Gastrointestinal Function and Metabolic Responses to Fasted Exercise; Implications for Food, Energy Intake and Obesity

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Gastrointestinal Function and Metabolic Responses to Fasted Exercise; Implications for Food, Energy Intake and Obesity

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Abstract

The increasing economic burden confronted by nation-states in the face of a global obesity epidemic is well documented. Intermittent fasting has become a popular intervention for metabolic health and combining intermittent fasting with exercise may support weight management programming. Therefore, fasted exercise has become an increasingly popular strategy in weight management practices. However, the effects of fasted exercise on gastrointestinal function, metabolic markers, and appetite regulation remains unclear. This thesis aimed to enhance understanding of fasted versus fed exercise on gastrointestinal function, metabolic responses, and appetite. This will support the development of novel non-pharmacological interventions for weight management. A series of studies on human volunteers are presented in this thesis. Firstly, the effect of brisk walking in the fasted versus fed state on the gastric emptying rate (GER), metabolic responses and appetite hormone responses in healthy men were determined. These findings suggest that GER, hunger and appetite regulatory hormones are not sensitive to low-intensity bouts of physical activity and, that fasted brisk walking holds positive implications for weight management practices. Following on from these findings, the diurnal influences of GER, appetite and metabolic responses when performing fasted and non-fasted exercise in healthy men was assessed. The findings suggest that evening fasted exercise delays GER, without changes in appetite. No compensatory effects were observed for appetite following fasted exercise and regardless of the time of day, fasted exercise induces a negative energy balance without a subsequent compensatory response in energy intake (EI). Following on from these results, the diurnal influences were further explored by measuring gastrointestinal hormones and metabolites, as well as GER and appetite following fasted versus non-fasted exercise in lean men. These findings suggested GER is slower in the evening; with subtle differences observed in hormonal and metabolic responses. No compensatory effects were found for appetite post-exercise or 24 h EI post-trial. To understand if these findings were consistent within other population groups, the study was repeated in overweight men. No differences were found in all variables. Overall, the findings within this thesis suggest fasted exercise may hold positive implications for weight management practices in healthy, lean, and overweight populations. Future work is required to assess the long-term effects of fasted exercise on metabolic health, energy balance and gastrointestinal function.

Table of Contents

Publications

McIver VJ, Mattin L, Evans GH, Yau AMW. (2018). The effect of brisk walking in the fasted versus fed state on metabolic responses, gastrointestinal function, and appetite in healthy men. Int J Obes (Lond). 43(9), pp.1691-1700. https://www.nature.com/articles/s41366-018-0215-x

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Conference presentations and proceedings

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List of Tables

List of Figures

List of Abbreviations and Symbols

°C	Degree Celsius
µl	Microlitre
µM	Micromole
$^{12}CO_2$	Carbon 12 carbon dioxide
$^{13}CO_2$	Carbon 13 carbon dioxide
Acetyl-coA	Acetyl coenzyme a
AG	Acylated ghrelin
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
AUC	Area under the curve
BM	Body mass
BMI	Body mass index
^{13}C	Carbon 13
CKK	Cholecystokinin
CNS	Central nervous system
DOB	Delta over baseline
DPP-IV	Dipeptidyl peptidase IV
EE	Energy expenditure
EI	Energy intake
et al.	*et alii*
Fat_{max}	Maximal fat oxidation
g	Gram
GH	Growth hormone
GHS-R	Growth hormones secretagogue receptor
GI	Gastrointestinal
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
GLP-1R	Glucagon-like peptide-1 receptor
GLU	Glucose
h	Hour
IR	Infrared
kcal	kilocalorie
kg	Kilogram

kJ	Kilojoules
L	Litre
Ltd	Limited
m	Metre
mg	Milligram
min	Minute
mL	Millilitre
mm	Millimetre
Mmol/L	Millimoles per litre
n	Participant number
NEFA	Non-esterified fatty acid
pmol/L	Picomoles per litre
P	Probability
PP	Pancreatic polypeptide
PYY	Peptide tyrosine tyrosine
RMR	Resting metabolic rate
RPE	Rating of Perceived Exertion
SD	Standard deviation
sec	Second
SEM	Standard error of the mean
SPSS	Statistical Package for Social Sciences
TAG	Triacylglycerol
$T_{1/2}$	Half emptying time
T_{lag}	Time of maximal emptying rate
U	Unit
UK	United Kingdom
USA	United States of America
VAS	Visual Analogue Scale
$\dot{V}O_2max$	Maximum oxygen uptake
WHO	World Health Organisation
y	Year

1.0. GENERAL INTRODUCTION

1.1. Obesity and energy balance

Obesity has become a worldwide epidemic, and its prevalence continues to rise. Obesity is one of the leading preventable causes of death within the United Kingdom (UK). Globally, is has been predicted that 1.12 billion adults will be obese by 2030 (Kelly et al., 2008). The terms overweight and obesity refers to excessive fat accumulated that presents a risk to health and development of chronic diseases such as type 2 diabetes, hypertension, hyperlipidaemia, coronary heart disease, and cancer (Hurt et al., 2010). Obesity is often viewed in energy balance terms and is a result of excessive food intake and/or insufficient physical activity (Hall et al., 2012; Hill et al., 2012). Several factors can influence energy balance, for instance, daily environment (behavioural/habitual lifestyle you are exposed to), genetic, metabolic, and physiological factors have all been implicated (Hall et al., 2012). These factors all interact to influence an individual's overall energy balance.

1.2. Anatomy and function of the gastrointestinal system

The gastrointestinal (GI) system comprises of major GI organs and accessory organs. It is responsible for ingesting and digesting food and is the largest endocrine organ within the human body (Ahlman & Nilsson, 2001). The GI tract is typically divided into an upper and lower section (Wang, 2012), with associated accessory organs. The upper GI tract is the mouth, the pharynx, the oesophagus, and the stomach. The lower GI tract includes the small intestine, the duodenum, the jejunum, the ileum, large intestine, the cecum, and the colon. The organs that are accessory to the alimentary canal of the GI tract are organs such as, the liver, gallbladder, and pancreas. These organs are utilised for secretory, storage, waste filtering and are also associated with hormonal glands (Hunt et al., 2015).

The GI tract digests ingested food for the absorption and delivery of nutrients and energy to the body, whilst also expelling waste material. The primary functions of the GI tract are characterised as four distinct processes; digestion, absorption, excretion and protection (Cheng et al., 2010).

Digestion involves both mechanical and chemical processes. Mechanical digestion reduces the size of the food to increase surface area and mobility. Mechanical digestion initially begins in the mouth with mastication, supporting the breakdown of food into smaller particles, which then goes into the stomach and segmentation in the small intestine (Boland, 2016). Peristalsis is the involuntary contraction of smooth muscles within the oesophagus, stomach, and intestines, and supports mechanical digestion to physically break down food substances into smaller particles (Gayer & Basson, 2009). Whereas, chemical digestion refers to the further break down of ingested food by the secretion of enzymes by the digestive tract, into a form that is absorbable (Boland, 2016; Cheng et al., 2010). There are three stages phases of gastric secretion that aid in chemical digestion. These stages are known as the are the cephalic, gastric, and the intestinal phase (Lloyd, 1994), these stages intersect and occur simultaneously. Firstly, the cephalic phase of gastric secretion takes place before the food arrives in the stomach, predominantly while food is being consumed (Merki et al., 1991). It results from the visual, olfactory (smell), auditory inputs, thought, or taste of food. Secondly, the gastric phase occurs which triggers the gastric activity in the stomach (Konturek et al., 1979). Following this, the duodenum responds to the incoming chyme, regulating gastric activity via hormones and neural reflexes, which is known as the intestinal phase (Browning & Travagli, 2014).

Absorption involves transferring digested nutrients, water and electrolytes from the lumen of the small intestine into the circulatory and lymphatic capillaries by active transport and passive diffusion through the epithelial cells of the intestinal villi (Matthews & Laster,

1965). This is followed by excretion, which eliminates undigested and unabsorbed, typically solid material, from the GI tract by defecation.

