood samples were collected after 20 min of supine rest at 1 and 3 h. Subjects did not consume any additional water between breakfast and lunch. An *ad-libitum* pasta lunch meal (1.25 ± 0.01 $kcal \cdot g^{-1}$) was served at 3.25 h, and subjects were permitted 20 min to eat. Subjective appetite measurements were obtained immediately before, immediately after, and 1 h after the eating period. Subjects did not consume any additional food or fluids until after the final appetite measurement was completed.

Breakfast Meals

During PLA, subjects consumed a viscous breakfast meal with a volume equating to 5 mL·kg body mass^{-1}. The meal consisted of 15% (0.75 mL·kg body mass^{-1}) low-energy flavoured squash (Vimto – No Added Sugar Squash, Vimto, UK), with the remainder made up of tap water. To thicken the solution and increase the perception of energy intake (Fiszman & Varela, 2013), 0.1 g·kg body mass^{-1} xanthan gum (My Protein, UK) was added, and the mixture was blended thoroughly. This resulted in a viscous mixture similar in consistency to soft-set jelly which was not possible for subjects to simply drink and was required to be consumed from a standard bowl with a standard spoon. During FOOD, subjects consumed a standardised meal consisting of puffed rice cereal (Rice Krispies, Kellogg's, UK), semi-skimmed milk (Sainsbury's UK), white bread (Hovis, UK), seedless strawberry jam (Hartley's, UK), and apple juice (Sainsbury's UK). This was selected to provide 20% of estimated energy requirements, determined by multiplying resting metabolic rate (Mifflin et al., 1990) by a physical activity level of 1.6. Subjects ate the cereal and milk in FOOD from a standard bowl using a standard spoon. During WAT, subjects consumed 8 mL·kg body mass^{-1} of plain tap water. Tap water was consumed alongside the PLA and FOOD meals, to ensure iso-volume total water content of all three meals. The nutritional contents of the breakfast meals are presented in **Table 7.2**.

Table 7.2. Nutritional contents of the breakfast meals.

	WAT		PLA		FOOD	
	Mean	SD	Mean	SD	Mean	SD
Carbohydrate (g)	0	0	1.4	0.1	114.9	5.9
Protein (g)	0	0	0.3	0	15.7	0.8
Fat (g)	0	0	0	0	5.1	0.3
Fibre (g)	0	0	4.8	0.4	2.4	0.1
Energy (kcal)	0	0	16	1	573	30
Energy (kJ)	0	0	68	6	2399	124
Water (mL)	618	54	618	54	618	54
Volume (g)	618	54	625	55	757	60

WAT, water-only control; PLA, placebo breakfast; FOOD, typical whole-food breakfast

Subjective Appetite Responses

Subjects rated sensations of hunger, fullness, desire to eat (DTE), prospective food consumption (PFC), and nausea on paper-based 100 mm visual analogue scales (VAS). Ratings of subjective sensations of alertness, satisfaction, tiredness, relaxation, and energy were included as decoy questions to distract subjects from the main study outcomes.

Blood Sampling and Analysis

Venous blood samples (~10 mL per sample) were collected after 20 min of supine rest and were treated and analysed for determination of acylated ghrelin and PYY concentrations, as described in **Chapter 3**. Capillary blood samples were collected by piercing the fingertip and were analysed for determination of glucose concentrations, as described in **Chapter 3**. Due to an issue with venous blood collection, one subject's venous blood samples were omitted from the final analysis.

Statistical Analyses

For subjective appetite-related variables and blood glucose concentrations, total area under the curve (AUC) values were calculated using the trapezoidal method. Data were analysed using the methods described in **Chapter 3**. For plasma acylated ghrelin concentrations, box plot analyses showed two consistently outlying subjects within the data set, exhibiting concentrations ~5 and ~10 SD greater than the mean of the eleven other subjects. Therefore, these subjects were removed from the analysis of acylated ghrelin data.

7.3. Results

Ad-libitum Food and Water Intake

Ad-libitum energy intake at lunch was significantly greater during WAT (1093 ± 249 kcal) compared to FOOD (981 ± 284 kcal; $dz = 0.91$; $P < 0.05$), with PLA (1062 ± 273 kcal) not different from WAT ($dz = 0.24$; $P = 1.000$) or food ($dz = 0.60$; $P = 0.088$). There was no effect of trial order on *ad-libitum* energy intake ($P = 0.696$). Combining energy intake at lunch with the energy contained in each breakfast meal, cumulative energy intake during FOOD (1554 ± 301 kcal) was greater than during PLA (1078 ± 274 kcal; $d_z = 3.49$; $P < 0.001$) and WAT (1093 ± 249 kcal; $d_z = 3.32$; $P < 0.001$). Cumulative energy intake was not different between PLA

and WAT (d_z = 0.11; P = 1.000; **Figure 7.1**). There were no differences in *ad-libitum* water intake at lunch between trials (PLA: 397 ± 211 mL; FOOD: 373 ± 171 mL; WAT: 376 ± 154 mL; P = 0.768).

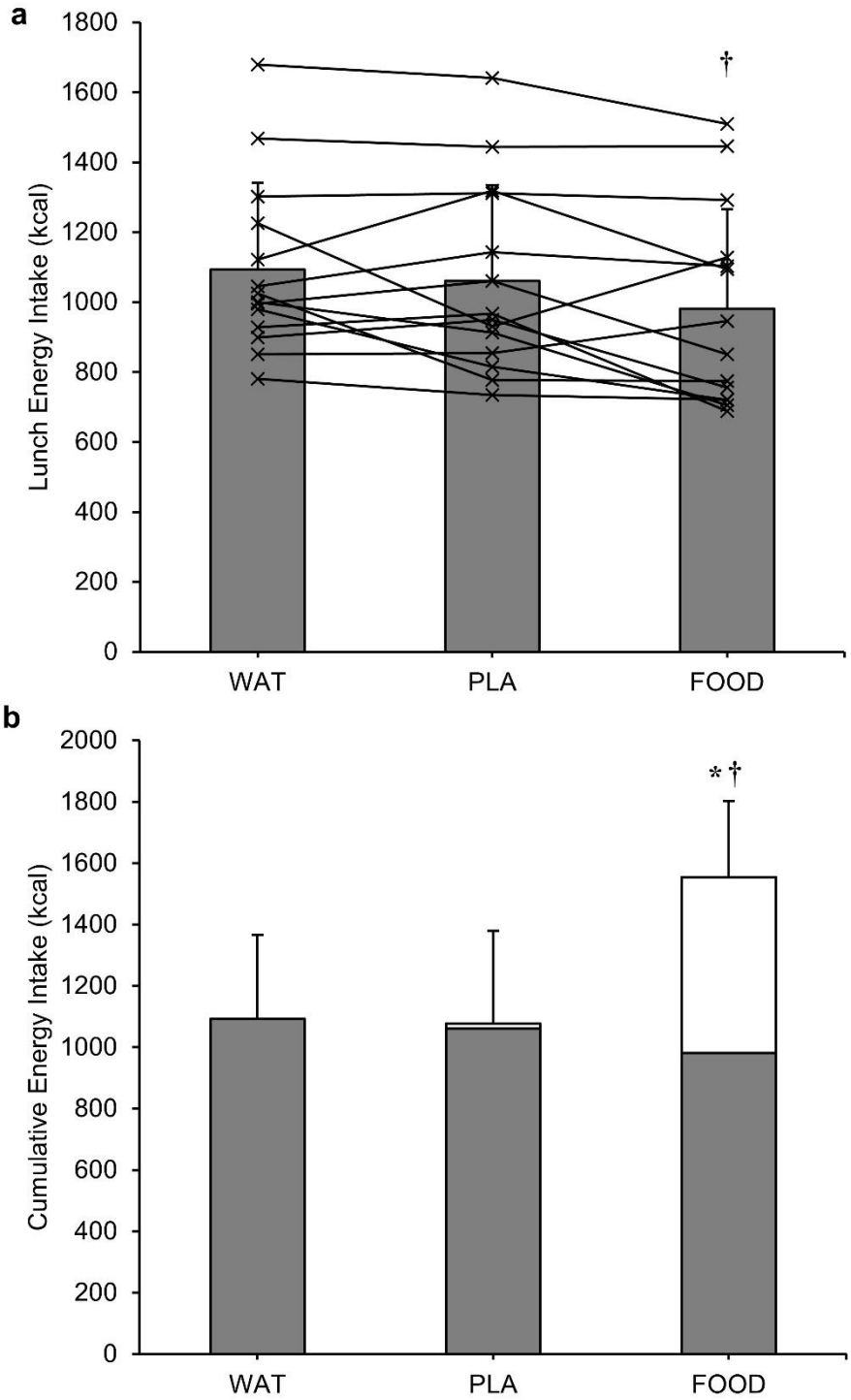

Figure 7.1. **(a)** *Ad-libitum* energy intake (kcal) at lunch and **(b)** cumulative energy intake (kcal) across the entire trial. The bars display mean values at lunch and breakfast, with vertical error bars representing SD. The lines display individual subjects' lunch energy intake for each experimental trial. † FOOD vs. WAT (P < 0.05); * FOOD vs. PLA (P < 0.05).

Subjective Appetite Responses

There were trial ($P < 0.001$), time ($P < 0.001$), and interaction ($P < 0.001$) effects for hunger, fullness, PFC, and DTE. There were no significant effects for nausea ($P \geq 0.081$). AUC for hunger ($d_z = 0.79$), DTE ($d_z = 0.69$), and PFC ($d_z = 0.80$) were lower, and fullness was higher ($d_z = 0.71$), during PLA compared to WAT ($P < 0.05$). AUC for hunger ($d_z = 1.63$), DTE ($d_z = 1.43$), and PFC ($d_z = 2.05$) were also lower, and fullness was also higher ($d_z = 1.38$), during FOOD compared to WAT ($P < 0.001$; **Figure 7.2**). Additionally, AUC for hunger was lower during FOOD compared to PLA ($d_z = 0.60$; $P < 0.05$). AUC for nausea was not different between trials ($P = 0.070$; **Figure 7.3**).

Following breakfast consumption, hunger was lower during PLA and FOOD, compared to WAT, for 1 h ($P < 0.05$) and remained lower in FOOD for 2 h ($P < 0.05$). Hunger was not different between trials immediately before lunch ($P \geq 0.091$). Fullness was higher in PLA compared to WAT immediately, and 0.5 h post-breakfast ($P < 0.05$). Fullness was significantly greater in FOOD compared to WAT at all time points until immediately before lunch ($P < 0.05$), except for 0.5 h ($P = 0.064$). PFC was lower in PLA and FOOD compared to WAT for 0.5 h post breakfast ($P < 0.05$) and remained lower in FOOD until 2 h ($P < 0.01$). DTE was lower in FOOD, compared to WAT, for 2 h after breakfast ($P < 0.01$), but there were no differences between PLA and FOOD ($P \geq 0.126$) or PLA and WAT ($P \geq 0.066$) at any time point.

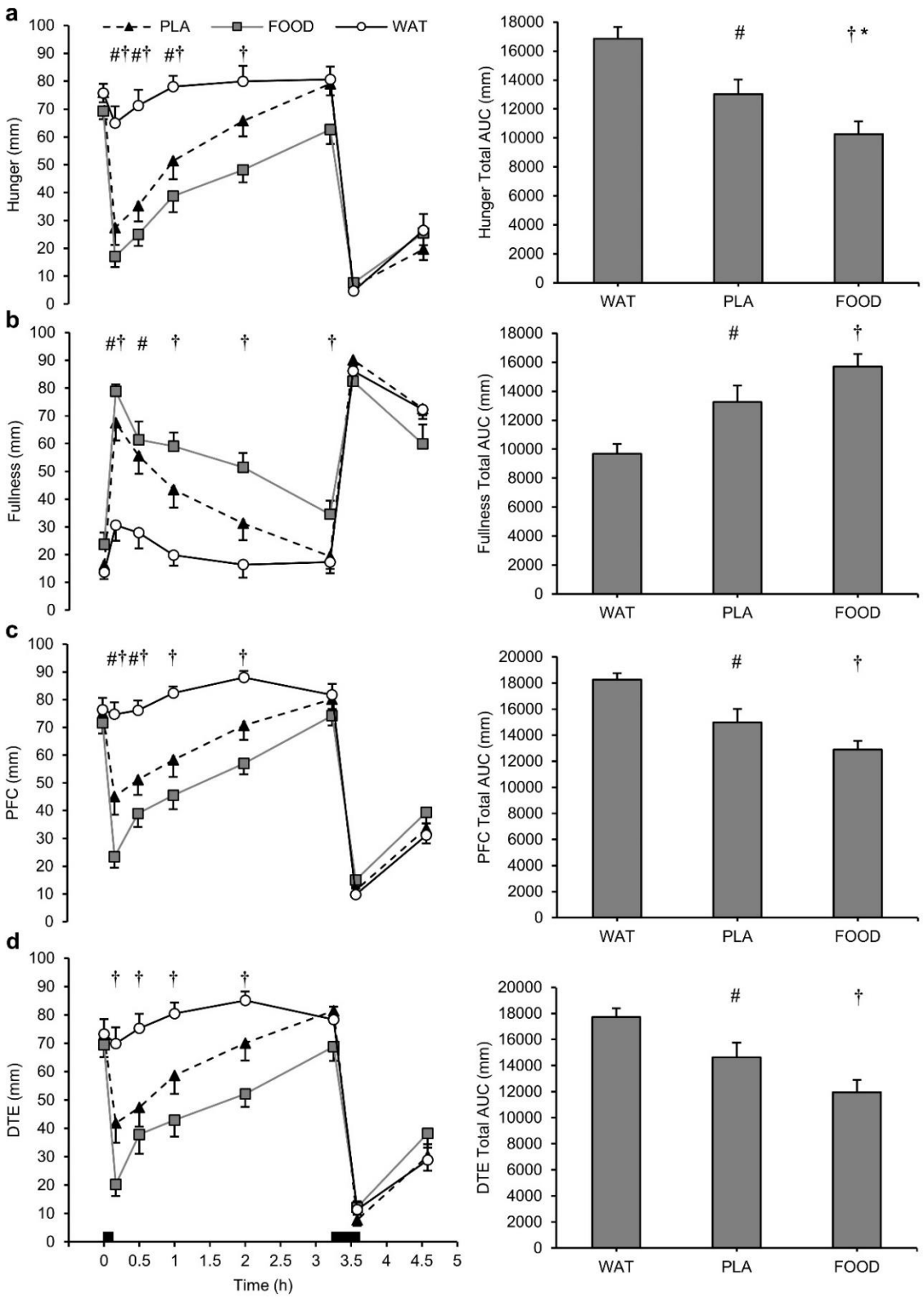

Figure 7.2. (a) Hunger, **(b)** fullness, **(c)** prospective food consumption (PFC), and **(d)** desire to eat (DTE) during WAT, PLA, and FOOD. Data are presented at each time point (left) and as total area under the curve (AUC) for each trial (right). † FOOD vs. WAT ($P < 0.05$); * FOOD vs. PLA ($P < 0.05$); # PLA vs. WAT ($P < 0.05$). Black rectangles represent breakfast and lunch. Data are mean ± SEM.

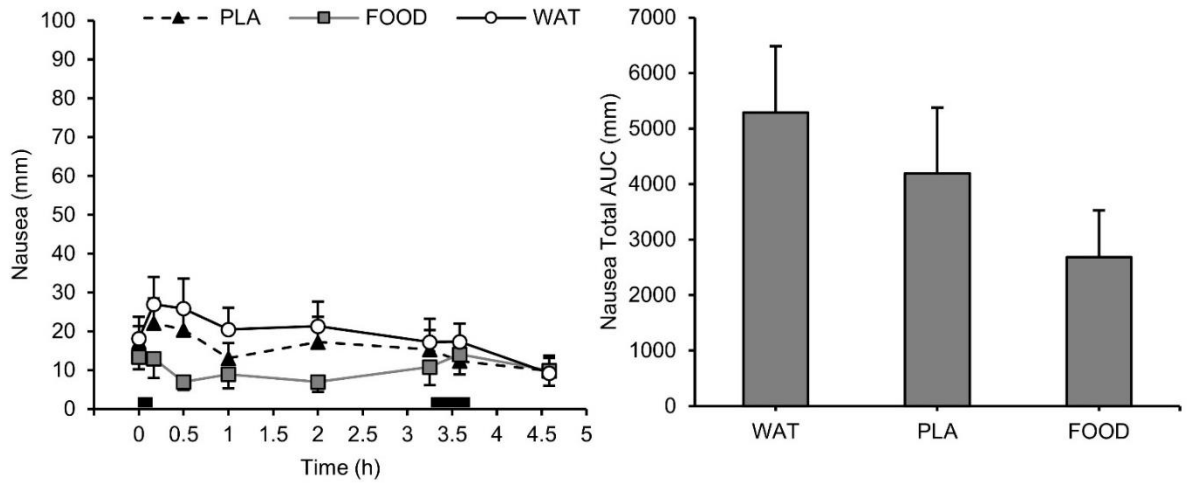

Figure 7.3. Nausea during WAT, PLA, and FOOD. Data are presented at each time point (left) and as total area under the curve (AUC) for each trial (right). Black rectangles represent breakfast and lunch. Data are mean ± SEM.

Blood Analyses

There were time ($P < 0.001$), trial ($P < 0.001$), and interaction ($P < 0.001$) effects for blood glucose concentrations. Compared to FOOD, blood glucose concentrations were lower during PLA and WAT at 0.5 ($P < 0.001$), 1.5 ($P < 0.01$), and 2 h ($P < 0.05$). Blood glucose concentrations increased after breakfast during FOOD and were significantly greater than baseline between 0.5 and 2 h ($P < 0.01$), returning to baseline concentrations at 3 h ($P = 0.501$). Blood glucose concentrations did not change from baseline during PLA ($P \geq 0.883$) or WAT ($P \geq 0.302$; **Figure 7.4**). AUC for blood glucose concentrations was significantly different between trials ($P < 0.001$). AUC was significantly higher in FOOD compared to PLA ($P < 0.001$) and WAT ($P < 0.001$). There was no difference in AUC for glucose between PLA and WAT ($P = 0.482$).

There were time ($P < 0.001$), trial ($P < 0.001$), and interaction ($P < 0.001$) effects for plasma acylated ghrelin concentrations. Acylated ghrelin concentrations were lower at 1 h during FOOD compared to PLA and WAT ($P < 0.05$). Acylated ghrelin concentrations were greater than baseline at 1 and 3 h in PLA ($P < 0.01$), and at 3 h in WAT ($P < 0.05$). Acylated ghrelin concentrations were lower than baseline at 1 h in FOOD ($P < 0.01$). Plasma PYY concentrations showed a main effect of time ($P < 0.001$), but there were no main effects of trial ($P = 0.187$), and no interaction effects ($P = 0.054$; **Figure 7.5**).

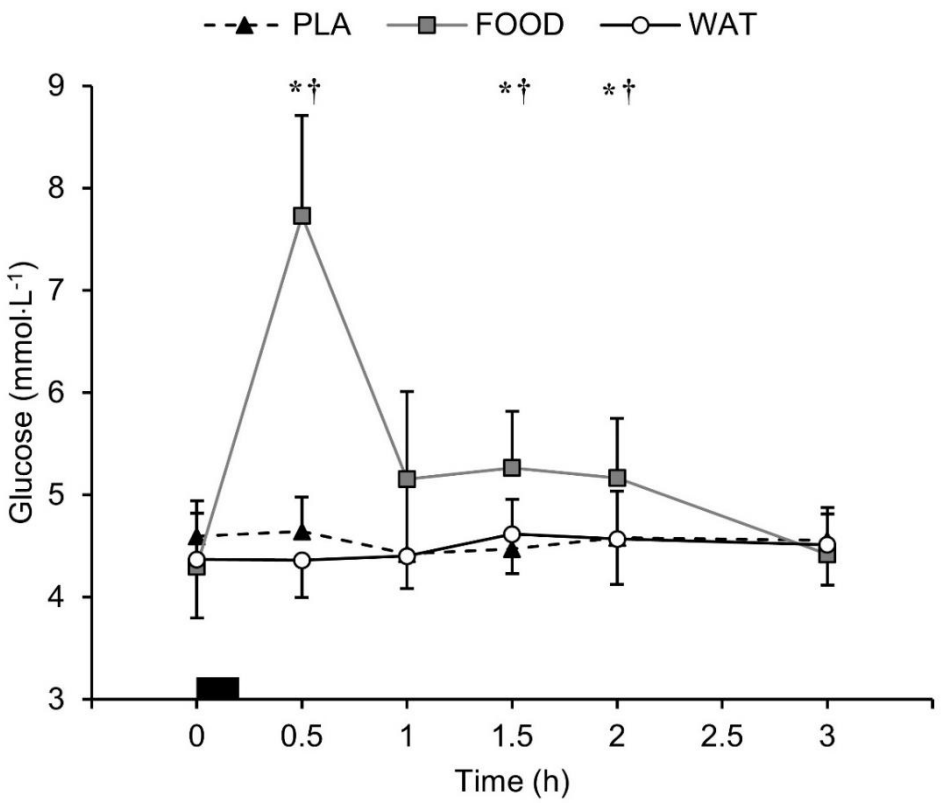

Figure 7.4. Blood glucose concentrations over the course of the trial during WAT, PLA, and FOOD. † FOOD vs. WAT ($P < 0.05$); * FOOD vs. PLA ($P < 0.05$). Black rectangle represents breakfast. Data are mean ± SD.

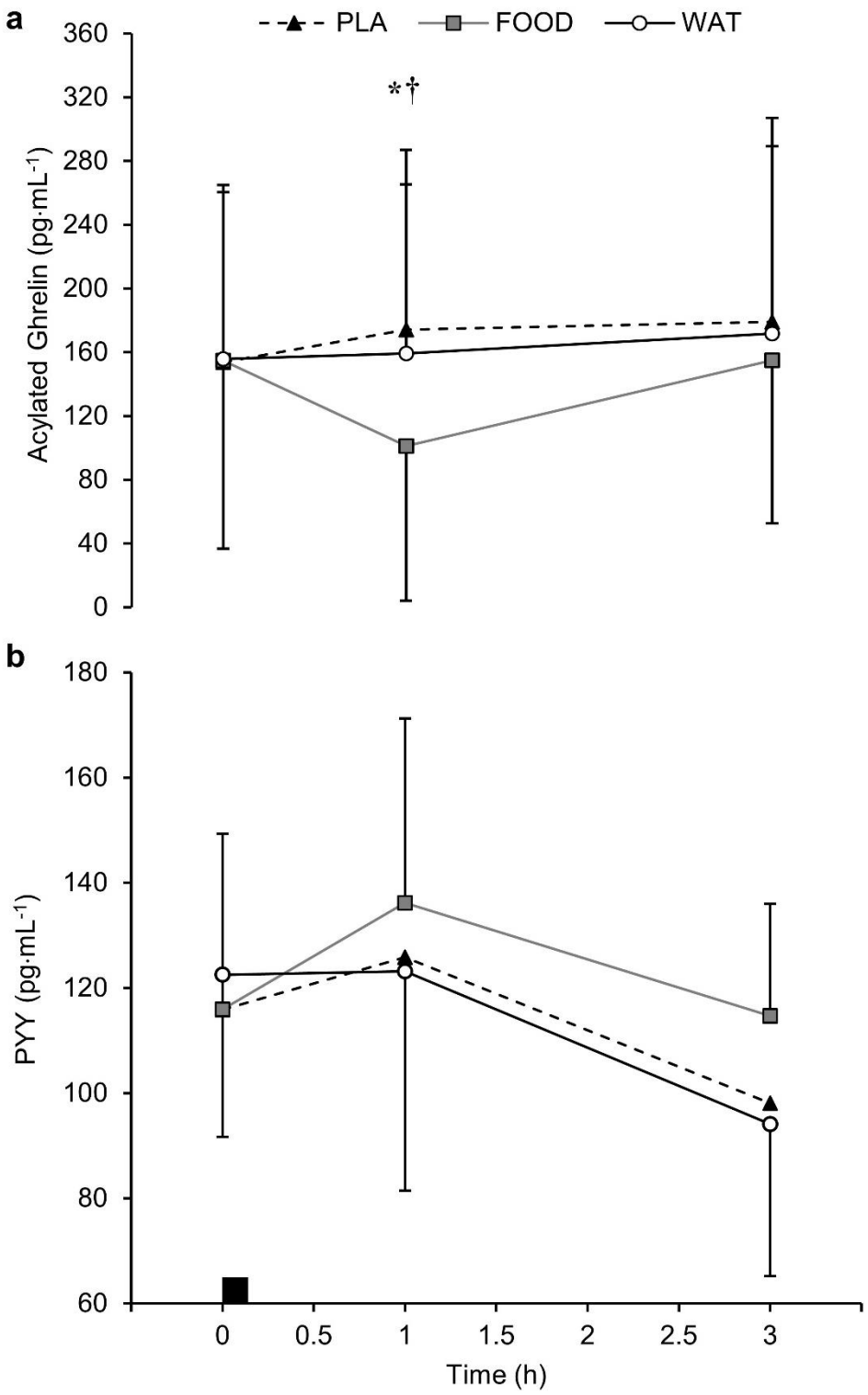

Figure 7.5. Plasma concentrations of **(a)** acylated ghrelin (*n*=11), and **(b)** PYY (*n*=13), over the course of the trial during WAT, PLA, and FOOD. † FOOD vs. WAT ($P < 0.05$); * FOOD vs. PLA ($P < 0.05$). Black rectangle represents breakfast. Data are mean ± SD.

7.4. Discussion

This study showed that a very low-energy, viscous placebo meal suppressed subjective appetite to a similar extent as a typical whole-food meal and more-so than a water-only control. However energy intake at lunch was only reduced after the whole-food meal, but not after the placebo meal, compared to the water-only control. Due to the very low energy content of the placebo meal, cumulative energy intake (breakfast plus lunch) was lower than the whole-food breakfast trial, and not different to the water-only trial. These results support the idea that morning fasting may successfully reduce energy intake over breakfast and lunch, but consuming a very low-energy, viscous placebo meal may attenuate the elevations in subjective appetite associated with morning fasting, potentially enhancing its efficacy by reducing the likelihood of mid-morning snacking and improving dietary adherence.

With the exception of one study which provided a notably small breakfast (~250 kcal) (Astbury et al., 2011), morning fasting studies show that the energy deficit created by fasting is not fully compensated for at lunch, resulting in a reduction in cumulative energy compared to when breakfast is consumed (Chowdhury et al., 2015; Chowdhury et al., 2016*a*; Clayton et al., 2015; Gonzalez et al., 2013; Levitsky & Pacanowski, 2013). This was also the case in the present study, as, compared to FOOD, cumulative energy intake was approximately 477 and 461 kcal lower during PLA and WAT. Whilst it is possible that further energy intake compensation may occur at subsequent meals, studies that have examined energy intake beyond a single meal have revealed no such compensation (Clayton et al., 2015; Levitsky & Pacanowski, 2013). Collectively, these studies suggest that the effects of morning fasting on *ad-libitum* energy intake are largely constrained to lunch. These findings demonstrate that total daily energy intake can be similarly reduced following both complete fasting during the morning and fasting instigated via a virtually energy-free meal.

We observed a 112 kcal increase in lunch energy intake following morning fasting, compared to when a ~575 kcal meal was consumed. This is consistent with previous studies reporting an increase in lunch energy intake of between 153–206 kcal following omission of a ~250–733 kcal breakfast (Astbury et al., 2011; Chowdhury et al., 2015; Clayton et al., 2015; Levitsky & Pacanowski, 2013). Some studies, however, have reported a similar energy intake at lunch following morning fasting and breakfast consumption (Chowdhury et al., 2016*a*; Gonzalez et al., 2013; Levitsky & Pacanowski, 2013). Inconsistencies in these findings may result from methodological differences between studies, such as differences in the time-interval between

breakfast and lunch, and/or the method employed to assess *ad-libitum* energy intake (*i.e.,* a homogenous, single-item meal versus a multi-item buffet meal).