1.2.1. Stomach

The stomach is a muscular, J-shaped organ in the higher part of the abdomen (Burdan et al., 2012) (Figure 1). The stomach is separated into five areas; cardia, fundus, corpus (body), antrum and pylorus (Tarpley et al., 1987). The primary function of the stomach is to assist with digestion. The roles of the stomach include ingesting food as a reservoir, secretion of acid, enzyme secretion and its part in GI motility (Hunt et al., 2015). In the stomach, food endures both mechanical (muscular contract to breakdown food) and chemical digestion (food further mixes with secreted acid and enzymes). The secretion of acid and enzymes are critical to aid with the digestion of food (Ramsay and Carr, 2011). Acid secretion is also a non-immunological defence that is important to fight against unwelcome pathogens (Hellström et al., 2006; Hunt et al., 2015). The stomach plays a crucial motility role involving both the proximal portion and a distal portion. The proximal region (cardia, fundus, and proximal one-third of the corpus) acts as the reservoir capacity of the stomach. The proximal regions provide low frequency, sustained contractions that are accountable for creating a basal pressure within the stomach (Soybel, 2005). The distal region (two-thirds of the corpus, antrum and pylorus) is muscular in comparison to the proximal region, which involves the mixing the contents in the stomach before delivering them to the duodenum (Hellström et al., 2006; Hunt et al., 2015). The motility in both the proximal and distal regions of the stomach are controlled by complex neural and hormonal signals (Goyal et al., 2019; Hunt et al., 2015). The stomach is a vital endocrine organ, secreting peptide hormones, such as, gastrin and ghrelin (Hellström et al., 2006; Hunt et al., 2015).

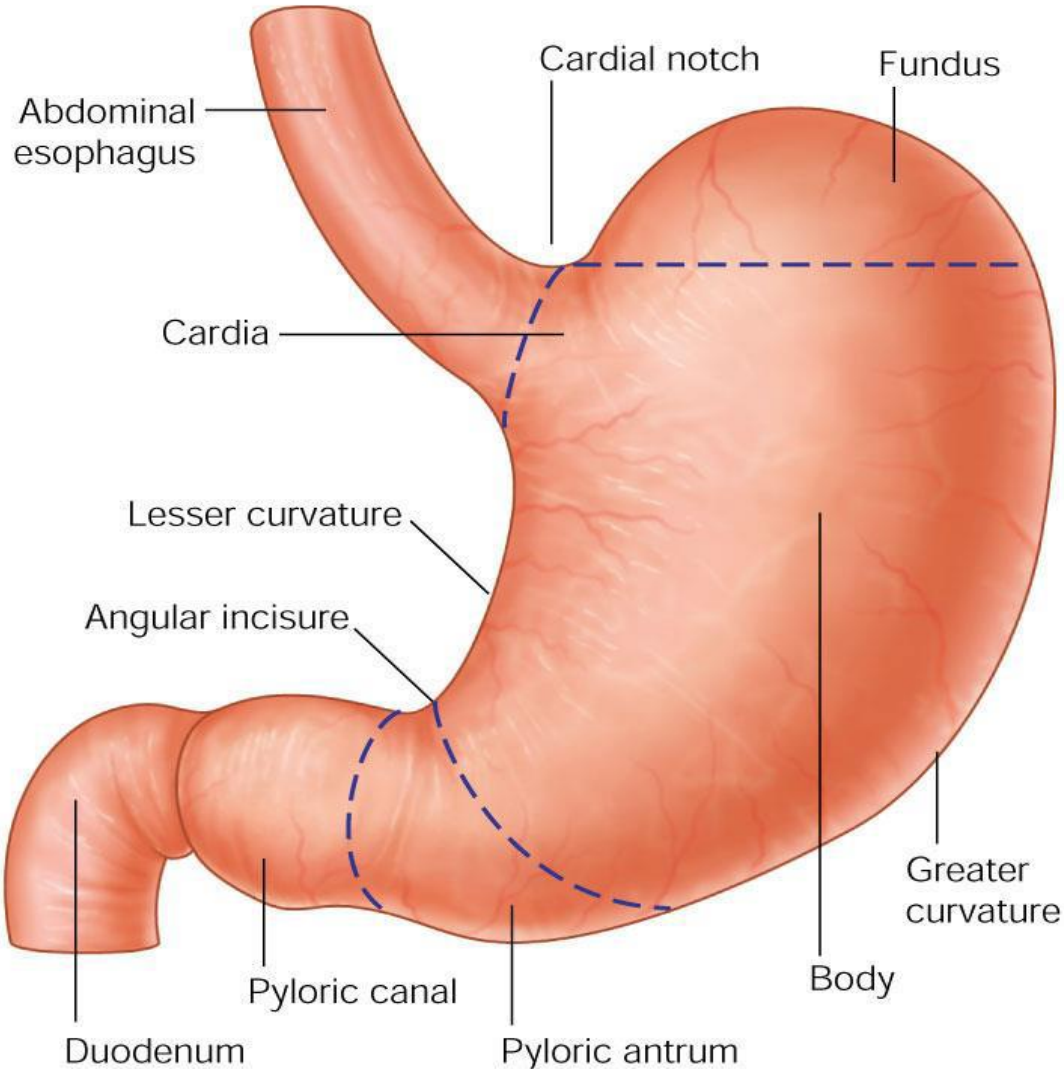

Figure 1: The anatomy of the stomach (Adapted from Drake et al., 2014).

1.3. Gastric emptying

Gastric emptying is the procedure by which the stomach empties its contents into the small intestine for the delivery and absorption of nutrients to the body (Varón & Zuleta, 2010). Gastric emptying is regulated by a complex set of neural and hormonal signals (Goyal et al., 2019; Hellström et al., 2006; Tambascia et al., 2014). The autonomic motor nerves signal the stomach to increase gastric secretion and motility when food is consumed in order for the stomach to empty (Browning & Travagli, 2014; Cooke, 1975; Hellström et al., 2006). The force and frequency of antral contractions determine the delivery of content into the duodenum (Cooke, 1975). Furthermore, to allow the stomach to empty its contents, the delivery of nutrients also depends on the opening of the pyloric sphincter (Indireshkumar et al., 2000). Vagal excitatory reflexes arbitrate this process, stimulated by gastric distension (Geliebter, 1988). The process of emptying of the stomach into the small intestine is affected by a magnitude of complex factors, some of which are described in more detail below.

1.3.1. Factors that affect gastric emptying

The gastric emptying of liquids is more rapid in comparison to the emptying of solids (Achour et al., 2001; Collins et al., 1991; Fisher et al., 1982). This is because liquids emptying from the stomach require no trituration (Collins et al., 1991; Houghton et al., 1988; Szarka & Camilleri, 2009a). On the other hand, emptying of solids requires an initial relaxation of the proximal stomach, followed by a linear increase in tonic contraction within the proximal stomach (Goyal et al., 2019; Janssen et al., 2011). Increasing the intragastric volume within a test meal accelerates gastric emptying, in both liquid and solids forms (Costill & Saltin, 1974; Doran et al., 1998; Hunt & Stubbs, 1975; Leiper, 2015). A previous study investigated the effects of increasing meal volume and energy densities on the rate of gastric emptying and found increasing either energy density or volume of the meal increased the rate of emptying at

all-time points (Hunt et al., 1985). The steady rate of energy delivery was associated with a greater meal volume and calorie density (Hunt et al., 1985).