Findings from longer-term studies suggest that some degree of adaptation may occur when morning fasting is repeated over consecutive days. In a crossover study, two weeks of daily fasting until 11:00 resulted in greater self-reported daily energy intake in a sample of healthy, lean females (Farshchi et al., 2005*a*). Furthermore, individuals with obesity were randomised to either fast until 11:00 or consume a 700-kcal breakfast before 11:00 daily for six weeks, with results showing that the compensatory increase in energy intake after morning fasting completely offset the difference in total daily energy intake between the groups (Chowdhury et al., 2016*b*). These data suggest that morning fasting over the long term may be associated with adaptations that drive an increase in appetite and energy intake to account for the energy omitted at breakfast.

The current study showed that appetite was supressed during PLA compared to WAT, which is similar to a previous study (Naharudin et al., 2020). Specifically, hunger, PFC and DTE were lower, and fullness was higher during the PLA and FOOD trials, compared to WAT. Dietary success is known to be influenced by persistently elevated appetite sensations (Polidori et al., 2016). In the present study, the suppression of appetite during PLA was most pronounced 0.5–1 h after breakfast, whereas FOOD suppressed appetite for longer. This indicates that consuming a placebo meal does not suppress appetite as strongly as after consuming a ~575 kcal whole-food meal. However, the transient suppression of appetite during PLA may be meaningful, as research has also linked morning fasting with increased impulsive snacking (Schlundt et al., 1992). Therefore, the immediate appetite suppressing effects of a very low-energy placebo meal may have the potential to improve dietary success by increasing restraint and reducing the temptation for snacking during fasting periods. Future research should aim to elucidate the effects of placebo meal consumption on dietary adherence and snacking behaviours in a free-living environment.

The viscosity of the PLA meal was increased by the addition of xanthan gum, a soluble fibre often used as a low-energy thickening agent (Habibi & Khosravi-Darani, 2017). The effects of several different viscous soluble fibres, including pectin, alginate, and β-glucan, on appetite and energy intake, have been examined in a number of studies with differing methodological designs (Fiszman & Varela; 2013; Ho et al., 2015). Typically, these studies compare the satiating properties of soluble fibre mixtures of varying viscosities (Bennet et al., 2009; Vuksan

et al., 2009), and/or nutritional contents (Marciani et al., 2001; Solah et al., 2010). It is generally agreed that increasing the viscosity of a liquid enhances its effects on satiety (Bennett et al., 2009; Marciani et al., 2000; Marciani et al., 2001; Solah et al., 2010) and food intake (Ho et al., 2015; Vuksan et al., 2009). The study of Marciani and Colleagues (2001) compared the appetite responses to test breakfast meals of both a high and low viscosity, which either contained ~323 kcal, or no energy. The meals of increased viscosity resulted in greater subjective satiety ratings, independent of the presence or absence of energy. Similar findings were observed by Solah et al. (2010), who showed that the viscosity of a test beverage had a greater effect on satiety than its protein content. These results suggest that the addition of soluble fibre to a meal may have more profound effects on appetite than its nutrient content.

Despite having a virtually identical energy and macronutrient content, PLA and WAT produced divergent appetite responses during the early post-meal period. The PLA meal contained a small amount of energy (16 ± 1 kcal), although data from our physiological variables indicate that this is unlikely to explain the differences in appetite between PLA and WAT. Acylated ghrelin and PYY respond predominantly to the ingestion of energy, rather than gastric distension (Oesch et al., 2006; Williams et al., 2003). Accordingly, the only changes observed in these physiological markers of appetite and blood glucose concentrations were after the energy-containing meal. Aligning with this, plasma concentrations of acylated ghrelin and PYY were not different between the WAT and PLA trials. Therefore, despite the whole-food meal inducing a hormonal response associated with increased satiety and reduced hunger, these physiological variables cannot explain differences in subjective appetite between the PLA and WAT trials. It should be noted that the physiological regulation of appetite is complex, and the effects of other hormones and/or neural signals on appetite during PLA cannot be ruled out.

Such discordant hormonal and subjective appetite responses have been observed previously following placebo meal consumption (Naharudin et al., 2020). Subjects in the present study and that of Naharudin et al. (2020) were self-reported regular breakfast consumers, and research suggests that morning fasting adversely affects appetite to a greater extent in habitual breakfast consumers than breakfast skippers (Thomas et al., 2015). Therefore, simply the knowledge of having consumed a meal, rather than the physiological responses to ingested nutrients, may mediate the satiating effects of breakfast consumption in these individuals. Additionally, consuming a volume of water immediately prior to a meal has been shown to reduce appetite and *ad-libitum* energy intake, likely via gastric distension (Corney et al., 2016), although the gastric emptying rate of water is rapid (Vist & Maughan, 1994), and its effects on

appetite are typically lost after 30 min in young individuals (Van Walleghen et al., 2007). Gastric emptying is, however, slowed in by the addition of soluble fibre (Yu et al., 2014). Because the addition of xanthan gum to PLA provided a small amount of fibre (~5 g), a delayed gastric emptying of PLA compared to WAT is a possible mechanism explaining the divergent appetite responses to the meals. Additionally, the oral processing of food which includes chewing and swallowing mediates the satiating effects of a meal via physiological and psychological mechanisms (de Graaf, 2012). As such, the prolonged oro-sensory exposure time of solid foods has been shown to elicit a greater and extended suppression of subjective appetite, compared to liquid foods (Mattes, 2005). This may also contribute to the differences in subjective appetite between PLA and WAT.

It has been previously reported that 6 weeks of morning fasting reduced habitual physical activity energy expenditure, which fully compensated for the reduction in energy intake, thus offsetting the energy deficit created by fasting (Betts et al., 2014). Whether a similar effect would be shown when fasting is instigated via the consumption of a very low-energy placebo meal is unknown. It is interesting to note, however, that two previous studies showed that endurance and resistance exercise performance were greater after a very low-energy placebo meal, compared to water-only (Mears et al., 2018; Naharudin et al., 2020). Therefore, it is plausible that the act of eating (rather than the specific content of the meal) in the morning is sufficient to maintain physical activity, and as such, a very low-energy placebo meal may present a more effective method of energy restriction. This warrants further investigation.

Herein we provide novel data demonstrating that an acute, single-exposure to placebo meal consumption can suppress subjective appetite compared to consuming water-only, and can reduce energy intake over breakfast and lunch, compared to a typical whole-food meal. These findings have practical implications for lean individuals looking to manage energy intake as a means of weight maintenance. Future studies should explore whether similar results would be observed following repeated placebo meal consumption over days and weeks, especially given the initial unfamiliarity of the viscous meal to subjects. Furthermore, to increase the application of this intervention, it would be prudent to examine responses to a placebo meal within an unblinded study design to account for potential demand effects resulting from knowledge of its lack of energy content. Finally, the effects of placebo meal consumption should be investigated in other population groups, specifically overweight or obese individuals, who have been shown to respond differently to acute and chronic morning fasting interventions (Betts et al., 2014; Chowdhury et al.,. 2015; Chowdhury et al., 2016*a*; Chowdhury et al., 2016*b*).

Conclusion

A typical, whole-food meal and a very low-energy placebo meal both reduced subjective appetite compared to water-only, but the placebo meal also reduced cumulative energy intake across breakfast and lunch. Therefore, placebo meal consumption may be an effective strategy for managing the elevations in appetite which often accompany fasting-based interventions, whilst still reducing cumulative energy intake and thus aiding weight management.

Chapter 8 – General Discussion

8.1. Summary of Key Findings

The studies presented within this thesis aimed to examine the appetite, energy intake, and metabolic responses to novel exercise and nutrition strategies. Ensuring that exercise and nutrition interventions can be conveniently embedded into lifestyles is fundamental in achieving adherence and long-term success. As such, this programme of work considered exercise timing and macronutrient manipulations of existing meals as important factors in study design and implementation. To do this, common exercise timing behaviours, preferences, and opportunities were first surveyed (**Chapter 4**), which subsequently informed the design of two laboratory studies that aimed to achieve fasted metabolic conditions via the manipulation of pre-exercise nutrition (**Chapters 5 and 6**). The final study of this thesis examined the utility of a novel, viscous meal, containing virtually no energy, as an alternative method to mitigate against some of the challenges associated with fasting-based regimes (**Chapter 7**). The main findings of this thesis are presented below:

Chapter 4

- Most people reported preferring to exercise in the morning, however, only a small proportion of people (31%) were able to exercise at their preferred time during the week.
- The most common time of day for people to exercise during the week was the early evening (16:00–19:59), whereas the morning (08:00–11:59) was the most common time for exercise during the weekend.
- 'Job/work commitments' was the main barrier preventing exercise at preferred times of the day. Accordingly, weekday exercise timing behaviours were associated with employment status, with the majority in full-time employment exercising in the early evening. This trend was inverted towards morning exercise in those who were unemployed/retired/self-employed, and in response to home-working as a result of COVID-19 lockdown restrictions.
- Weekday temporal restrictions resulting from full-time employment appears to be a primary factor governing exercise timing behaviours, with the early evening (16:00–19:59) identified as the ideal time of day in which there is opportunity for exercise to be incorporated with people's lives.

Chapter 5

- Fasting for 7 h increased fat oxidation and reduced carbohydrate oxidation during evening exercise compared to exercising 2 h after a meal.
- Fasting before evening exercise increased post-exercise appetite and energy intake. However, the increase in energy intake did not compensate for the omission of the pre-exercise meal. Accordingly, net energy intake was lower when evening exercise was performed following a 7 h fast.
- Motivation to exercise, voluntary exercise performance, and enjoyment of exercise sessions was reduced when exercising after a 7 h fast.
- The benefits associated with overnight-fasted morning exercise might be attained by individuals unable to exercise in the morning, however, the chronic success of this intervention may be compromised by elevations in appetite and subsequent energy intake, as well as reductions in the motivation to exercise and lower enjoyment of exercise.

Chapter 6

- A low-carbohydrate, high-protein meal consumed 3 h before evening exercise increased fat oxidation and reduced carbohydrate oxidation compared to a high-carbohydrate, lower-protein meal, albeit to a lesser extent than fasting for 8 h prior to exercise.
- Increased rates of fat oxidation after the low-carbohydrate, high-protein meal and fasting were accompanied by increased plasma concentrations of non-esterified fatty acids, and reduced plasma concentrations of glucose and insulin, although glycerol was only elevated during fasted exercise.
- The low-carbohydrate, high-protein meal also suppressed subjective appetite and induced a peripheral appetite hormone profile conducive to reduced hunger and increased satiety (*i.e.,* increased GLP-1 and PYY and reduced acylated ghrelin) compared to a high-carbohydrate, lower-protein meal, and an 8 h pre-exercise fast. Accordingly, post-exercise energy intake was reduced by 22% compared to a high-carbohydrate, lower-protein meal, and 27% compared to fasting.
- A low-carbohydrate, high-protein pre-exercise meal may offset some of the difficulties associated with fasted evening exercise, whilst also achieving much of the beneficial metabolic response.

Chapter 7

- Compared to overt morning fasting, both a very low-energy placebo meal and a typical whole-food meal reduced subjective appetite during the morning, although the whole-food meal suppressed appetite for longer (~2 h) compared to the placebo meal (~1 h).
- Only the whole-food meal altered circulating concentrations of peripheral appetite hormones, suppressing plasma concentrations of acylated ghrelin and elevating concentrations of PYY compared to the very-low-energy placebo and water-only.
- Energy intake at lunch was lower only following the whole-food meal, but not the very low-energy placebo meal, compared to water-only. Nevertheless, due to the very low energy content of the placebo meal, cumulative energy intake (breakfast plus lunch) was lower in the very low-energy placebo trial, compared to the typical, whole-food trial.
- A placebo meal may be an effective strategy for managing the elevations in appetite that often accompany fasting-based dietary regimes.

8.2. Discussion of Key Findings

In **Chapter 4**, a cross-sectional survey of 512 adults revealed that the early evening (16:00–19:59) was the most frequently reported time for exercise to commence on a typical weekday (Monday–Friday). Despite the morning (08:00–11:59) representing the preferred time of day for the majority, there was a lack of opportunity to engage in morning exercise during the week. Amongst other commitments such as 'spending time with family' and 'providing care for children', 'job/work commitments' was the most commonly cited barrier preventing exercise at preferred times. As such, there is clearly a discordance between when people prefer to exercise and when they have opportunity to exercise, and opportunity appears to be the primary determinant of exercise timing behaviours. Therefore, future studies should consider evening exercise a primary target for the implementation of exercise interventions. Several facets of human physiology and behaviour, such as macronutrient metabolism, appetite, and components of energy balance, are subject to circadian (24-h) fluctuations (Gerhart-Hines & Lazar, 2015), meaning findings from morning exercise may not directly apply to other times of the day.

It has been known for decades that the nutritional state in which exercise is performed can influence the metabolic, hormonal, and molecular responses (Coyle et al., 1985; Enevoldsen et al., 2004; Wallis & Gonzalez, 2019), and an abundance of sports performance research has

been conducted to elucidate the optimal nutritional conditions in which training should occur to maximise exercise adaptation (Burke et al., 2011; Impey et al., 2018). Whilst most previous research is aimed towards improving sports performance, the potential for interactions between nutrition status and exercise to be harnessed to optimise the metabolic health and weight management responses is a topic of increasing research interest (Frampton et al., 2022; Hansen et al., 2017). Data from **Chapter 4** highlights that when individuals exercise after midday, they typically do so after consuming a meal or snack 1–2 h before commencing exercise (29.4–55.6% of subjects), and only a very small proportion (≤6.5% of subjects) exercise >5 h after eating. However, consuming a meal in close proximity to exercise may blunt some of the adaptations that are attained, compared to exercising after a period of fasting. For example, 6 weeks of regular exercise after an overnight fast has been shown to enhance improvements in insulin sensitivity in both lean individuals (Van Proeyen et al., 2010), and in individuals with overweight/obesity, compared to the same exercise performed 1.5–2 h after a meal (Edinburgh et al., 2020). Almost all research on fasted exercise has been undertaken in the morning, likely because the overnight fast offers a practical and convenient opportunity to achieve a fasted state, meaning the responses to fasted exercise at other times of day are not well known.

8.2.1. Metabolic Outcomes

A reduced capacity to oxidise fat as a substrate is associated with the development of obesity and type 2 diabetes. For example, an elevated 24 h respiratory quotient (indicating low fat oxidation) is associated with an increased risk of weight gain (Zurlo et al., 1990) and weight re-gain after weight loss (Ellis et al., 2010). Additionally, individuals with obesity and type 2 diabetes have a reduced capacity to oxidise fat (Blaak et al., 2000; Kelley & Simoneau, 1994; Kelley et al., 1999), and an elevated resting respiratory exchange ratio has been linked with an increased presence of metabolic risk factors (Rosenkilde et al., 2010). Accordingly, exercise training interventions that improve capacity for fat oxidation have been associated with reciprocal improvements in insulin sensitivity in individuals with overweight/obesity (Edinburgh et al., 2020; Goodpaster et al., 2003; Venables & Jeukendrup, 2008). An increased capacity to oxidise fat during exercise has also been associated with elevated 24 h fat oxidation and enhanced markers of insulin sensitivity in lean and healthy males (Robinson et al., 2015*a*). Therefore, strategies designed to elevate fat oxidation during exercise (such as fasted exercise) might offer beneficial metabolic adaptations in the long term.

Informed by data collected in **Chapter 4**, **Chapters 5 and 6** examined fasted exercise performed in the early evening. Prior fasting for 7–8 h increased fat oxidation during exercise compared to exercise performed 2–3 h after a meal. Comparable increases in fat oxidation have also been shown during morning and evening exercise performed after 9 h of fasting (McIver et al., 2019*b*). It is well-established that exercising after a 10–12 h overnight fast increases fat oxidation (Edinburgh et al., 2020; Vieira et al., 2016), and despite circadian variation in substrate oxidation (Rynders et al., 2020; Zitting et al., 2018), findings from this thesis and a previous study (McIver et al., 2019*b*) demonstrate that a shorter, 7–9 h period of afternoon fasting also increases fat oxidation during evening exercise.

In **Chapter 5**, fasting for 7 h increased total fat oxidation by 3.25 g during 30 min moderate-intensity evening cycling, and in **Chapter 6**, fasting for 8 h increased fat oxidation by 11.66 g over 60 min evening cycling. In **Chapter 6**, NEFA and glycerol concentrations were also measured, and were elevated when exercising after fasting, indicating increased adipose tissue lipolysis (Robinson et al., 2016). These findings are especially relevant considering that the enhanced metabolic adaptations to fasted exercise training may be mediated, in part, by pathways linked to free-fatty acid signalling, as described in **Chapter 2.4.4** (Edinburgh et al., 2022). For example, elevated concentrations of plasma NEFA can act as ligands for peroxisome proliferator-activated receptors (PPARs), which are key regulators of fatty acid oxidation (Howard & Margolis, 2020). PPARs can upregulate the expression of proteins involved in fat oxidation, including CPT-1 (Dressel et al., 2003), and thus increase fat oxidative capacity. Previous studies suggest an ~3 g increase in fat oxidation over 30 min moderate-intensity exercise can enhance insulin sensitivity (Venables & Jeukendrup, 2008; Vieira et al., 2016). These findings, therefore, suggest that the benefits of overnight-fasted exercise might also be attained by incorporating a period of fasting prior to evening exercise, which may be of particular interest to individuals unable to exercise in the morning. It should be noted that the study of Venables & Jeukendrup (2008) recruited a sedentary population living with obesity, so whether this threshold translates to a lean and healthy cohort is currently unknown.

Despite the potential metabolic health benefits of fasted exercise, the main benefits from exercise are likely to be driven by the volume and intensity of exercise performed (Foulds et al., 2014), and long-term adherence (Stonerock & Blumenthal, 2017). In **Chapter 5**, the perceptual responses to fasted evening exercise were examined to gauge the potential acceptability and practicality of the intervention. Specifically, subjects rated motivation and readiness to exercise, tiredness, and energy, before commencing exercise. Exercise enjoyment

was assessed post-exercise (PACES-8; Raedeke, 2007), and an exit survey was completed once subjects had finished the study to gather further subjective and perceptual responses. Fasting before evening exercise reduced motivation, readiness, and energy immediately pre-exercise, and reduced exercise enjoyment, indicating a suboptimal psychological state for maximising the volume or intensity of voluntary exercise. Accordingly, work completed during a 15-min performance test was reduced by ~3.8% after fasting, and 15 of the 16 subjects felt they had performed worse during fasted versus fed evening exercise. Because of the short duration of the test (15 min), the reduction in voluntary performance was very small (~6 kcal), meaning it was unlikely to alter overall energy balance. However, the purpose of the performance test was not to detect a meaningful change in energy expenditure, but to capture subjects' motivation and willingness to perform voluntary exercise. If motivation to exercise and self-selected intensity and/or duration of exercise is curtailed, as this reduction in performance might imply, this could dramatically impact the success of exercise training programmes. Interestingly, perceived effort and exercise enjoyment do not appear to be affected when fasted exercise is performed in the morning (Frampton et al., 2022), which corresponds with responses to the exit survey in **Chapter 5**, revealing that 12 of the 16 subjects found fasted evening exercise more difficult than their previous experiences of overnight-fasted morning exercise. Therefore, these findings highlight potential difficulties with adopting a fasted exercise regime in the evening.

The negative perceptions of fasted evening exercise may have been mediated, in part, by the requirement for subjects to undergo large deviations from habitual dietary patterns (Hills et al., 2013), as individuals exercising after midday typically consume a meal or snack 1–2 h before commencing exercise (**Chapter 4**). In accordance with this, responses to the exit survey revealed that 10 of the 16 subjects found fasting for 7 h before evening exercise either 'difficult' or 'very difficult'. Therefore, whilst the promising metabolic responses to fasted evening exercise made a chronic intervention study the next logical step, the overall negative perceptual, performance, and appetite responses (discussed later) to this intervention in the acute setting redirected the focus of the subsequent studies.

The study in **Chapter 6** also examined evening exercise performed 3 h after a low-carbohydrate (0.2 g·kg body mass^{-1} carbohydrate), high-protein meal. The rationale for this study was based on the knowledge that consuming carbohydrate increases plasma glucose and insulin concentrations (Coyle et al., 1997), which inhibit hormone-sensitive lipase (Saltiel & Kahn, 2001), and stimulate fatty acid re-esterification in adipose tissue (Enevoldsen et al.,

2004; Frayn et al., 1995). This ultimately reduces fatty acid availability for oxidation during exercise in a carbohydrate-fed state (Coyle et al., 1997; Vieira et al., 2016). Accordingly, consuming a low-carbohydrate, high-protein meal has been shown to increase fat oxidation during exercise compared to a high-carbohydrate meal (Furber et al., 2021; Oliveira et al., 2021; Rothschild et al., 2021), possibly by a similar extent to fasting (Impey et al., 2015; Rothschild et al., 2021; Taylor et al., 2013). Therefore, this strategy might provide some of the metabolic benefits of fasted exercise, whilst mitigating some of the challenges of fasted evening exercise identified in **Chapter 5**. The low-carbohydrate, high-protein meal (1186 ± 140 kcal; 18 ± 2 g carbohydrate) increased fat oxidation by 8.00 g during 60 min moderate-intensity evening exercise compared to an isocaloric high-carbohydrate, lower-protein meal (163 ± 19 g carbohydrate). NEFA concentrations were also greater after the low-carbohydrate, high-protein meal, which, as alluded to earlier, might mediate some of the metabolic benefits of fasted exercise training (Edinburgh et al., 2022; Zbinden-Foncea et al., 2013). It should be noted that the increase in fat oxidation after the low-carbohydrate, high-protein meal was smaller than that following an 8-h fast (+11.66 g), and NEFA concentrations were also greater after fasting compared to the low-carbohydrate, high-protein meal. Nevertheless, **Chapter 6** provides novel data on an intervention whereby carbohydrate intake may need only be restricted acutely before exercise to increase fat oxidation, without needing to endure extended fasting. Longer-term studies are required to elucidate whether repeated exposures to these acute manipulations are sufficient to drive long-term changes in metabolic health.

Plasma glycerol concentrations, which can be used as a marker of adipose tissue lipolysis, were elevated only during fasted exercise. This suggests that different mechanisms may explain the elevated fat oxidation in the low-carbohydrate, high-protein trial, and the fasting trial. Because the fat content of the low-carbohydrate, high-protein meal and the high-carbohydrate, lower-protein meal was closely matched (difference of ~6 g, or ~4% energy from fat), it is unlikely that differences in fat oxidation were due to increased dietary fat appearance. Therefore, it is more likely that increased fat oxidation during the low-carbohydrate, high-protein trial was facilitated by intramuscular triglyceride utilisation (Coyle et al., 1997; van Loon et al., 2003). Increased intramuscular triglyceride turnover is another mechanism through which fasted exercise may enhance improvements in insulin sensitivity (Edinburgh et al., 2022; Gemmink et al., 2020), and these findings suggest that this may also be possible with exercise performed after a low-carbohydrate, high-protein meal. However, from the static measurement of plasma glycerol concentrations in **Chapter 6**, it cannot be determined whether these concentrations

reflect differences in lipolysis or glycerol uptake (Robinson et al., 2016; van Hall et al., 2002). Previous studies report elevated NEFA and glycerol concentrations during exercise after smaller doses of protein (Erdmann et al., 2010; Impey et al., 2015; Oliveira et al., 2021), which suggests the high protein dose, and resultant high insulin concentrations, provided in the low-carbohydrate, high-protein meal in **Chapter 6**, might have reduced rates of lipolysis and fat oxidation, compared to fasting.

An interesting outcome not assessed within this thesis is the impact of fasting and a low-carbohydrate, high-protein meal on postprandial glycaemic control at a post-exercise meal. This could not be assessed in the studies described in this thesis, as the measurement of post-exercise *ad-libitum* energy intake meant that it would not have been possible to determine whether any changes in glycaemic control were due pre-exercise nutrition status or simply due to differences in energy intake. This topic has been subject to considerable debate with regards to fed and fasted exercise (Chacko, 2014; Chacko, 2017; Haxhi et al., 2013; Hansen et al., 2017; Heden & Kanaley, 2019), with most studies showing lower glucose concentrations after a single meal when consumed before, compared to after, exercise (Colberg et al., 2009; Gaudet-Savard et al., 2007; Heden et al., 2015; Larsen et al., 1997; Larsen et al., 1999; Poirier et al., 2000, 2001; Sacchetti et al., 2021). This evidence led to the proposal that exercise should be performed within 30–90 min after consuming a meal to maximise reductions in postprandial glycaemia in response to that meal (Chacko, 2014; Chacko, 2017). Blood glucose and insulin concentrations rise after consuming carbohydrate, but these subsequently decrease to basal levels at the onset of exercise due to increased glucose uptake and oxidation within skeletal muscle (Coyle et al., 1985). Therefore, because the aforementioned studies were only concerned with the glycaemic response to a single meal consumed before or after exercise, it is perhaps no surprise that exercising after a meal appears favourable. However, the finding that regular pre-meal (fasted) exercise enhances improvements in glycaemic control in the long term compared to post-meal (fed) exercise (Edinburgh et al., 2019; Van Proeyen et al., 2010) suggests that caution should be applied when translating these acute responses to long-term changes in glycaemic control.

Using a more relevant study design to the fasted exercise paradigms adopted in this thesis, postprandial metabolism has also been assessed in response to fasted exercise induced by meal omission versus consumption (Edinburgh et al., 2018; Gonzalez et al., 2013). In the first of these studies, exercise after a meal increased postprandial glucose and insulin concentrations in response to an oral glucose tolerance test conducted after exercise, compared to fasted

exercise, implying worsened postprandial glucose control after fed exercise (Gonzalez et al., 2013). In a follow-up study utilising dual stable isotope tracers and muscle biopsies to assess plasma glucose kinetics, despite glucose rates of appearance being increased after fed exercise, these were offset by increased glucose uptake, ultimately resulting in similar postprandial glucose responses after fed and fasted exercise (Edinburgh et al., 2018). Importantly, the increased glucose uptake following fed exercise occurred despite lower insulin concentrations compared to fasted exercise, suggesting improved glucose tolerance in this trial. This corroborates with data at rest showing improved glucose tolerance at subsequent meals in a phenomenon termed the 'second meal effect' (Hamman & Hirschman, 1919; Staub, 1921). Therefore, in light of these data, it is plausible that fasted evening exercise might have worsened subsequent postprandial glucose control compared to fed evening exercise, at least in the acute setting in which the study was conducted.

Contrastingly, a low-carbohydrate, high-protein meal before exercise (*i.e.*, **Chapter 6**) has the potential to improve postprandial glucose control compared to exercise performed after a typical, high-carbohydrate meal. Several studies have shown that consuming a whey (Jakubowicz et al., 2014; King et al., 2018; Ma et al., 2009; Smith et al., 2021; Smith et al., 2022; Watson et al., 2019; Wu et al., 2016) or soy (Kashima et al., 2016; Konya et al., 2019; Silva Ton et al., 2014) protein 'preload' before meals can reduce postprandial glucose concentrations, possibly due to an increased insulin response and delayed gastric emptying with elevated release of incretins GIP and GLP-1 (Akhavan et al., 2014; Gillespie et al., 2015; Jakubowicz et al., 2014; Ma et al., 2009). In **Chapter 6**, GLP-1 concentrations were elevated throughout the 5-h postprandial period following the low-carbohydrate, high-protein meal, and persisted into the post-exercise period, suggesting a subsequent improvement in postprandial glucose control may have been possible via this mechanism. This was not measured within this thesis but represents an interesting avenue for future research.

8.2.2. Appetite and Energy Intake Outcomes

Only one previous study has examined the effects of fasting prior to evening exercise (McIver et al., 2019*b*). Fasting for 9 h before evening exercise increased appetite in the pre-exercise period, although appetite was offset by exercise, resulting in no differences in post-exercise appetite, irrespective of whether it was performed 1 h (fed) or 9 h (fasted) after a meal. Similar findings have been reported when exercise is performed in the morning following an overnight

fast (Gonzalez et al., 2013; McIver et al., 2019*a*), although others have shown that the elevated appetite prior to fasted exercise remains higher after exercise (Bachman et al., 2016; Griffiths et al., 2020). Findings from **Chapters 5 and 6** align with the latter, demonstrating that the elevated appetite following 7–8 h of fasting extended into the post-exercise period. Accordingly, in **Chapter 5**, energy intake was ~100 kcal greater at the first meal after exercise (dinner) when exercise was conducted in the fasted state.