Calorie and/or energy density of the food plays a vital role in the regulation of gastric emptying (Calbet & MacLean, 1997). A greater energy content of solutions (Costill & Saltin, 1974; Coyle et al., 1978; Foster et al., 1980; Maughan & Leiper, 1996) and meals (Peracchi et al., 2000; Hunt & Stubbs, 1975) slows down gastric emptying. Irrespective of the relative contributions of energy from fat, carbohydrate, and protein (Hunt, 1980) which at isoenergetic quantities slows down the rate of gastric emptying in equal amounts (Hunt & Stubbs, 1975). For instance, water empties rapidly from the stomach, however, as the solution becomes more calorie-dense, then the rate of gastric emptying will be slower (Vist and Maughan, 1994; Beckers, Leiper and Davidson, 1992). This fast emptying of water would be mostly controlled by the passage of propagated waves passing along the stomach to the duodenum with the little role required for the pylorus relaxing to obtain food stuff from the proximal antrum (Goyal et al., 2019; Muller et al., 2018). Whereas, if dextrose was added in the water or lipid into a solution, gastric emptying rate is slower (Mihai et al., 2018). This slowing of emptying is due to a diminution of propagated pressure waves in the stomach and an increase in pyloric closure, with both effects tending to keep fluid in the stomach (Goyal et al., 2019). Therefore, calorie content of a test solution has a great influence on gastric emptying, thus, increasing energy density slows down the rate of gastric emptying (Hunt et al., 1985; Hunt & Stubbs, 1975).

Osmolality has been suggested to influence gastric emptying rate (Hunt, 1960; Meeroff, Go & Phillips, 1975; Vist and Maughan 1995), especially for solutions with no nutrient content, however, this is not always the circumstance for energy-dense solutions (Rehrer et al., 1993; Leiper, 2015). Previous studies have substituted glucose polymers for glucose monomers to sustain the carbohydrate content but reducing the osmolality of a test solution. Most studies

have found minimal differences in the rate of gastric emptying following isoenergetic solutions of glucose monomers compared with glucose polymers, regardless of the differences in osmolality (Maughan, 1997; Brouns et al., 1995). In contrast to this, Sole and Noakes (1989) found gastric emptying was faster with a 15% glucose polymer solution compared to a 15% glucose monomer solution, although no differences were observed in the rate of gastric emptying from 5 or 10% of these two glucose solutions. Overall, high osmolality typically slows down gastric emptying (Vist and Maughan 1995; Leiper, 2015).

Nerve conduction and muscle motility are influenced by temperature. Gastric emptying rate has been suggested to be affected by the temperature of liquids and solids consumed (Mishima et al., 2009) Previous studies have found only acute changes in gastric emptying rate with liquids of different temperatures (Costill and Saltin, 1974; Lambert and Maughan, 1992; Sun et al., 1988). However, the findings on the effect of temperature on gastric emptying rate are inconsistent. Some studies have demonstrated a low calorie-dense liquid meal in both colder and warmer temperatures emptied the stomach slower than at body temperature (Sun et al., 1995; 1998), whilst others demonstrate a delay in emptying of liquids is obtained only with a warmer meal (Troncon and Iazigi, 1988). Other studies have reported that a hot meal significantly accelerates gastric emptying (Mishima et al., 2009). Due the inconsistencies in findings from previous investigations, it is difficult to draw firm conclusions, however it could be suggested that the rate of recovery of intragastric temperature can be influenced to some degree by the temperature of the liquid beverage and the thermal capacity of the solution, alongside the volume consumed (Leiper, 2015).

Gastric emptying has also been observed to be influenced by blood glucose concentrations (Chang, Rayner, Jones, Horowitz, 2010; Rayner, Samsom, Jones, Horowitz, 2001). Acute changes in blood glucose concentrations have a major impact on gastric motor

function and gastric emptying rate (Marthe et al., 2013; Rayner et al., 2001). Acute hyperglycaemia (blood glucose concentration approx. 15 mmol/L) delays gastric emptying (MacGregor, Gueller, Watts, Meyer, 1976; Oster-Jorgensen, Pedersen, Larsen, 1990; Russo et al., 2005), whereas, hypoglycaemia accelerates gastric emptying, which has been found in both healthy and type 1 diabetes individuals (Schvarcz et al., 1997). Overall, the elevation of blood glucose concentrations has shown to slow down gastric emptying rate.

The effect of posture and body position affecting gastric emptying has received some attention, however, the existing literature is conflicting. Some studies have shown that gastric emptying rate is decreased in a head-down tilted position (Holwerda et al., 2016). Similarly, Moore et al., (1988) found supine position compared to sitting, standing, or combined sitting-standing postures significantly slowed gastric emptying. Similar findings were also observed within previous studies (Asada et al., 1989; Mannell and Esser, 1984; Queckenberg and Fuhr, 2008). Horowitz et al., (1993) reported that intragastric distribution was affected by gravity, however, but not so much for total stomach emptying, whilst other studies observed no change of effect on gastric emptying despite changes in body position (Doran et al., 1998; Steingoetter et al., 2006). The inconsistencies may be due to differences in test meals ingested, the sample size of participants and protocols used to measure gastric emptying rate. Overall, it seems food ingestion in an upright position (sitting or standing) accelerates gastric emptying, whereas supine position can significantly slow down gastric emptying rate (Asada et al., 1989; Mannell and Esser, 1984; Queckenberg and Fuhr, 2008)

Gastric emptying rate is also affected by the time of day, with a diurnal variation having been observed (Kentish et al., 2013). Goo et al., (1987) compared morning (AM) and afternoon (PM) gastric emptying rates in healthy men and found that AM gastric emptying was significantly faster compared to PM gastric emptying rate. More recently, Grammaticos et al.,

(2015) reported more than 220% delay on the rate of gastric emptying half time when they received toast at 23:00 as compared to the same meal at 08:00.

To summarise, there are many factors, both physical and chemical characteristics of a solution/meal that influence the rate of gastric emptying. Additional factors are the endocrine system of the GI tract and performance of exercise, which will be discussed in depth further on. It is important to understand and acknowledge the effect various factors play on the physiology of gastric emptying. For instance, as gastric emptying rate is accelerated this could potentially diminish the negative feedback satiety signals (Cardoso et al., 2007). However, it is still unknown whether exercise in a fasted vs fed state may alter gastric emptying rate of a subsequent meal. Therefore, investigations on the effects of fasted exercise on gastric emptying rate may provide useful information for both general and clinical populations.

1.3.2. The measurement of gastric emptying

There are a variety of methods available for the assessment of gastric emptying (Maughan & Leiper, 1996). The method utilised will depend on a number of factors such as, whether the test meal is a solid or liquid substance, the extent of invasiveness that the participant can withstand, level of precision required and the facilities and/or budget accessible (Maughan & Leiper, 1996). It is vital to acknowledge the strengths and weaknesses of the various methods, and the large inter-individual variability and of the factors known to influence gastric emptying. Some of the commonly used techniques available for gastric emptying assessment are discussed below.

Gastric emptying scintigraphy, first described in 1966 (Griffith et al., 1966), is commonly used for both clinical and research purposes. Scintigraphy uses gamma cameras to create two-dimensional images and is non-invasive, reproducible, quantitative and is considered the gold standard for the evaluation of gastric emptying of both liquids and solids (Hellström et al.,

2006; Seok, 2011; Szarka & Camilleri, 2009b, 2009a). Findings between studies can fluctuate depending on factors such as, institution carrying out the technique, the choice of meal composition, positioning of the participant, frequency of measurement and duration (Abell et al., 2008). These differences in the method can often make it difficult to compare between studies (Abell et al., 2008; Seok, 2011). Therefore, standardisation procedures should be carried out to ensure accurate findings (Szarka & Camilleri, 2009a). For example, the radiotracer must be tightly bound to the solid meal, to accurately quantify emptying. In addition to this, the individual should be advised to conduct the assessment test after at least a 4 h fast (Vasavid et al., 2014).

The double sampling gastric aspiration technique of George (1968), modified by Beckers et al. (1988) is a another method of measuring of gastric emptying. This method measures the change in gastric volume whilst accounting for the volume of gastric secretion at the different time intervals (Leiper, 2015). This technique is reliable and accurate (Hardy et al., 1987). Advantages of this technique are that it enables multiple measurements of composition of the gastric contents and total gastric volume in one experiment (Beckers et al. 1988; Maughan and Leiper, 1996) and has been successfully used within previous exercise studies (Evans et al., 2016; Gant et al., 2007; Leiper et al., 2005; Rehrer et al., 1990). However, there are some disadvantages, the technique can only be used for liquids (Maughan & Leiper, 1996), with reasonably large test volumes requiring to be ingested, in which all participants cannot endure the aspiration tube (Leiper et al., 2001).