Contrastingly, in **Chapter 6**, post-exercise dinner energy intake was not different following evening exercise performed after an 8 h fast, or 3 h after a high-carbohydrate meal. Post-exercise dinner energy intake was, however, ~262 kcal greater when exercise was performed fasted, compared to 3 h after a low-carbohydrate, high-protein meal. These differences were likely due to the high-carbohydrate meal inducing shorter-lived effects on appetite compared to the low-carbohydrate, high-protein meal because of its lower protein content (discussed later). Because the interval between the pre-exercise meal and *ad-libitum* dinner was longer in **Chapter 6** compared to **Chapter 5** (5 h versus 3 h), pre-dinner appetite was not different between the high-carbohydrate and fasting trials. Nevertheless, data from both **Chapters 5 and 6** suggest a potential disparity in post-exercise energy intake responses between fasted exercise when performed in the morning and in the evening, with fasted evening exercise appearing to provoke compensatory eating. This may be due to the fasting period occurring during waking hours, meaning subjects are likely more aware of their increased appetite compared to when the fasting period occurs during sleeping, as is common with fasted morning exercise studies. Additionally, the fasting period before evening exercise aligns with circadian-related increases in appetite and energy intake, which are both generally greater later in the day (NHANES, 2016; Scheer et al., 2013). Therefore, initiating a period of fasting later in the day may be more challenging than in the morning, where appetite is naturally at its lowest. However, despite differences in energy intake compensation between morning and evening exercise, in both cases, the increase in energy intake following fasted exercise does not compensate for the omission of the pre-exercise meal. As such, net energy intake over the course of the day is lowest with fasted exercise, regardless of whether it is performed in the morning or evening.

Information regarding post-exercise energy intake compensation within this thesis is restricted to the immediate post-exercise period, so it is not known whether divergent eating behaviours would have occurred outside of this. Although it may be easy to assume that reducing the number of hours available for eating is the primary reason for the reduction in daily energy intake following fasted exercise, data from studies examining fasted exercise performed in the

morning implicate another potential mechanism. For example, whilst seemingly paradoxical, studies using weighed food packages to track energy intake over 24 h following morning exercise have shown reduced energy intake in the evening when the exercise was performed after an overnight fast, compared to after a meal (Bachman et al., 2016; Edinburgh et al., 2019). This serves to exacerbate the energy deficit created by fasting.

In contrast, McIver et al. (2019*b*) reported no differences in self-reported energy intake during the 24 h following fed and fasted evening exercise, although self-reported measurements of energy intake are prone to misreporting (Dhurandhar et al., 2015; Rennie et al., 2007) and may have lacked the sensitivity to detect such differences. However, in **Chapters 5 and 6**, no differences in appetite measured on the morning after fed or fasted evening exercise were reported, suggesting the compensatory appetite effects of fasted evening exercise are limited to the first meal (*i.e.*, dinner). This agrees with findings showing that the elevated appetite following morning fasting is offset by the first meal (*i.e.*, lunch), and despite 24 h energy intake being reduced compared to breakfast consumption, appetite remains offset on the following morning (Clayton et al., 2015; Clayton et al., 2016*a*). Therefore, although the subsequent day likely represents the greatest opportunity for energy intake compensation following evening exercise, the overnight fast may limit any further differences between fed and fasted evening exercise. Interestingly, energy intake may also increase in anticipation of exercise (Barutcu et al., 2021) and/or energy restriction (James et al., 2020), possibly alongside concomitant reductions in spontaneous physical activity (James et al., 2020). Therefore, energy compensation is also possible on the same day as evening exercise before exercise commences. This could not be assessed in the studies within this thesis, as food intake and physical activity were controlled to ensure similar metabolic conditions at the start of trials.

The finding that fasted morning exercise can reduce evening energy intake observed by Bachman et al. (2016) and Edinburgh et al. (2019) is consistent with a theory that appetite and energy intake might be regulated by endogenous carbohydrate stores (Flatt et al., 1996; Flatt et al., 2001). This theory originated from the observation that individuals with higher rates of whole-body carbohydrate oxidation at rest and during exercise had higher *ad-libitum* energy intakes (Almeras et al., 1995; Burton et al., 2010; Hopkins et al., 2014; Pannacciulli et al., 2007; Snitker et al., 1997). Tissue-specific utilisation of carbohydrate has more recently been explored in relation to appetite and energy intake (Edinburgh et al., 2019). In the study of Edinburgh et al. (2019), glucose appearance and clearance (indicative of hepatically-derived glucose utilisation) were positively correlated with post-exercise energy intake, but were not

correlated with muscle glycogen utilisation, whole-body fat utilisation, or energy expenditure. These findings led to the hypothesis that hepatic carbohydrate availability specifically, may be linked to post-exercise appetite control (Gonzalez et al., 2019; Hopkins, 2019). Therefore, the increased utilisation of fat and reduced reliance upon carbohydrate during fasted exercise may be an additional mechanism through which fasted exercise creates an energy deficit. Future studies are required to elucidate whether hepatic carbohydrate availability is causally linked to energy balance in humans by examining candidate mechanisms, as findings in humans are currently limited to correlational studies.

Although the study in **Chapter 5** was not initially designed to detect sex-related differences, some interesting data emerged which warrant some discussion. Specifically, the increase in post-exercise *ad-libitum* energy intake following fasted evening exercise compared to fed evening exercise was driven predominantly by males, with seemingly no such compensation occurring in females. Previous research has generally shown similar appetite and energy intake responses to acute exercise between males and females (Dorling et al., 2018), although the effects of pre-exercise nutrition status have not been considered (Frampton et al., 2022), so the sex-specific responses to fasted exercise are not well understood. Findings from **Chapter 5** suggest that fasted evening exercise may not provoke a compensatory energy intake response in females, potentially making it a more effective weight management strategy for females than males. Because changes in sex hormone concentrations during the menstrual cycle can cause fluctuations in appetite and energy intake (Buffenstein et al., 1995), it was ensured that female subjects completed all experimental trials within in the same phase of the cycle. However, concentrations of sex hormones were not measured in the study in **Chapter 5**, meaning future studies with larger sample sizes of both males and females, along with the measurement of sex hormone concentrations in female subjects, are required to further explore these preliminary findings.

Appetite was elevated during the fasting periods in **Chapters 5 and 6** and extended into the post-exercise period. Poor appetite control can worsen dietary adherence (Drapeau et al., 2007; Polidori et al., 2016; Vogels & Westerterp-Plantenga, 2005) and influence food choices (Read & van Leeuwen, 1998; Tal & Wansink, 2013). Therefore, elevated appetite resulting from fasted evening exercise may cause individuals to make poorer food choices, such as selecting high-energy foods or snacking. Whilst less of a problem in the laboratory-based studies within this thesis, when performed in a free-living environment where food choice and eating occasions are not controlled, poor food choices could offset the energy deficit created by

exercise. Regarding this, it is particularly interesting to note that the low-carbohydrate, high-protein pre-exercise meal investigated in **Chapter 6**, was not only successful in increasing fat oxidation, but also reduced appetite and subsequent energy intake compared to a high-carbohydrate, lower-protein pre-exercise meal. Oliveira et al. (2021) similarly reported lower post-exercise hunger when performed after a high-protein breakfast. These responses are likely related to the protein content of the meal, as there is evidence that protein intake suppresses subjective appetite and improves weight management (Belza et al., 2013; Leidy et al., 2015; Rolls et al., 1988). Anorexigenic hormones GLP-1 and PYY were greater after the low-carbohydrate, high-protein meal compared to the high-carbohydrate, lower protein meal, which is consistent with findings from a previous study (Oliveira et al., 2021). GLP-1 and PYY increase dose-dependently with protein content (Belza et al., 2013; van der Klaauw; 2013), meaning the appetite suppressing effects of a low-carbohydrate, high-protein meal may be mediated by GLP-1 and PYY. As the majority of people who exercise in the evening likely do so after eating 1–2 h prior (**Chapter 4**), a low-carbohydrate, high-protein meal may offer a more practical solution to increasing fat oxidation by mitigating some of the appetite-related challenges associated with fasted evening exercise.

Because consuming a low-carbohydrate, high-protein meal meant that fasting still resulted in the lowest net energy intake in **Chapter 6**, **Chapter 7** examined an alternative strategy to manage fasting-induced elevations in appetite without providing energy. In this study, the effects of a very low-energy, viscous 'placebo' meal on subjective appetite, appetite regulatory hormones, and subsequent energy intake were examined. Previous studies have shown that a very low-energy placebo meal can suppress subjective appetite compared to fasting (Naharudin et al., 2020), and can maintain exercise performance at levels seen after consuming a meal (Mears et al., 2018; Naharudin et al., 2020). Therefore, a placebo meal could be harnessed as a strategy to mitigate difficulties with fasted exercise (*i.e.*, elevated appetite and reduced voluntary exercise performance). It is unknown, however, how these responses compare to those following a more typically consumed, whole-food meal. Morning fasting increases subjective appetite (Chowdhury et al., 2015; Chowdhury et al., 2016*a*; Clayton et al., 2015; Clayton et al., 2016*a*), and increases energy intake at lunch (Astbury et al., 2011; Chowdhury et al., 2015; Clayton et al., 2015; Levitsky & Pacanowski, 2013), compared to breakfast consumption. Because of this well-established response, the effects of a very low-energy, viscous placebo meal were first examined within this paradigm, relative to the consumption of a more typically consumed, whole-food meal, and a water-only control.

The very low-energy placebo meal reduced subjective appetite compared to water-only, similar to previous work (Naharudin et al., 2020), although this response was shorter-lived (~1 h) compared to that following a more typically consumed, whole-food meal containing ~575 kcal (~2 h). Accordingly, energy intake at lunch (~3 h later) was suppressed only by the whole-food meal. Nevertheless, the transient suppression of subjective appetite following a very low-energy, placebo meal could increase the efficacy of fasting-based interventions by increasing dietary adherence and reducing the temptation for consuming high-energy foods, without providing calorie-containing nutrients and thus sustaining the energy deficit.

The suppressed subjective appetite following the placebo meal occurred without any changes in appetite regulatory hormones compared to fasting. Specifically, acylated ghrelin and PYY concentrations were not different following the placebo meal and fasting, but the whole-food meal suppressed acylated ghrelin, and elevated PYY concentrations, compared to both trials. Therefore, despite the whole-food meal inducing a hormonal response associated with increased satiety and reduced hunger, these physiological variables cannot explain differences in subjective appetite between the placebo and fasting trials. Firstly, increasing the viscosity of a liquid enhances its effects on satiety (Bennett et al., 2009; Marciani et al., 2000; Marciani et al., 2001; Solah et al., 2010) and food intake (Ho et al., 2015, Vuskan et al., 2009). Therefore, the increased viscosity of the very low-energy placebo meal via the addition of a soluble fibre (xanthan gum) might have increased its effects on satiety compared to consuming water-only. Secondly, the addition of soluble fibre slows gastric emptying (Yu et al., 2014), ultimately prolonging the duration of gastric distension, which is typically very short-lived after consuming water-only (Van Walleghen et al., 2007; Vist & Maughan, 1994). Because adding xanthan gum to the placebo meal provided a small amount of fibre (~5 g), a delayed gastric emptying of this meal compared to water-only is a possible mechanism explaining the divergent appetite responses. Finally, the oral processing of food, including chewing and swallowing, mediates the satiating effects of a meal via physiological and psychological mechanisms (de Graaf, 2012). Specifically, the prolonged oro-sensory exposure time of solid foods elicits a greater and extended suppression of appetite compared to liquid foods (Mattes, 2005). This, therefore, may also have contributed to the differences in subjective appetite following the placebo meal and water-only.

Although in **Chapters 5 and 6**, skipping the pre-exercise meal and fasting resulted in the greatest reduction in net 24-h energy intake, fasting before evening exercise also reduced motivation to exercise, exercise enjoyment, and voluntary exercise performance in **Chapter 5**,

and pre-exercise energy levels in **Chapter 6**. Therefore, fasting appears to elicit a psychological state characterised by a more general reduction in the motivation to be active. Accordingly, previous studies have shown that incorporating periods of fasting into a dietary regime reduces spontaneous physical activity, which can profoundly alter energy balance (Chowdhury et al., 2016*b*; Templeman et al., 2021*b*). In **Chapter 6**, pre-exercise energy levels were greater after the low-carbohydrate, high-protein meal compared to fasting, meaning a low-carbohydrate, high-protein meal may help maintain a more optimal psychological state for engaging in exercise, whilst still increasing fat oxidation. Interestingly, it has previously been shown that both endurance and resistance exercise performance was greater after consuming a very low-energy placebo meal compared to fasting (Mears et al., 2018; Naharudin et al., 2020). Therefore, simply the act of eating a very low-energy placebo meal, rather than the energy content of the meal, could be sufficient to maintain physical activity levels and/or exercise performance, without offsetting the energy deficit created by fasting. This warrants further investigation, particularly in the context of evening exercise where fasting appears more challenging.

8.3. Practical Implications

The findings in this thesis have implications for the design and implementation of future exercise interventions. The time of day in which individuals exercise is dictated by several logistical and personal factors including job/work commitments and family-related responsibilities, which ultimately curtail the time available for exercise. Although the morning was the preferred time of day for exercise, most people reported exercising in the early evening during the week, but in the morning during the weekend. Exercise studies are typically conducted in the morning due to the practicalities regarding diet and physical activity control, however, circadian variation in human physiology and behaviour (Gerhart-Hines & Lazar, 2015; Smith & Betts, 2022) mean that findings from morning exercise might not directly apply to other times of day. Additionally, adherence may be enhanced by performing exercise at a consistent time of day, irrespective of the exercise timing preference (Brooker et al., 2019). This is termed ‘temporal consistency’, and is the idea that performing a behaviour, *i.e.*, exercise, at a specific time regularly, creates a protected time for exercise habits (Kaushal & Rhodes, 2015; Schumacher et al., 2019; Schumacher et al., 2020). Therefore, in order to maximise ecological validity and utility, future studies should be designed so that exercise

timing coincides with when most people have the opportunity to exercise consistently, which findings from this thesis would indicate is the early evening.