Imaging techniques such as, magnetic resonance imaging (MRI) and ultrasound are other methods that can be used for the assessment of gastric emptying and results have been shown to correlate well to those obtained by the scintigraphy or the double sampling gastric aspiration techniques (Maughan & Leiper, 1996). MRI is a validated method for gastric emptying

measurement (Curcic et al., 2015; Sauter et al., 2012; Schwizer et al., 2006; Treier et al., 2008). MRI allows visualising the volume and distribution of the ingested meal as well as of gastric secretion (Maughan & Leiper, 1996). In addition to the MRI method, ultrasound imaging has been shown to be a safe non-invasive technique for the measurement of gastric emptying (Bateman & Whittingham, 1982).

Breath testing is another method for gastric emptying assessment which is performed using a standardised meal labelled with Carbon-13 (^{13}C). The ^{13}C substrate (in the test meal) passes through the stomach into the duodenum to be absorbed, metabolised in the liver and $^{13}CO_2$ exhaled in the breath that is measured (Waseem, Moshiree and Draganov, 2009). The transit of the meal through the stomach is the rate-limiting step in the process, assuming normal bowel, liver and pulmonary function (Camps et al., 2018). The breath test method is a valid, non-invasive, and relativity cheap method of measuring indirectly gastric emptying rate for both liquids and solids (Camps et al., 2018; Ghoos et al., 1993; Sanaka & Nakada, 2010). A previous study advocated that a sampling period of at least 4 hours is required to allow precise curve fitting (Sanaka & Nakada, 2010). However, this duration for sample collection may have practical limitations and burden the participants or patients involved. Recovery variations are observed within 2 hours post-ingestion within liquid foods (Bluemel et al., 2015; Camps et al., 2018). Liquid meals empty somewhat rapidly, particularly if the macronutrient content of the test solution is low (Camps et al., 2018). Therefore, lower calorie-dense/semi-solid meals may empty a lot quicker and thus, a smaller breath sampling duration may be adequate to detect differences (Bluemel et al., 2015).

1.4. Hormones involved in the regulation of appetite, food intake and gastric emptying

The central nervous system (CNS) plays a significant part in GI motility, providing extrinsic neural inputs that regulate, modulate, and control the GI functions (Browning and Travagli, 2014; Hunt et al., 2015). The CNS also plays an important part in energy balance. The CNS detects signals from the periphery about metabolic status; the CNS then processes this information to deliver appropriate responses to ensure the state of a positive or negative energy balance occurs (Smith and Ferguson, 2008). The feelings of hunger and satiety are controlled in the CNS via the brain-gut axis (Klok et al., 2006).

Hunger is defined as a strong desire or need for food, while satiety is defined as feeling full or satisfied (Smith and Ferguson, 2008). Hunger and satiety control meal by meal eating behaviour (Rui, 2013). During the cephalic phase of appetite control, food, and food-related signals control anticipatory meal activity and meal initiation (Rui, 2013). Hunger levels are enhanced by information regarding food availability and palatability being transferred to the brain by olfactory, and visual signals through polymodal sensory pathways (Rui, 2013). Once the food is consumed and enters the stomach and GI tract, the food components regulate the secretion of polypeptide hormones from the GI enteroendocrine cells (Sam et al., 2012; Rui, 2013). These hormones act as signals to influence hunger and satiety (Druce and Bloom, 2006).

There are a collection of episodic hormones secreted from endocrine cells within the GI tract. The gut releases over 20 peptide hormones, some of which target the brain to regulate appetite. A variety of these gut peptide hormones influence hunger and satiety, and therefore, energy intake (Horner et al., 2011). In addition to this, it is well recognised that gut hormones play a vital part in regulating gastric emptying rate (Druce, and Bloom, 2006; Hellstrom et al.,

2006; Rui, 2013). The release and secretion of these peptide hormones to influence gastric emptying rate can depend on the chemical composition of the chyme entering the duodenum, for example, a greater fat content of the chyme, releases cholecystokinin (CCK), and thus reducing gastric emptying (Jolliffe, 2009). As food enters the duodenum, it is stretched, activating stretch receptors and inhibiting the vagus nerve, vagal tone and mobility, slowing down the rate of gastric emptying (Browning et al., 2017; Jolliffe, 2009). The neural and hormonal processes initially trigger the duodenum, feedback to slow down the rate of gastric emptying and constitute the entero-gastric reflex (Jolliffe, 2009). This process regulates the transport of the chyme into the duodenum, which allows the absorption of nutrients within the small intestine to be expanded (Goyal et al., 2019; Jolliffe, 2009). Some of the primary appetite-related hormones that also influence gastric emptying rate are discussed below.

1.4.1 Ghrelin

Ghrelin is a 28-amino-acid peptide, discovered as the endogenous ligand of the growth hormone secretagogue receptor (GHS-R) (Pradhan et al., 2014; Delporte, 2013). Ghrelin is secreted predominantly from the stomach, in humans, the P/D_1 cells in the fundus (Date et al., 2000; Sakata & Sakai, 2010). Ghrelin is also expressed in many tissues such as, in the duodenum, jejunum, colon, pancreas, and hypothalamus (Hosoda et al., 2000; Ghreardoni et al., 2006; Delporte, 2013). There are two forms of ghrelin, acylated ghrelin and des-acylated ghrelin. Acylated ghrelin, which is the octanoylated form of ghrelin, has orexigenic activity and is recognised as the "active form" of ghrelin (Nakai et al., 2003; Delporte, 2013). Des-acylated ghrelin, which is the non-octanoylated form, is anorexigenic, and typically known as the "inactive form". Octanoylation is produced by ghrelin O-acyltransferase (GOAT) (Hosoda, et al., 2000; Wysokinski, et al., 2014). Ghrelin is a key regulator for appetite, and also promotes the secretion of growth hormone as it binds to growth hormone secretagogue receptor (Holliday

& Blannin, 2017). Ghrelin is well recognised for increasing hunger (often referred as the 'hunger hormone'), food intake and accelerating the rate of gastric emptying (Hellstrom et al., 2006; Hosoda et al., 2000; Ghreardoni et al., 2006; Delporte, 2013). Ghrelin does this by mediating information to the activation of neurons in the brainstem, to the hypothalamic arcuate nucleus, which is embroiled in the control of feeding behaviour (Cowley et al., 2003). Research has found that subcutaneous ghrelin administration increases appetite and energy intake in both lean and obese participants (Macke et al., 2009; Druce et al., 2005; Rezaie et al., 2015; Takaya et al., 2000; Lawrence et al., 2002; Wren et al., 2001), and increases the gastric emptying rate in healthy and diabetes populations (Murray et al., 2005; Hellstrom et al., 2006).

Ghrelin was initially isolated from the stomach, but research in the last decade has shown that ghrelin has regulatory roles in many organs and systems, such as peripheral tissues, pancreas, ovary and adrenal cortex (Pradhan et al., 2014; Klok et al., 2006). The secretion of ghrelin by the stomach is dependent on the nutritional state, for example, ghrelin elevates during the pre-prandial period, stimulating energy intake via the vagus nerve, the brainstem, and the hypothalamic arcuate nucleus, while during the post-prandial period ghrelin levels are decreased (Delhanty et al., 2012).