Unlike fasted morning exercise, fasted evening exercise may be associated with elevated appetite and reduced voluntary performance, as well as reduced motivation to exercise and exercise enjoyment. This further supports the concept that interventions may differ based upon the time of day in which they are employed. The low-carbohydrate, high-protein meal investigated in **Chapter 6** offers a practical strategy whereby nutritional composition can be acutely manipulated before exercise to attain much of the benefits associated with fasted exercise, whilst overcoming some of the challenges related to appetite and motivation to exercise. If the acute responses observed in **Chapter 6** persist chronically and materialise as tangible improvements in health, then this intervention may offer an alternative to those requiring significant alterations to existing dietary habits, such as chronic low-carbohydrate/low-energy diets or fasting-based interventions.

Elevated appetite has been linked with poor dietary adherence (Drapeau et al., 2007; Polidori et al., 2016; Vogels & Westerterp-Plantenga, 2005), meaning any strategies which offset elevations in appetite during energy restriction might improve adherence (Gibson & Sainsbury, 2017). The very low-energy placebo meal investigated in **Chapter 7** offers a novel strategy to reduce appetite, but without providing nutrients that would interrupt the fasted metabolic state and offset the energy deficit created by fasting. This concept could be adapted to generate a commercial product. Dietary strategies incorporating meal replacements are a popular topic of investigation and may offer benefits to consumers due to convenience and reducing the decision-making process related to making 'correct' food choices (Astbury et al., 2019; Heymsfield et al., 2003; Kruschitz et al., 2017). Investigating the effects of a very low-energy placebo meal within the context of exercise training represents an interesting avenue for future research.

Data presented in **Chapter 7** and in previous work (Chowdhury et al. 2015; Chowdhury et al., 2016*a*; Clayton et al., 2016*b*; Naharudin et al., 2020) demonstrate divergent responses between subjective markers (*i.e.,* hunger, fullness, desire to eat, prospective food consumption, energy intake) and hormonal regulators (*i.e.,* acylated ghrelin, PYY, and GLP-1) of appetite. Appetite and food intake are complex phenomena and are influenced by a host of psychological, behavioural, and metabolic factors (Blundell et al., 2010). Therefore, there remains the question

as to whether the measurement of these hormones provides any further insight into appetite regulation and eating behaviour beyond the measurement of subjective markers.

8.4. Limitations and Directions for Future Research

It is imperative that the effects of dietary and exercise strategies are first examined acutely before commencing chronic intervention studies, however, the short-term nature of the studies in this thesis means that findings cannot be extrapolated beyond this acute intervention and monitoring period. The findings of **Chapters 5 and 6** demonstrate that both fasting and consuming a low-carbohydrate, high-protein meal before evening exercise increase fat oxidation and thus have the potential to induce metabolic health benefits with training. Future studies should investigate the effects of repeated exposure to these interventions on markers of metabolic health.

Regarding weight management outcomes, despite studies within this thesis and previous work showing acute reductions in net daily energy intake following fasted exercise (Bachman et al., 2016; Edinburgh et al., 2019; Gonzalez et al., 2013; Griffiths et al., 2020), chronic studies (*i.e.*, 4–12 weeks) do not generally show changes in body weight or composition (Brinkmann et al., 2019; De Bock et al., 2008; Edinburgh et al., 2020; Gillen et al., 2013; Schoenfeld et al., 2014; Van Proeyen et al., 2011; Verboven et al., 2020). This suggests that either more than 12 weeks are required to elucidate chronic effects, or that some degree of adaptation or behavioural compensation occurs beyond the acute monitoring period. Due to its ability to reduce appetite and compensatory energy intake compared to fasting, it is possible that a low-carbohydrate, high-protein pre-exercise meal may be more effective than fasted exercise at eliciting long-term changes in body weight or composition, although this requires further study. Additionally, the appetite, energy intake, and metabolic responses to a very low-energy placebo meal need exploring following multiple exposures over days and weeks, especially given the initial unfamiliarity of the viscous placebo meal and the potential for demand effects resulting from knowledge of its lack of energy content.

A second limitation is that the studies in this thesis recruited only lean and metabolically healthy subjects. Weight gain occurs progressively throughout life, with most weight gain occurring during mid-adulthood (Østbye et al. 2011). Therefore, many individuals classified as lean today may become overweight or obese later in life. Given that the energy balance system defends more strongly against weight loss than weight gain (Hill et al., 2012), early intervention

in lean individuals to prevent weight gain might be a more efficacious approach than attempting to reduce obesity once established (Monnier et al., 2021). Additionally, prolonged postprandial exposures to hyperglycaemia may have negative health consequences even for lean individuals (Ceriello et al., 2008; Levitan et al., 2004). Therefore, it is still important to examine these interventions within a lean and healthy cohort. However, the findings presented in this thesis should not be directly applied to other population groups. For example, individuals with obesity and type 2 diabetes have impaired metabolic flexibility, which is characterised by a reduced capacity to alternate between oxidising carbohydrate and fat (Goodpaster & Sparks, 2017). Therefore, the metabolic benefits of fasted exercise and exercise after a low-carbohydrate, high-protein meal, which are likely mediated via increases in fat oxidation, may be blunted in these individuals. Accordingly, lean individuals and individuals with overweight or obesity have been shown to respond differently to fasting-based interventions (Betts et al., 2014; Chowdhury et al., 2015; Chowdhury et al., 2016*a*; Chowdhury et al., 2016*b*; Gonzalez et al., 2018). Future studies should, therefore, explore the interventions presented within this thesis in other population groups.

To control for factors that could confound subjective appetite and to allow for measurements of metabolism/hormonal markers of appetite regulation, the studies in this thesis were conducted within a laboratory environment. This enabled a greater degree of experimental control to be imposed and increased the precision of certain measures (*i.e.*, energy intake) compared to free-living study designs. However, eating behaviour is influenced by external factors, which, alongside changes in spontaneous physical activity that are not possible within the confines of a laboratory, may serve to alter the responses to these interventions in a free-living environment. Future research should examine these interventions with subjects in their habitual setting, where there is likely greater opportunity for compensatory energy balance behaviours to occur.

Although the studies presented within **Chapters 5 and 6** of this thesis investigated the responses to fasted exercise at a novel time of day (*i.e.*, the evening), the absence of an overnight-fasted morning exercise trial means that direct comparisons of fasted morning and evening exercise could not be made. Fasted morning exercise is typically performed after a longer (10–14 h) overnight fast, compared to the 7–8 h daytime fast investigated in this thesis. The duration of the fast has a well-established influence on metabolism. For example, glycerol concentrations have been shown to increase in direct proportion to the duration of the fast (Montain et al., 1991). Therefore, whilst fasting for ≥6 h before exercise appears to favour fat

oxidation regardless of the time of day (McIver et al., 2019*b*; Montain et al., 1991), the metabolic effects of a shorter, daytime fast, may not necessarily mimic those of a longer, overnight fast. Further studies directly comparing responses to fasted morning and evening exercise are still needed.

The composition of the low-carbohydrate (0.2·g·kg body mass^{-1}), high-protein meal investigated in **Chapter 6** was constructed based on changes made to the respective high-carbohydrate (2·g·kg body mass^{-1}), lower-protein meal. A carbohydrate content of 2·g·kg body mass^{-1} was selected based on previous findings showing that this is sufficient to suppress fat oxidation during 60 min exercise when consumed 3 h prior (Stevenson et al., 2006; Wu et al., 2003). To minimise the confounding effects of differences in dietary fat ingestion on substrate oxidation, the low-carbohydrate, high-protein meal was created by reducing the carbohydrate content and increasing the protein content of the high-carbohydrate, lower-protein meal, thus holding fat consistent between trials. Additionally, the meal was designed to provide an ecologically valid energy content typical to this mealtime (~1/3 total daily energy intake, assuming 3 meals are consumed during the day), with 35–40% estimated energy requirements chosen based on its use as a standardised lunch meal in previous studies (Clayton et al., 2016*a*; Smeets et al., 2008; Smeets & Westerterp-Plantenga, 2009). Therefore, ensuring meals were isocaloric resulted in a very high protein content, which likely exceeded the protein intake that would typically be consumed at a single meal in the real world. Future studies should aim to determine whether this meal can be manipulated and refined to improve acceptability and long-term adherence within a chronic intervention study. This could include alterations to the energy content of the meal and/or the distribution of energy intake over a series of meals with restricted carbohydrate intake (*e.g.*, lunch and a pre-exercise snack).

Similarly, the very low-energy, viscous placebo meal studied in **Chapter 7** was a proof-of-concept study design and requires further refinement to increase its ecological validity. The meal took the form of a soft-set jelly, in that it was not possible to simply drink, and had to be eaten from a bowl with a spoon. This was an important characteristic in order for it to be clearly distinguished from the water-only control trial, in an attempt to mimic the properties of a 'meal', rather than a drink. If the objective were to translate this concept to the design of a commercial product, then future work would be required to increase palatability and acceptability. Further to this, in the real world, the purchasing consumer would likely be aware of the lack of energy content. To increase the application of this intervention, it would be prudent to examine responses to a very low-energy 'placebo' meal within an unblinded study

design to account for potential demand effects resulting from knowledge of its lack of energy content.

It should be noted that the rates of energy expenditure and substrate oxidation presented within this thesis are not corrected for rates of protein oxidation because urine samples were not collected during experimental trials. Rates of protein oxidation are typically estimated by measuring urea in urine to estimate nitrogen excretion, with classical papers estimating 6.25 g of protein per g of nitrogen (Jeukendrup & Wallis, 2005). During exercise, the oxidation of branched chain amino acids, such as leucine, have been shown to contribute minimally to energy expenditure (<1%), and during extreme conditions of high-intensity exercise lasting 5 hours, protein oxidation only accounted for ~5–10% of total oxygen consumption (Rowlands & Hopkins, 2002*b*). Therefore, it is generally assumed that protein oxidation during exercise is negligible (Wagenmakers, 1989). Indeed, 24 h protein oxidation increases when adhering to a high protein diet (Bray et al., 2015; Griffen et al., 2022), and post-exercise protein oxidation is increased in proportion to increasing dietary protein intake (Bolster et al., 2005). Therefore, it is possible that an increase in protein oxidation during exercise after the low-carbohydrate, high-protein meal in **Chapter 6** was unaccounted for. However, because the respiratory quotient of protein falls between that of carbohydrate and fat, even at the upper ends of protein oxidation (*i.e.*, 5–10% of energy expenditure), it is unlikely to have a meaningful effect on the respiratory exchange ratio and would ultimately lead to a proportional reduction in absolute carbohydrate and fat oxidation (Jeukendrup & Wallis, 2005).

8.5. Translation into Practice

It is important to reflect on the overall bigger picture of this research and consider how the findings may translate into the everyday lives of the population in relation weight and health management. Firstly, data from **Chapter 4** highlight that work commitments are a fundamental determinant of exercise behaviours for many, often preventing people from exercising when they would like to. Given the considerable amount of time that full-time workers spend at work, the workplace presents itself as a key setting for public health promotion (National Institute for Health and Care Excellence, 2008). Employers and policy makers are encouraged to use this opportunity to support physical activity within the workforce, and the findings of this thesis suggest that structuring working schedules to permit exercise at preferred times may be beneficial. This lends support to the suggestion that adopting flexible working policies may be

an effective strategy to increase opportunity for exercise (The National Institute for Health and Care Excellence, 2008). Such approaches may include flexible working hours, remote working where possible, and encouraging physical activity breaks during the working day. The power of effective workplace intervention is evidenced by initiatives such as the cycle to work schemes across the UK, the Healthy Working Wales Programme, and the Active People, Healthy Nation initiative in the United States (Centers for Disease Control and Prevention, 2023). Therefore, workplace interventions with a focus on the temporal structure of the working day may be particularly effective at improving public health through facilitating regular exercise.

Integrating nutrition and exercise interventions has the potential to optimise the benefits that are achieved compared to when implemented in isolation. Acute data from this thesis indicate that both fasting and consuming a low-carbohydrate, high-protein meal before exercise may acutely increase fat oxidation and facilitate a reduction in net daily energy intake. However, the potential long-term weight management and health benefits of implementing these interventions within lives outside of the laboratory are currently unknown. It has previously been shown in individuals with overweight and obesity that the acute increase in fat oxidation which occurs during overnight-fasted morning exercise persisted throughout each exercise session (3 times per week) over a 6-week training period, ultimately resulting in improved insulin sensitivity (Edinburgh et al., 2020). Interestingly, these benefits occurred despite subjects returning to habitual diet and activity patterns in between exercise sessions. It is, therefore, plausible that the observed acute increase in fat oxidation during evening exercise performed after a period of afternoon fasting (**Chapter 5**) or a low-carbohydrate, high-protein lunch (**Chapter 6**) may drive similar metabolic benefits if performed 2–3 times per week, possibly without the need to alter food intake on non-exercising days.

Regarding the effectiveness of these interventions for the prevention and treatment of obesity, there are some challenges which may arise when attempting to translate the findings from the laboratory-based studies that comprise this thesis into real-world settings. For example, appetite and food intake are influenced by several external factors which were effectively removed within the laboratory-controlled environment. Social pressures and environmentally determined factors such as the widespread availability of highly palatable, energy dense foods within today's obesogenic environment make interventions involving food restriction notoriously challenging. This may result in the observed acute energy deficits induced by fasted exercise and placebo meal consumption being offset, as is commonly seen in translational

studies (Betts et al., 2014; Chowdhury et al., 2016*b*; Brinkmann et al., 2019; Edinburgh et al., 2020; Verboven et al., 2020). Furthermore, the effects that these interventions have on other lifestyle behaviours, such as sleep and physical activity, which were not measured within this thesis, have the potential to modulate energy intake responses in the real world (Brondel et al., 2010; Shi et al., 2008). Changes to body weight or composition are popular reasons for engaging in nutrition and exercise interventions, with a lack of detectable change in these tangible outcomes often leading to dropouts and poor adherence (Bazrafkan et al., 2021; Ortner Hadžiabdić et al., 2015). Therefore, despite potential health improvements, if these interventions were to result in limited or no changes in body weight/composition, then they may lack long-term efficacy. Due to its ability to reduce appetite and compensatory energy intake compared to skipping the pre-exercise meal completely and undergoing an extended period of fasting, it is possible that regularly consuming a low-carbohydrate, high-protein pre-exercise meal may be more effective than fasted exercise at eliciting long-term changes in body weight or composition and facilitating long-term adherence.