1.4.2. Glucagon-like peptide-1 (GLP-1)

Glucagon-like peptide-1 (GLP-1) is a 30-amino acid peptide hormone, secreted from the L-cells in the duodenum, small and large intestine of the GI tract (Lim and Brubaker, 2006). GLP-1 is primarily secreted in two forms, GLP-$1^{(7\text{-}36\ \text{amide})}$ with smaller amounts of the bioactive form GLP-$1^{(7\text{-}37)}$. GLP-1 ($^{1–37}$ and $^{1–36\ \text{amide}}$) also exist but exhibit little biological activity (Fava et al., 2016). GLP-$1^{(9\text{-}36\ \text{amide})}$ is formed by dipeptidyl peptidase IV (DPP-IV) rapidly degrading GLP-$1^{(7\text{-}36\ \text{amide})}$ (Gupta, 2013). The primary role of GLP-1 is to work as an incretin hormone, potentiating insulin secretion in the presence of high plasma glucose

concentrations (Graaf et al., 2016; Doyle and Egan, 2008). GLP-1 has also been shown to suppress appetite and promote satiety (Dailey and Moran, 2014), reduce glucagon secretion, and induce beta-cell neogenesis (Zhang et al., 2019), increase insulin secretion (Meloni, DeYoung, Lowe, Parkes, 2013), and inhibit beta-cell apoptosis (Ferilla et al., 2003). Additionally, GLP-1 has also been shown to decrease the rate of nutrient absorption into the blood by slowing down gastric emptying rate (Deane et al., 2010). Previous investigations have conducted GLP-1 based therapies and found reduced appetite and inhibitory effects on the rate of gastric emptying from infusing GLP-1 in healthy (Nuack et al., 1997; Little et al., 2006; Delgado-Aros et al., 2002), obese (Nasland et al., 1998) and type 2 diabetic populations (Meier et al., 2003). In addition to this, infusion of GLP-1 has also been shown to slow down gastric emptying of both solid and liquid components of a meal (Little et al., 2006). GLP-1 secretion is reliant on meal composition, increasing in response to a meal, especially within high protein and high-fat meals (Pais et al., 2016; Parvaresh et al., 2018). Changes in GLP-1 can stimulate neurotransmitter receptors located in the hypothalamus, influencing appetite and subsequent energy intake (van Bloemendaal et al., 2014)**.**

1.4.3. Peptide Tyrosin Tyrosin (PYY)

Peptide tyrosin tyrosin (PYY) is a 36-amino-acid peptide and is related to the pancreatic polypeptide (PP-fold) peptide family along with PP and neuropeptide Y (NPY) (Batterham et al., 2002). PYY is secreted from the GI L-cells in the small intestine, existing in two endogenous forms, $PYY^{(1-36)}$ and $PYY^{(3-36)}$. These bind and activate three Y receptor subtypes (Y1, Y2, Y3), though $PYY^{(3-36)}$ acts mainly via the Y2 receptors (Y2R) (Batterham and Bloom, 2003), which are peripherally found in the liver, intestine, spleen, muscle and adipose tissue and centrally in the arcuate nucleus of the hypothalamus (Wynne and Bloom, 2006). PYY is known to be a satiety peptide released in response to feeding, with large concentrations found

in the colon and rectum (Karra et al., 2009). Studies have found PYY to slow down gastric emptying rate (El-Salhy et al., 2013; Marathe et al., 2013), and suppress appetite (Karra et al., 2009; Morinigo et al., 2006). PYY levels are low after overnight fasting but following a meal circulating levels of $PYY^{(3-36)}$ typically elevate within 15 minutes, peaking within the second hour after meal initiation and slowly decrease within six hours (Koegler et al., 2005). The magnitude of the rise will depend on the calories ingested (De Silva and Bloom, 2012). Previous studies have found lower PYY concentrations in obese patients (Roth et al., 2005). Batterham et al., (2003) found a 30% reduction in calorific value of a meal consumed two hours post PYY infusion and 33% reduction in food consumption over a 24 h period in both lean (n=31) and obese individuals (n=30). This may suggest peak PYY in post-prandial responses is dependent on meal calorie value and/or population (lean vs obese).

1.4.4. Pancreatic Polypeptide (PP)

Pancreatic polypeptide (PP) is a 36-amino acid that is primarily secreted by F cells of the pancreas, found in the Islets of Langerhans (Śliwińska-Mossoń et al., 2017). The hormone PP has effects on metabolism and energy intake and is also known to slow down gastric emptying rate (Strommer et al., 2005). The vagus nerve is the primary stimulator that causes the pancreas to rapidly release PP after a meal and remains elevated for approximately 4-6 h in humans (Schwartz, 1983; Belinova et al., 2017). Former investigations found that the secretion of PP is decreased in cases of obesity and increased in cases of anorexia nervosa (Shi, et al., 2015; Ramracheya *et al.,* 2016; Lin et al., 2004), suggesting PP may play a regulatory part in energy balance and relative contributions of obesity (Asakawa et al., 2003).

1.4.5. Insulin

Insulin is a peptide hormone that is secreted by the β cells of the pancreatic islets of Langerhans (Da Silva Xavier, 2018). Insulin is a hormonal regulator of appetite, and an

essential hormone that affects carbohydrate, protein, and lipid metabolism (Qaid & Abdelrahman, 2016). Insulin regulates blood glucose concentration (Röder et al., 2016). Insulin is rapidly secreted in response to a meal, with well-characterised hypoglycaemic effects (Polonsky, Given, and Van Cauter, 1988). The secretion of insulin following a meal is due to the absorbed glucose into the bloodstream, decreasing the mobilisation from glycogen stores and adipose tissue, and increasing uptake of ingested substrates to fat depots for storage (Dimitriadis, et al., 2011; Frayn and, Karpe, 2014).

1.4.6. Other hormones

Cholecystokinin (CCK) is a hunger suppressant and was the first GI hormone discovered for its role (Gibbs, Young & Smith, 1973; Chaudhri et al., 2006). CCK has been shown to control a variety of physiological effects, including regulation of nutrient delivery to the intestines, the stimulation of gallbladder contraction and pancreatic and gastric acid secretion (Dockray, 2014; Amamska et al., 2014). CCK is mainly synthesised and released from in the I cells of the duodenum and small intestine (Dockray, 2012). CCK acts to relax the proximal stomach and enhance contractions in the distal stomach (Okonkwo and Adeinka, 2019). CCK secretion typically increases within approximately 15 min after a meal (Chearskul et al., 2008; Amamska et al., 2014; Moehlecke et al., 2016), and is predominantly stimulated by high fat and protein meals (Stewart, Feinle-Bisset, Keast, 2011; Mushref and Srinvasan, 2013; Pesta and Samuel, 2014). Therapeutic approaches for CCK have also been applied, as CCK administration has been known to reduce energy intake by reducing meal size and duration (Bilski et al., 2009). Previous investigations have found that intermittent CCK infusions resulted in decreased ingestion of meal size in obese populations (Smith, 2006; Kim et al. 2011). CCK along with GLP-1 and PYY inhibits the rate of gastric emptying (Hellstrom et al., 2006; Dockray, 2012; Dockray, 2012; Amamska et al., 2014).

Leptin, a 167-amino-acid product of the human leptin gene, is secreted into the bloodstream by adipose tissue to regulate energy homeostasis and is associated with body fat (Zhou and Rui, 2013). The release of leptin by adipose tissue is affected by numerous factors such as gender, age, exercise, and glucose uptake. Leptin secretion can reflect the amount of energy stored in fat and influence acute variations in food intake, suppressing food intake (Park and Ahima, 2015; Klok et al., 2006). On the other hand, decreased levels of leptin can prompt increased feeding and suppress energy expenditure (Ahima et al., 1996; Welt et al. 2004; Chan et al. 2003; Farooqi et al. 2002; Ahima, 2008).

1.5. The effect of acute exercise on gastric emptying rate, gastrointestinal hormones and appetite regulation

Over recent years, more studies have been conducted on acute exercise to determine the effect this has on GI hormones, GER, and subjective feeling of appetite and subsequent food intake. Research has found the intensity of exercise and mode of exercise being performed may affect appetite regulation, secretion of gut hormones and the rate of gastric emptying differentially. However, these findings are inconsistent within the literature, with some studies observing differences, whilst others do not.

Previous investigations have reported a suppression in appetite during acute exercise at or greater than 60% peak oxygen uptake ($\dot{V}O_{2peak}$) (Vatansever-Ozen et al., 2011). However, this suppression typically returns to resting control levels within 30-60 min in physically active individuals performing resistance exercise (Broom et al., 2008; Laan et al., 2010), treadmill running (King et al., 2015; King et al., 1997), cycling (Martins et al., 2007) and in obese men performing cycling exercise (Ueda et al., 2009). In contrast, Holliday & Blannin, (2017)

observed no significant decrease in the subjective feeling of appetite when endurance-trained men completed a high-intensity aerobic exercise bout (cycling at 76% maximum oxygen uptake ($\dot{V}O_{2max}$)). This is consistent with other studies, following extended bouts of continuous exercise at 65–70% $\dot{V}O_{2max}$ in trained individuals (Deighton et al., 2014; King, Wasse, & Stensel, 2011; King et al., 2011; Wasse et al., 2012). The exercise-induced suppression of appetite may be different between athletic and non-athletic populations, with some research suggesting various levels of habitual physical activity may modify appetitive responses (Beaulieu et al., 2016; Blundell et al., 2015). However, no exercise-induced suppression of appetite following high-intensity cycling exercise (70% $\dot{V}O_{2peak}$) was observed with recreationally active men (Mattin et al., 2018). Conversely, the immediate decrease in appetite following a high-intensity exercise bout reported in previous studies (>70% $\dot{V}O_{2max}$), has not been found when performing exercise at lower intensities (<60% $\dot{V}O_{2max}$) (King et al., 2010; Imbeaultet al., 1997; Pomerleauet al., 2004; Unick et al., 2010). King et al., (2010) found no differences in perception of appetite between a brisk walking and control trial. Consistent with these findings, Ueda et al., (2009) reported no effect on appetite immediately following 60 min cycling at 50% $\dot{V}O_{2max}$.

The appetite suppression effect observed in high-intensity exercise (>70% $\dot{V}O_{2max}$) does not seem to provoke changes in macronutrient or energy intake later on in the day post-exercise, irrespective of the increased metabolic demands associated with high-intensity exercise compared to low-intensity exercise (<60% $\dot{V}O_{2max}$), especially when a meal is ingested 60 min post-exercise (Balaguera-Cortes et al., 2011; Jokisch et al., 2012; King, Wasse, & Stensel, 2011; King et al., 2010; King et al., 1994; King et al., 1997; Martins et al., 2007; Mattin et al., 2018; Schubert et al., 2013; Stensel et al., 2010; Thompson et al., 1988). One of the most typically used methods to assess energy intake is by *ad libitum* meals (Holliday & Blannin,

2017; King et al., 2010; King et al., 1997; Ueda et al., 2009). However, energy intake within *ad libitum* meal can be as high as ~5500 kJ, thus, providing this after exercise may be unrealistic and not provide a precise indication of compensatory effects post-exercise (King et al., 2013). Limitations to the existing literature are the acute measure of energy intake post-exercise. Research suggests that compensatory effects of energy intake can occur not only on the day of exercise but the day post-exercise, with Rocha et al., (2013) reporting an increase in food intake two days post-exercise. The effect of increased food intake may be dependent on physical activity status. For that reason, measuring energy intake not only over the remainder of the day of exercise but maybe over the following 48 h would be insightful (Holliday & Blannin, 2017).

Previous investigations have typically focused on orexigenic hormone acylated ghrelin, and the anorexigenic signals PYY, GLP-1, and PP in response to nutrient ingestion post-moderate-intensity exercise (Martins et al., 2007; Wasse et al., 2013). The effects of exercise on ghrelin and overall appetite regulation have been studied extensively with studies showing that aerobic exercise at intensities at or greater than 60% $\dot{V}O_{2max}$ suppresses circulating concentrations of acylated ghrelin (Broom et al., 2007; Broom, Batterham, King, & Stensel, 2008; Deighton, Barry, Connon, & Stensel, 2013; Holliday & Blannin, 2017; King et al., 2011; Vatansever-Ozen, Tiryaki-Sonmez, Bugdayci, & Ozen, 2011). Parallel to this decrease in ghrelin, an increase in satiety hormone concentrations such as GLP-1, PYY, and PP have been observed during aerobic exercise (Sliwowski, Lorens, Konturek, Bielanski, & Zoladz, 2001; Balaguera-Cortes et al., 2011; Kawano et al., 2013; Larsen et al., 2017; Martins et al., 2007; Ueda, Yoshikawa, Katsura, Usui, Nakao, et al., 2009; Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009). These hormonal fluctuations observed during and immediately post-exercise are typically short-lived, returning back to resting control levels within 60 min post-exercise

(Broom et al., 2007; King, Miyashita, et al., 2010; Martins et al., 2007; Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009; Ueda, Yoshikawa, Katsura, Usui, Nakao, et al., 2009). In contrast to these findings, Mattin et al., (2018) observed no significant differences for ghrelin, GLP-1, PYY, PP or insulin following a bout of low and high-intensity cycling (60 min at 40% and 70% $\dot{V}O_{2peak}$). However, the lack of differences may be due to the relativity small sample size (n=8) (Mattin et al., 2018). The hormonal fluctuation shown post-exercise during higher intensity exercise has not always been observed following low and moderate-intensity exercise (King et al. 2010; Imbeault et al., 1997; Thompson et al., 1988).

The observations discussed above on the secretion of some gut hormones in relation to exercise intensity may also affect the regulation of GER. Therefore, the role of gut-derived hormones in the regulation of gastric emptying has received significant attention. A reduction in GER has been observed during exercise exceeding 70% $\dot{V}O_{2max}$; this has been suggested to be due to a reduction in splanchnic blood flow (Evans et al., 2016). Previous investigations observed that GER of liquids is reduced during high-intensity exercise (70% $\dot{V}O_{2max}$) in comparison to steady-state moderate intensity exercise or rest (Costill & Saltin, 1974; Leiper, Broad, & Maughan, 2001; Leiper, Prentice, Wrightson, & Maughan, 2001; Matsuzaki et al., 2016). However, other previous investigations have found exercise at a moderate intensity to increase the rate of gastric emptying (Neufer et al., 1989). This could be due to the increase in intragastric pressure from the contractile activity of the abdominal muscles (Neufer et al., 1989). In contrast, Evans et al., (2016) found the intensity of exercise had minimal effect on gastric emptying rate following ingestion of a glucose solution. Similarly, Mattin et al., (2018) also observed no effect on the rate of gastric emptying of a semi-solid meal following exercise intensities at 40% and 70% $\dot{V}O_{2peak}$. However, most of the aforementioned studies measured gastric emptying during exercise (Costill & Saltin, 1974; Leiper, Broad, & Maughan, 2001;

Leiper, Prentice, Wrightson, & Maughan, 2001; Matsuzaki et al., 2016), whilst others measured gastric emptying after exercise in the rest period (Evans et al., 2016; Mattin et al., 2018). The findings on the effect of exercise on gastric emptying rate appear to be dependent on the intensity of exercise performed and the macronutrient content of the test meal provided. Typically, a slower emptying rate is observed during higher intensity and with increased nutrient content. It is also important to note that all these studies performed either cycling or sprint intervals as their mode of exercise. Thus, it would be interesting to investigate whether the gastric emptying rate is affected by other exercise modes and at different intensities than just the common exercise intensity of 40% or 70% $\dot{V}O_{2max.}$

1.6. Benefits of fasting

Fasting is defined as restraining from consuming food and/or energy intake over a period of time (Longo & Panda, 2016). There are various approaches to intermittent fasting interventions, typically categorised as, time-restricted feeding, alternate-day fasting (ADF) and modified fasting regimens, i.e. the 5:2 diet (Patterson et al., 2015; Templeman et al., 2018). Intermittent fasting has gained a lot of attention over recent years and has become increasingly popular, however, there is a lack of scientific studies to confirm if intermittent fasting should be encouraged from a weight management standpoint. The subsequent section will discuss the current understanding of intermittent fasting interventions, breakfast consumption versus breakfast skippers and fasting versus a non-fasted exercise in relation to overall appetite regulation and weight management.