Finally, a common theme throughout this thesis is the effect of timing on the responses to exercise interventions. Public health guidelines provide evidence-based advice with regards to the duration, intensity, and type of exercise that individuals should engage in (National Health Service, 2021), however, there remains the question of whether recommendations should also consider exercise timing. The number of people already achieving physical activity guidelines is low (Guthold et al., 2018), and therefore, the inclusion of additional factors runs the risk of overcomplicating public health messages. Furthermore, recommendations relating specifically to exercise timing may be particularly problematic when considering the hectic schedules led by many in today's society, which may make temporally consistent exercise (exercising at the same time regularly) practically challenging. Therefore, at present, guidelines should remain simplified to the key message that exercise is beneficial, regardless of time of day, and individuals should exercise at a time that aligns with their schedule and/or preference (Janssen et al., 2022; Mansingh & Handschin, 2022). Rather than the findings within this thesis contributing to recommendations or guidelines, the interventions studied represent additional weight and health management tools which may be conveniently incorporated into the lives of some individuals (*i.e.*, those who already exercise in the afternoon/evening), but not others.

8.6. Conclusion

Exercise timing behaviours amongst the population are variable across the week and are dictated by several logistical and personal factors. The evening was the most common time of day for exercise to occur during the week, likely determined by a working lifestyle. This thesis has expanded on the existing knowledge base surrounding fasted exercise, showing that when performed in the evening, fasting for 7 h increased fat oxidation and reduced net 24 h energy intake, aligning with overnight-fasted morning exercise studies. However, fasted evening exercise elevated appetite and reduced voluntary exercise performance, as well as the motivation to exercise and exercise enjoyment. A low-carbohydrate, high-protein meal also increased fat oxidation compared to a high-carbohydrate meal, whilst reducing appetite and subsequent energy intake compared to both a high-carbohydrate meal and fasting. Therefore, this strategy may offer some of the metabolic benefits associated with fasted exercise without the need to endure daytime fasting. A very low-energy placebo meal also reduced subjective appetite relative to fasting and, therefore, may offer an alternative strategy to offset the challenges to appetite and compensatory eating behaviour associated with fasting. Further work is needed to explore this in the context of evening exercise.

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Appendices

Appendix A

Statement of Consent to Participate in the Study within Chapter 4

Background Information

- It is well understood that regular exercise is an effective strategy for weight management and for improving metabolic health.
- A significant proportion of the population fall short of government physical activity guidelines, often due to having a lack of time to exercise because of work, family, and social commitments.
- The timing of exercise with respect to both the time of day, and in relation to food intake, may alter the benefits that are gained from exercise.
- The current exercise and nutrition behaviours of the population need establishing in order for successful future exercise interventions to be developed.
- Taking into consideration the impact of COVID-19 social distancing measures will provide insight into what happens to these behaviours when work, family, and social constraints are altered.

What is involved?

- Completion of a short online questionnaire about your exercise/nutrition behaviours and preferences before and after the onset of the COVID-19 pandemic.
- Should take between 5 - 15 minutes to complete. With your consent, you may also be contacted in the future with regards to your potential involvement in follow-up research studies in this area.

Who can take part?

- You must be aged 18 years or older.
- You must have been engaging in some form of planned and/or structured physical exercise:

1) At least **once per week for at least 6 months prior to** the implementation of COVID-19 social distancing measures in your country

and/or

2) At least **once per week since** the implementation of COVID-19 social distancing measures in your country

- If you are unsure whether you are eligible to take part, please contact the research team.

Participation and withdrawal

- Participation is voluntary, so it is up to you to decide whether or not to take part.
- You should **read the full information sheet by clicking here** and if you have any questions you should ask the research team.
- If you choose to participate, you are free to withdraw your data without giving a reason for up to two weeks after completion of the questionniare. If you wish to withdraw your data after data collection, you may do so by contacting one of the researchers and providing your unique ID code (generated at the start of the questionnaire), at which point all of your data will be destroyed.

Confidentiality, data storage and access

- Data will be stored securely in password protected files on a secure cloud server.
- Only the research team who are directly involved in the study will have access to identifiable data.
- Any data shared publicly will non-identifiable.

If you agree to participate in the project outlined above, please complete the following screening questions and the informed consent form, before continuing to the questionnaire.

1. I confirm that I am aged 18 years or older.
2. I confirm that I was engaging in some form of planned and/or structured physical exercise at least once per week during the 6 months prior to the implementation of social distancing measures in my country.
3. I confirm that I have been engaging in some form of planned and/or structured physical exercise at least once per week since the implementation of social distancing measures in my country.
4. I have read the participant information sheet provided and agree to participate in this project which involves the completion of an online questionnaire.
5. I confirm that I have been provided with the contact information of the researchers and have had the opportunity to ask questions about the study and, where I have asked questions, these have been answered to my satisfaction.
6. I am aware that I am free to withdraw my consent to participate in the study without giving a reason within two weeks of completing the questionnaire by providing my unique ID code (this will be generated on the next page). Following withdrawal, my personal data will be destroyed.
7. I understand that any personal information regarding me, gained through my participation in this study, will be treated as confidential and only handled by individuals relevant to the performance of the study and the storing of information thereafter. Where information concerning myself appears within published material, my identity will be kept anonymous.
8. I hereby fully and freely consent to my participation in this project.

Appendix B

Statement of Consent to Participate in the Study within Chapter 5

1) I, …… agree to partake as a participant in the above study.

2) I understand from the participant information sheet, which I have read in full, and from my discussion(s) with *Mr Tommy Slater* that this will involve me *completing 2 familiarisation trials involving a maximal exercise test on an exercise bicycle. After this, I will complete three experimental trials. Based on the trial, I will report to the Nottingham Trent University laboratory at a specific time of day to complete some subjective responses questionnaires and an exercise performance test on an exercise bicycle. On the day before the first trial, I will complete a weighted food and drink diary and replicate this before the following trials. I will also not eat or drink (other than plain water) past 8.30pm on the day before trials. During experimental trials, I will eat a pre-prepared breakfast, lunch, dinner, and set of snacks as and when instructed outside the laboratory. I will attend the laboratory on 5 separate occasions for approximately 1.5–2 hours each time, with a total laboratory time commitment of about 9 hours.*

3) It has also been explained to me by *Mr Tommy Slater* that the risks and side effects that may result from my participation are as follows: *I may experience mild increases in hunger. I may experience nausea, dizziness, and light-headedness during and following maximal exercise. However, an experienced investigator will oversee all testing session. I may experience muscle soreness in the 48 hours following exercise testing, which I can alleviate with gentle stretching.*

4) I confirm that I have had the opportunity to ask questions about the study and, where I have asked questions, these have been answered to my satisfaction.

5) I undertake to abide by University regulations and the advice of researchers regarding safety.

6) I am aware that I can withdraw my consent to participate in the procedure at any time and for any reason, without having to explain my withdrawal and that my personal data will be destroyed and that my medical care or legal rights will not be affected.

7) I understand that any personal information regarding me, gained through my participation in this study, will be treated as confidential and only handled by individuals relevant to the performance of the study and the storing of information thereafter. Where information concerning myself appears within published material, my identity will be kept anonymous.

8) I confirm that I have had the University's policy relating to the storage and subsequent destruction of sensitive information explained to me. I understand that sensitive information I have provided through my participation in this study, in the form of *questionnaires* will be handled in accordance with this policy.

9) I confirm that I have completed the health questionnaire and know of no reason, medical or otherwise that would prevent me from partaking in this research.

10) I understand that the information collected about me will be used to support other research in the future and may be shared anonymously with other researchers.

11) I confirm that I am aware that I need to complete a COVID19 symptom questionnaire daily in the 7 days prior to every trial in the study / visit to the University's research facilities.

12) It has been explained to me that there may be additional risks arising from the current COVID pandemic. I have read the NTU recommendations for undertaking 'Research with human participants' and undertake to abide by the special measures which have been explained to me for this study together with such Government Guidelines that are at the time prevailing.

Participant signature: Date:

Independent witness signature: Date:

Primary Researcher signature: Date:

Appendix C

Statement of Consent to Participate in the Study within Chapter 6

1) I, …… agree to partake as a participant in the above study.

2) I understand from the participant information sheet (Dated June 2021, Version 1), which I have read in full, and from my discussion(s) with *Mr Tommy Slater* that *this will involve me completing 2 preliminary trials, one of which involves a maximal exercise test on an exercise bicycle. After this, I will complete three experimental trials. I will report to the pre-arranged Nottingham Trent University laboratory at 12.15–12.45pm after having consumed a pre-prepared breakfast and volume of water at home. I will then either consume one of two lunch meals or remain fasted. At 4pm, I will cycle for 1 hour at a moderate intensity on an exercise bicycle. At 6pm, I will consume a pre-prepared dinner in the laboratory, before leaving at 6.30pm with some snacks that I can chose whether or not to eat during the evening. On the day before the first trial, I will complete a weighted food and drink diary and replicate this before the following trials. I will also not eat or drink (other than plain water) past 8pm on the day before trials. I will attend the laboratory on 5 separate occasions for approximately 1.5 hours in the first two visits, and 6 hours in the remaining three visits, with a total laboratory time commitment of about 21 hours. Before, during, and after exercise, I will provide a series of expired gas samples whilst wearing a face mask and breathing into a tube connected to a Douglas bag for 2–5 minutes at a time. I will also provide eight, ~11 mL blood samples per experimental trial, equating to ~88 mL in total, per trial.*

3) It has also been explained to me by *Mr Tommy Slater* that the risks and side effects that may result from my participation are as follows: *I may experience mild increases in hunger. I may experience nausea, dizziness, and light-headedness during and following maximal exercise and although it is extremely unlikely, high intensity exercise has been known to reveal unsuspected heart or circulation problems and very rarely these have had serious or fatal consequences. However, an experienced investigator will oversee all testing session. I may experience muscle soreness in the 48 hours following exercise testing, which I can alleviate with gentle stretching. I may feel dizzy and there is a chance of fainting during the blood samples. There is also a chance of getting a small bruise from the needle. If I feel any dizziness during the samples, I will alert the trained researcher who is taking the sample.*

4) I confirm that I have had the opportunity to ask questions about the study and, where I have asked questions, these have been answered to my satisfaction.

5) I undertake to abide by University regulations and the advice of researchers regarding safety.

6) I am aware that I can withdraw my consent to participate in the procedure at any time and for any reason, without having to explain my withdrawal and that my personal data will be destroyed and that my medical care or legal rights will not be affected.

7) I understand that any personal information regarding me, gained through my participation in this study, will be treated as confidential and only handled by individuals relevant to the performance of the study and the storing of information thereafter. Where information concerning myself appears within published material, my identity will be kept anonymous.

8) I confirm that I have had the University's policy relating to the storage and subsequent destruction of sensitive information explained to me. I understand that sensitive information I have provided through my participation in this study, in the form of *questionnaire responses and blood samples* will be handled in accordance with this policy.

9) I confirm that I have completed the health questionnaire and know of no reason, medical or otherwise that would prevent me from partaking in this research.

10) I understand that the information collected about me will be used to support other research in the future and may be shared anonymously with other researchers.

11) It has been explained to me that there may be additional risks arising from the current COVID pandemic. I have read the NTU recommendations for undertaking 'Research with human participants' and undertake to abide by the special measures which have been explained to me for this study together with such Government Guidelines that are at the time prevailing.

Participant signature: Date:

Independent witness signature: Date:

Primary Researcher signature: Date:

Appendix D

Statement of Consent to Participate in the Study within Chapter 7

1) I, …… agree to partake as a participant in the above study.

2) I understand from the participant information sheet, which I have read in full, and from my discussion(s) with *Mr Tommy Slater* that this will involve me *completing a preliminary trial to familiarise myself to the procedures included in the main trials. Subsequent to this, I will complete three trials, each taking place between 08:15–13:00. Within these trials, I will report to the Nottingham Trent University laboratory following an overnight fast, where breakfast and lunch will be provided. I will consume these meals as and when instructed within the laboratory. I will attend the laboratory on 4 separate occasions for between 1–5 hours each time, with a total laboratory time commitment of about 16.5 hours. I will provide three, 15mL blood samples per experimental trial, equating to 45mL in total per trial.*

3) It has also been explained to me by *Mr Tommy Slater* that the risks and side effects that may result from my participation are as follows: *I may experience mild increases in hunger. It is also a possibility that the breakfast provided to me may cause some stomach discomfort. However, an experienced investigator will be present at all times, whom I can alert to any discomfort, which I can alleviate by withdrawing from the study and consuming additional food. I may feel dizzy and there is a chance of fainting during the blood samples. There is also a chance of getting a small bruise from the needle. If I feel any dizziness during the samples, I will alert the trained researcher who is taking the sample.*

4) I confirm that I have had the opportunity to ask questions about the study and, where I have asked questions, these have been answered to my satisfaction.

5) I undertake to abide by University regulations and the advice of researchers regarding safety.

6) I am aware that I can withdraw my consent to participate in the procedure at any time and for any reason, without having to explain my withdrawal and that my personal data will be destroyed and that my medical care or legal rights will not be affected.

7) I understand that any personal information regarding me, gained through my participation in this study, will be treated as confidential and only handled by individuals relevant to the performance of the study and the storing of information thereafter. Where information concerning myself appears within published material, my identity will be kept anonymous.

8) I confirm that I have had the University's policy relating to the storage and subsequent destruction of sensitive information explained to me. I understand that sensitive information I have provided through my participation in this study, in the form of *questionnaires and blood samples* will be handled in accordance with this policy.

9) I understand that as part of this study I will be consuming a supplement. I am aware that elite sports people (i.e. international or national standard) may undergo either out-of or in-competition (or both) doping tests and appreciate that the supplement being studied could be contaminated with a substance that appears on the banned lists.