1.6.1. Advantages of breakfast consumption versus non-breakfast consumption

Breakfast can often be described as the main or most significant meal of the day, with the theory that consuming breakfast will effectively set you up for the day ahead. Nutritionist

Adelle Davis famously stated in the 1960s: "Eat breakfast like a king, lunch like a prince and dinner like a pauper." (Spence, 2017). Previous investigations have later supported this theory with several studies reporting the important role of breakfast consumption for health-related quality of life (Ferrer-Cascales et al., 2018). Breakfast omission has previously been considered a factor of an unhealthy lifestyle that may serve for further health-risk behaviours, including smoking, alcohol consumption, reduced academic performance, a sedentary lifestyle, and also reduced motor-skills and depressive symptoms (Ferrer-Cascales et al., 2018; Keski-Rahkonen et al., 2003).

Breakfast skippers, typically defined as no dietary intake between 05:00 and 09:59 (Ahola et al., 2019), have been linked with chronic stress and an increased risk of developing cardio-metabolic disease (Neumark-Sztainer et al., 2008; Neumark-Sztainer & Hannan, 2000; Witbracht et al., 2015). Cahill et al., (2013) reported a 27% increase in coronary heart disease in men who frequently skipped breakfast. It has been recommended that we should aim to consume about 15–25% of daily calorie intake at breakfast (i.e. 300–500 calories for women and 375–625 for men (Spence, 2017), though there is cultural variation on what an individual may choose to consume for their breakfast (Patterson et al., 2015; Spence, 2017).

Many dietary recommendations support regular breakfast consumption for weight management (Sievert et al., 2019). These recommendations are typically based on the theory that breakfast omission would lead to overcompensation of energy intake later on in the day (Garaulet & Gómez-Abellán, 2014). However, previous studies have found that individuals that ate breakfast had a greater energy intake in comparison to individuals who skipped breakfast, irrespective of whether they were regular breakfast consumers or not (Astbury et al., 2011; Clayton et al., 2015; Farshchi et al., 2005; Yoshimura et al., 2017). It has been proposed that breakfast consumption can play a positive part in weight loss with the assumption that, due

to the expenditure of calories earlier in the day, this prevents overcompensating later in the day (Bo et al., 2015; Sievert et al., 2019). However, investigations assessing this theory have reported no significant difference in metabolic rates between those who consumed breakfast versus and breakfast skippers (Betts et al., 2014; Chowdhury et al., 2016; Farshchi et al., 2005; Thomas et al., 2015). In addition, a number of previous studies have also reported no significant differences between the breakfast consumers and breakfast-skippers for leptin (Betts et al., 2014; Chowdhury et al., 2016), ghrelin (Betts et al., 2014; Chowdhury et al., 2016; Thomas et al., 2015) and insulin concentrations (Astbury et al., 2011; Betts et al., 2014; Chowdhury et al., 2016; Clayton et al., 2015; Thomas et al., 2015). Thus, skipping breakfast may not lead to overcompensation of food later in the day (Sievert et al., 2019). Previous studies have found total daily energy intake was higher in those consuming breakfast compared to no breakfast, in both habitual breakfast eaters and skippers (Betts et al., 2014; Chowdhury et al., 2016; Levitsky & Pacanowski, 2013). Breakfast omission may be a positive strategy to decrease total energy intake, without causing a greater perception of hunger throughout the day.

Another theory is that individuals who consume breakfast would be more physically active throughout the day, thus, producing a negative energy balance by increasing energy expenditure compared to those who skip breakfast (Bo et al., 2015; Sievert et al., 2019). However, Thomas et al., (2015) and LeCheminant et al., (2017) have observed no significant differences in physical activity (PA) levels between those who consumed breakfast and those who skipped breakfast. In contrast, Betts et al., (2014) and Yoshimura et al., (2017) reported in lean indiviudals increased PA levels associated with breakfast consumption, mostly occurring in the morning, with total thermogenesis higher in the breakfast group compared to the non-breakfast group for daily PA.

The common belief that breakfast is the most important meal of the day, combined with the recommendations of breakfast consumption for weight management remains debateable. Much of the guidance available is based upon findings from observational studies (Bjornaråa et al., 2014; Cho et al., 2003; Purslow et al., 2008), increasing the risk of selection bias and confounding by socio-economic aspects and healthy regimes (Sievert et al., 2019). Regular breakfast consumers versus breakfast skippers may differ in many ways, including personal preferences, socioeconomic status, occupation (e.g., night / shift workers) and other health related behaviours. In addition, there is, presently, no universal definition recognised for breakfast (Betts et al., 2014; Spence, 2017; Templeman et al., 2018). The term "breakfast" can be defined differently by one person to the next, e.g. an individual may not consume their first meal until after noon, and although technically this meal is breaking their fast, which is where the term breakfast originates from, the individual may not recognise this as consuming breakfast (Betts et al., 2016). Previous evidence from randomised control trials demonstrate that breakfast consumption does not generally have beneficial effects on weight loss in obese humans (Chowdhury et al., 2016) or normal weight women (LeCheminant et al., 2017).

Therefore, inconsistencies within the literature make it challenging to understand whether breakfast consumption or breakfast skipping are either beneficial tools for weight management and appetite regulation, or whether it comes down to personal preference. For example, advising breakfast could adversely affect weight control by increasing daily calories intake to a person's day, especially in those who have established eating behaviours (e.g. older populations etc.)(Sievert et al., 2019). Therefore, to provide a more precise and informative understanding of the advantages or disadvantages of breakfast consumption for weight management and appetite regulation, much more work is required to account for various lifestyles factors, different population groups, and timing of consumption

1.6.2. Fasted exercise

Intermittent fasting has become an increasingly popular intervention for the purpose of weight loss. The majority of people conduct an 8-10 h fast; this period occurs when one is sleeping, 'overnight fasting' (Maughan et al., 2010). During this fasting period, ketone bodies, non-esterified fatty acids (NEFA), and glucose derived from liver glycogen and gluconeogenesis are the main energy sources (Cahill, 2006; Vieira et al., 2016). Therefore, if this overnight fast is extended and exercise is conducted, it is speculated that this could further promote fat metabolism, and therefore be an effective strategy for weight loss compared to consuming breakfast before exercise. This question has raised some attention over recent years with studies investigating the effect of fasted versus fed exercise on a variety of metabolic and appetite markers.

Previous studies have found an increase in GLP-1 levels during acute exercise in a fed state (Douglas et al., 2016; Hallworth, 2017), suggesting GLP-1 may play a vital part in the acute control of energy homeostasis involving consumption of breakfast and exercise (Gonzalez et al., 2013; Douglas et al., 2016). Gonzalez et al., (2013) investigated the effect of consuming breakfast prior to exercise compared to remaining fasted and conducting exercise, on post-prandial appetite, metabolism, and macronutrient balance in active men. The study reported that irrespective of consuming breakfast or remaining fasted, the exercise bout created a reduced positive energy balance after *ad libitum* lunch consumption. Recently, Edinburgh et al., (2019) investigated the effect of 24 h energy balance of breakfast versus no-breakfast prior to exercise. The study reported no compensatory effects post-exercise of energy expenditure following fasted exercise, suggesting that skipping breakfast prior to exercise can produce a greater negative energy balance. This may hold positive implications to create an energy deficit for the day.

Intervention studies have been carried out to investigate the effects of fasted versus fed exercise on body composition. Van Proeyen et al., (2010) investigated this on active men while maintaining an energy-matched hyper-caloric diet. The participants conducted a mixture of both cycling and running for 60–90 min at moderate intensity, four times per week for six weeks. After the intervention, the fed group resulted in a greater body mass by 1.4 ± 0.4 kg compared to the fasted exercise group. A following study from the same laboratory, using the same methods but with participants following an isocaloric diet, revealed no differences in body composition between the fasted and fed group post-test (Van Proeyen et al., 2011).

In addition to this, Gillen et al., (2013) carried out a study investigating the effect of fasted versus fed exercise on body composition in overweight/obese women. The participants performed high-intensity interval exercise three times per week for six weeks. The authors found no differences between groups in body composition between fasted and fed conditions from baseline but both intervention groups had lower body fat at the end of the six weeks. Similar findings were also observed in a study by Schoenfeld et al., (2014) who explored the effect of body composition in response to fasted versus fed exercise in healthy women who followed a hypocaloric diet and performed aerobic exercise three times per week four weeks. The study reported significant reductions in body composition in both groups but no differences between the two conditions.