10) I confirm that I have completed the health questionnaire and know of no reason, medical or otherwise that would prevent me from partaking in this research. *I understand the potential risk of food allergens and I confirm I have no allergies.*

11) I confirm that I am aware that I need to complete a COVID19 symptom questionnaire prior to every trial in the study / visit to the University's research facilities.

12) I confirm that I recognise that my involvement with this research could result in an increased risk of me contracting COVID19, despite all the mitigation employed by the researchers.

Participant signature: Date:

Independent witness signature: Date:

Primary Researcher signature: Date:

Appendix E

Health Screen Questionnaire

Please complete this brief questionnaire to confirm fitness to participate:

1. **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 ☐

2. **In the past two years,** have you had any illness which require you to:

(a)	consult your GP	Yes ☐	No ☐
(b)	attend a hospital outpatient department	Yes ☐	No ☐
(c)	be admitted to hospital	Yes ☐	No ☐

3. **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)	Heart problems	Yes ☐	No ☐
(i)	Problems with bones or joints	Yes ☐	No ☐
(j)	Disturbance of balance / coordination	Yes ☐	No ☐
(k)	Numbness in hands or feet	Yes ☐	No ☐
(l)	Disturbance of vision	Yes ☐	No ☐
(m)	Ear / hearing problems	Yes ☐	No ☐
(n)	Thyroid problems	Yes ☐	No ☐
(o)	Kidney or liver problems	Yes ☐	No ☐
(p)	Allergy to nuts, alcohol etc.	Yes ☐	No ☐
(q)	Any problems affecting your nose e.g. recurrent nose bleeds	Yes ☐	No ☐
(r)	Any nasal fracture or deviated nasal septum	Yes ☐	No ☐

4. **Has any, otherwise healthy,** member of your family under the age of 50 died suddenly during or soon after exercise? Yes ☐ No ☐
5. Are there any reasons why blood sampling may be difficult? Yes ☐ No ☐
6. Have you had a blood sample taken previously? Yes ☐ No ☐
7. Have you had a cold, flu or any flu like symptoms in the last month? Yes ☐ No ☐
8. Have you ever tested positive for COVID-19? Yes ☐ No ☐
9. When did you receive this positive test, if applicable?

(DD/MM/YYYY)……..

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

….....
….....
….....
….....

Allergy Information

10. Are you allergic to any food products? Yes ☐ No ☐

If YES please provide additional information

….....
….....
….....
….....

11. Are you intolerant to any food products? Yes ☐ No ☐

If YES please provide additional information

….....
….....
….....
….....

NB Please note that in the 7-day period prior to any visit to the University to undertake a trial in a research study or to visit a University research facility YOU WILL NEED TO COMPLETE a **DAILY** COVID-19 symptom questionnaire. Please DO NOT come to the University if you have not completed this questionnaire and the member of research staff supervising the research study has not confirmed you should attend.

Female Subjects Only:

12. Are you pregnant, trying to become pregnant or breastfeeding? Yes ☐ No ☐

13. Are you currently using, or have you previously used, a contractive pill? Yes ☐ No ☐

If YES please provide additional information (*i.e.*, duration of use, brand etc.):

….....
….....
….....
….....

Appendix F

Physical Activity Questionnaire

During a typical week, how many times on average do you spend doing the following kinds of exercise **for more than 15 minutes**?

1. **Strenuous exercise (heart beats rapidly)**

   For example, running, jogging, squash, hockey, football, rugby, vigorous swimming, vigorous long-distance cycling:

   _____ times per week.

2. **Moderate exercise (not exhausting)**

   For example, fast walking, tennis, casual cycling, badminton, casual swimming, dancing:

   _____ times per week.

3. **Mild exercise (minimal effort)**

   For example, yoga, archery, fishing, bowling, golf, casual walking:

   _____ times per week.

Appendix G

Three-Factor Eating Questionnaire

Part 1: Please answer true or false.

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.**

   True □ False □

2. **I usually eat too much at social occasions, like parties and picnics**.

   True □ False □

3. **I am usually so hungry that I eat more than three times per day.**

   True □ False □

4. **When I have eaten my quota of calories, I am usually good about not eating any more.**

   True □ False □

5. **Dieting is so hard for me because I just get too hungry**

   True □ False □

6. **I deliberately take small helpings as a means of controlling my weight.**

   True □ False □

7. **Sometimes things just taste so good that I keep eating even when I am no longer hungry.**

   True □ False □

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.**

   True □ False □

9. **When I feel anxious, I find myself eating.**

   True □ False □

10. **Life is too short to worry about dieting.**

    True □ False □

11. **Since my weight goes up and down, I have gone on reducing diets more than once.**

    True □ False □

12. **I often feel so hungry that I just have to eat something**

    True □ False □

13. When I am with someone who is overeating, I usually overeat too.

True □ False □

14. I have a pretty good idea of the number of calories in common food.

True □ False □

15. Sometimes when I start eating, I just can't seem to stop.

True □ False □

16. It is not difficult for me to leave something on my plate.

True □ False □

17. At certain times of the day, I get hungry because I have gotten used to eating then.

True □ False □

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.

True □ False □

19. Being with someone who is eating often makes me hungry enough to eat also.

True □ False □

20. When I feel blue, I often overeat.

True □ False □

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

True □ False □

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

True □ False □

23. I often stop eating when I am not really full as a conscious means of limiting the amount that I eat.

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

27. When I feel lonely, I console myself by eating.

True □ False □

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

True □ False □

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

True □ False □

30. I eat anything I want, anytime I want.

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

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

True □ False □

Part 2: Please answer the following questions by circling the number with the response that is appropriate to you.

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

1 (rarely) 2 (sometimes) 3 (usually) 4 (always)

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

1 (not at all) 2 (slightly) 3 (moderately) 4 (very much)

39. How often do you feel hungry?

1	2	3	4
(only at meal times)	(sometimes between meals)	(often between meals)	(almost always)

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

1 (never) 2 (rarely) 3 (often) 4 (always)

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

1 (easy) 2 (slightly difficult) 3 (moderately difficult) 4 (very difficult)

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

1 (not at all) 2 (slightly) 3 (moderately) 4 (extremely)

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

1 (almost never) 2 (seldom) 3 (usually) 4 (almost always)

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

1 (unlikely) 2 (slightly unlikely) 3 (moderately likely) 4 (very likely)

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

1 (never) 2 (rarely) 3 (often) 4 (always)

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

1 (unlikely) 2 (slightly likely) 3 (moderately likely) 4 (very likely)

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

1 (unlikely) 2 (seldom) 3 (at least once a week) 4 (almost every day)

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

1 (unlikely) 2 (slightly likely) 3 (moderately likely) 4 (very likely)

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

1 (never) 2 (rarely) 3 (sometimes) 4 (at least once a week)

50. On a scale of 0 to 5, where 0 means no restraint in eating (eating whatever you want, whenever you want it) 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 'give 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 myself to start dieting again tomorrow.'

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

Scoring

One point is given for each item in Part 1 and for each item (numbered question) in Part 2. The correct answer for the true/false items is described below. **In part 1, an 'incorrect' response results in zero point being added to that factor. 'Correct' answers receive one point.** The direction of the question in Part 2 is determined by splitting the responses at the middle. If the item is labelled '+', those responses above the middle are given a zero. Vice versa for those with a '-'. For example, scoring 3 or 4 on the first item of Part 2 (no. 37) would receive one point. Anyone scoring 1 or 2 would receive a zero.

Key:

Question number	Correct Answer	Score	Factor concerning
1	True		DH
2	True		DH
3	True		H
4	True		DR
5	True		H
6	True		DR
7	True		DH
8	True		H
9	True		DH
10	False		DR
11	True		DH
12	True		H
13	True		DH
14	True		DR
15	True		DH
16	False		DH
17	True		H
18	True		DR
19	True		H
20	True		DH
21	False		DR
22	True		H
23	True		DR
24	True		H
25	False		DH
26	True		H
27	True		DH
28	True		DR
29	True		H

30	False		DR
31	False		DH
32	True		DR
33	True		DR
34	True		H
35	True		DR
36	True		DH
37	+		DR
38	+		DR
39	+		H
40	+		DR
41	+		H
42	+		DR
43	+		DR
44	+		DR
45	+		DH
46	+		DR
47	-		H
48	+		DR
49	+		DH
50	+		DR
51	+		DH

	Tally	Score	Boundaries
Dietary restraint (DR)			0-10 low 11-13 high 14-21 clinical
Dietary disinhibition (DH)			0-8 low 9-11 high 12-16 clinical
Hunger (H)			0-7 low 8-10 high 11-14 clinical

Source

Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *Journal of Psychosomatic Research*, *29*(1), 71–83. https://doi.org/10.1016/0022-3999(85)90010-8

King, J. A., Wasse, L. K., & Stensel, D. J. (2013). Acute exercise increases feeding latency in healthy normal weight young males but does not alter energy intake. *Appetite*, *61*, 45–51. https://doi.org/10.1016/j.appet.2012.10.018

Appendix H

Food Preferences and Allergies

Diets will be formulated, and foods will be supplied during the study. Please indicate in the table below whether there are any foods that you are **ALERGIC** to or **DISLIKE**.

Food	**Allergy (Yes/No)**	**Level of preference (1-5)** **1=enjoy eating** **5=will not eat**	**Additional Comments**
White bread			
Brown bread			
Tuna			
Spread (Flora)			
Mayonnaise			
Cooked meats (ham/chicken)			
Apple			
Satsuma			
Semi-skimmed milk			
Soy Protein (chocolate)			
Chocolate milkshake			
Strawberry milkshake			
Crisps			
Pasta			
Bolognese sauce			
Olive oil			
Cream			
Cereal bars			
Chocolate			
Yoghurt			
Cream			
Rice Krispies Cereal			
Porridge Oats			
Apple Juice			

Appendix I

Menstrual Cycle Questionnaire

(All information is confidential)

Name or number:

Date of birth: Age:

What day are you on today (day 1 = first day of bleeding)? Please circle below:

2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28

29 30 31 32 33 34 35 36 37 38 39 40

1) **Have you had regular periods in the last two years?** Yes ☐ No ☐

2) **How long is your menstrual cycle, from day 1 of bleeding to day 1 of the next bleed?** _____ DAYS

3) **How many days does your menstrual (blood) flow last?** _____ DAYS

---

4) **Do you currently take contraceptive pills?** Yes ☐ No ☐

If YES, please state what, when, and for how long?

What_____

Brand: _____

When (time of day): _____

Duration (months/years): _____

5) **Have you ever taken contraceptive pills?** Yes ☐ No ☐

If YES, please state what, when, and for how long?

What_____

Brand: _____

When (time of day): _____

Duration (months/years): _____

Appendix J

Subjective Appetite Questionnaire

* 1. Please enter your participant number and initials (E.g. 01TS)

* 2. How hungry do you feel?

Not hungry at all — Extremely hungry

* 3. How full do you feel?

Not full at all — Extremely full

* 4. How strong is your desire to eat?

No desire at all — Extremely strong

* 5. How much food do you think you could eat?

None at all — A lot

* 6. How nauseated do you feel?

Not nauseas at all — Extremely nauseas

Appendix K

Pre-Exercise Subjective Responses Questionnaire

Please enter your participant number and initials (E.g. 01TS)

How motivated do you feel to exercise?

Not motivated at all — Extremely motivated

How tired do you feel?

Not tired at all — Extremely tired

How energetic do you feel?

Not energetic at all — Extremely energetic

How ready do you feel to exercise?

Not ready at all — Extremely ready

Appendix L

Positive and Negative Affect Schedule (PANAS)

This scale consists of a number of words that describe different feelings and emotions. Read each item and then circle the appropriate number next to that word. Indicate to what extent you feel this way right now, that is, at the present moment. Use the following scale to record your answers:

1	**2**	**3**	**4**	**5**
Very, slightly	A little	Moderately	Quite a bit	Extremely
Distressed	1	2	3	4	5
Excited	1	2	3	4	5
Upset	1	2	3	4	5
Strong	1	2	3	4	5
Guilty	1	2	3	4	5
Scared	1	2	3	4	5
Hostile	1	2	3	4	5
Enthusiastic	1	2	3	4	5
Proud	1	2	3	4	5
Irritable	1	2	3	4	5
Alert	1	2	3	4	5
Ashamed	1	2	3	4	5
Inspired	1	2	3	4	5
Nervous	1	2	3	4	5
Determined	1	2	3	4	5
Attentive	1	2	3	4	5
Jittery	1	2	3	4	5
Active	1	2	3	4	5
Afraid	1	2	3	4	5

Source:

Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *Journal of Personality & Social Psychology*, *54*(6), 1063–1070. https://doi.org/10.1037/0022-3514.54.6.1063

Appendix M

Physical Activity Enjoyment Scale (PACES-8)

Please rate how you feel *at the moment* about the physical activity you have just completed

*I enjoyed it							I hated it
	1	2	3	4	5	6	7
I disliked it							I liked it
	1	2	3	4	5	6	7
*I found it pleasurable							I found it unpleasurable
	1	2	3	4	5	6	7
It was no fun at all							It was a lot of fun
	1	2	3	4	5	6	7
I felt as though I'd rather be doing something else							I felt as though there was nothing else I'd rather be doing
	1	2	3	4	5	6	7
*I was very absorbed in the activity							I was not at all absorbed in the activity
	1	2	3	4	5	6	7
*It was very pleasant							It was very unpleasant
	1	2	3	4	5	6	7
I felt bored							I felt interested
	1	2	3	4	5	6	7

*Item is reversed scored (*i.e.*, 1=7 points, 2=6 points … 6=2 points, 7=1 point).

Source:

ORIGINAL: Kendzierski, D., & DeCarlo, K. J. (1991). Physical activity enjoyment scale: Two validation studies. *Journal of Sport & Exercise Psychology, 13*(1), 50–64. https://doi.org/10.1123/jsep.13.1.50

SHORTENED: Raedeke, T. D. (2007). The relationship between enjoyment and affective responses to exercise. *Journal of Applied Sport Psychology*, *19*(1), 105–115. https://doi.org/10.1080/10413200601113638