Based on the current literature, it can be proposed that there are different benefits to conducting fasted and fed exercise. There appears there may be additional benefits of fasted exercise on weight loss and body composition, though not always, and the mechanisms are unclear. No studies have investigated fasted exercise on GER, which is an important factor in appetite regulation, thus, future studies are warranted.

1.6.3. Substrate utilisation

The nutritional state of an individual is an important determinant of substrate utilisation. Carbohydrate ingestion within the hours before an exercise bout reduces the rate of fat oxidation, whereas fasting prior to exercise (> 6 h) optimises fat oxidation (Coyle, 1995). The role of food intake prior to exercise has been well studied within the literature. Throughout exercise, NEFA play a substantial role in energy metabolism (van Hall, 2015). This is triggered by greater levels of adrenaline and lower levels of insulin within the blood (Jeukendrup, 2003; Vieira et al., 2016). Fasting stimulates low levels of insulin and hepatic glycogen (Maughan et al., 2010). Therefore, as an individual conducts aerobic exercise under fasting conditions, the energy required is predominantly provided by oxidation of plasma fatty acids (Aird et al., 2018; Coyle, 1995; Vieira et al., 2016). Carbohydrate intake pre-and during exercise increases exogenous substrate supply to the body cells, decreasing plasma fatty acid mobilisation and oxidation (Coyle, 1995; De Bock et al., 2005; Gregory et al., 2011; Horowitz et al., 1997). Carbohydrate ingestion causes an increase in plasma insulin concentrations, which acts as an inhibitory influence on adipose tissue triacylglycerol lipase and hormone-sensitive lipase (Spriet, 2014), inhibiting the breakdown of intramuscular TAG and reducing the availability of NEFA for oxidation (Achten & Jeukendrup, 2004; Coyle et al., 1985, 2017; Coyle et al., 1978; Spriet, 2014).

Aerobic exercise and resistance exercise in the fasted state has been known to increase fat oxidation. Frawley et al. (2018) found that resistance exercise in a fasted state relied more on fat metabolism than carbohydrate. In addition to this, investigations observed fat oxidation is increased at rest from 9 h (Burton et al., 2010) to 24 h (Shimada et al., 2013; Iwayama, Kawabuchi, et al., 2015; Iwayama, Kurihara, et al., 2015) after exercise when performed in the

fasted state compared to a fed state. This greater utilisation of fat as an energy source at rest observed within previous studies may stimulate adaptations for fat loss.

Reciprocal shifts in substrate utilisation correspond to exercise intensity; fat oxidation decreases, and carbohydrate oxidation increases in a linear manner to increased exercise intensity (Achten et al., 2002; Kang et al., 2007; Romijn et al., 2000). It is well-established that as exercise intensity gradually increases from low (25% $\dot{V}O_{2max}$) to moderate (65% $\dot{V}O_{2max}$) intensity, fat oxidation increases, whereas at higher intensities (85% $\dot{V}O_{2max}$), fat oxidation declines (Van Loon et al., 2001; Achten et al., 2003; Melanson et al., 2009). During high-intensity exercise, as the decline in fat oxidation occurs, carbohydrate becomes the primary fuel source to support high-intensity exercise workloads (Jeukendrup, 2014). The reason for decreased fat oxidation rates at higher exercise intensities has been unclear, with studies suggesting mechanisms which aid to explain the decreased fat oxidation rates during higher exercise intensities. For example, fatty acid oxidation is limited throughout the exercise to an extent by the decreased availability of plasma free fatty acids owing to a reduction in blood flow to adipose tissue, as well as, or a reduced capacity to produce adenosine triphosphate (ATP) from the oxidation of plasma free fatty acids (Achten et al., 2002; Achten & Jeukendrup, 2004; Melanson et al., 2009).

Performing aerobic exercise under fasted conditions is a recommended strategy to enhance fat oxidation throughout the exercise. Most studies investigating the effect of fasted exercise on fat oxidation have predominately focused on cycling, running and graded treadmill protocols, with typically trained populations. Therefore, research focusing on low to moderate exercise intensity exercise in the general population is required to clarify the acute and chronic adaptations to fasted versus fed exercise on substrate utilisation.

1.7. Objectives of this Thesis:

The aims of this research thesis were to assess the GI function, metabolic and appetite responses to fasted and non-fasted exercise. This was achieved through the following research study objectives:

1) To assess the effect of fasted versus non-fasted brisk walking on appetite-regulatory hormones, metabolic responses and GER of a subsequent meal post-exercise in healthy men

2) To assess the influence of diurnal variation of fasted and non-fasted state brisk walking on metabolic responses, appetite and GER of a subsequent meal and 24 h post energy intake in healthy men

3) To determine the influence of diurnal variation of fasted versus non-fasted cycling on appetite-regulatory hormones, metabolic responses and GER of a subsequent meal in lean men.

4) To determine the influence of diurnal variation of fasted versus non-fasted cycling on appetite-regulatory hormones, metabolic responses and GER of a subsequent meal in overweight men.

2.0. GENERAL METHODS

2.1. Participant criteria and ethical approval

All participants were male, had no history of respiratory, cardiovascular, or chronic gastrointestinal disease, and not taking regular medication as assessed by a medical screening questionnaire. Female participants were excluded due to evidence suggesting that periodic changes in sex hormones during different phases of the menstrual cycle can cause variations in appetite regulatory hormones, and energy intake (Brennan et al., 2011; Brennan et al., 2009; Lissner et al., 1988; Wade & Jones, 2004). All participants were free from musculoskeletal injury, non-smokers, and were not dieting. Participants were also normotensive (systolic blood pressure $\leq$ 140 mmHg and diastolic blood pressure $\leq$ 90 mmHg). Participants were not involved in shift work and did not report any disturbances to their normal sleep-wake cycle during the week prior to data collection. This was ensured by participants recording a 7-day habitual sleep diary leading up to each trial, and the midpoint of sleep (half-way point between sleep and wake time) was calculated. Participants were also classified as moderate or intermediate chronotypes according to the Munich Chronotype Questionnaire by Roenneberg, Wirz-Justice, Merrow (2003) (Appendix A). This ensured the exclusion of participants with an early diurnal phase, also known as extreme morning chronotypes and extreme evening chronotypes, since it is known that morning and evening types differ in the phase of their endogenous circadian rhythms (Roenneberg, Wirz-Justice, Merrow, 2003). Participants also completed the Pittsburgh Sleep Quality Index questionnaire (Buysse et al., 1989) (Appendix B), and the Epworth Sleepiness Scale questionnaire (Murray, 1991) (Appendix C).

All participants completed a physical activity (PA) habits questionnaire. The questionnaire was adapted from the General Practice Physical Activity Questionnaire (GPPAQ) which is a validated screening tool used in main care to evaluate the PA levels of

adults (16 to 74 years) (Departmental of Health, 2013) (Appendix H). This was to ensure participants were recreationally active men. This questionnaire is designed to understand the participants' habitual PA routines in their everyday life. For example, the amount of PA conducted on average per week, occupation e.g. office job/physical labour etc., at what part of the day would they typically perform an exercise. The amount of time spent conducting PA on an average per week were calculated and presented as number of minutes within the participant section with each experimental chapter.

All participants were informed of the details of the study procedures, both verbally and in writing prior to providing their written informed consent. Individual sample size estimates were performed using G*Power 3.1 (Faul et al., 2007) (see individual experimental chapter methods for specific details). All power calculations were performed with 80% power, and significance level set at 5%. The effect sizes were considered as low, medium, or high using Cohen's (1988) criteria. Data for GER and fat oxidation were used in all sample size estimates, specific details of which can be found in each specific chapter. Additional variables were also used in various chapters and are detailed in the methods of the experimental chapters. Overall, each study recruited twelve subjects. All studies within the thesis were granted approval by the Science and Engineering Research Ethics and Governance Committee of Manchester Metropolitan University prior to commencement (Appendix D to G).

2.2. Preliminary trial

All participants conducted a preliminary trial visit at least 7 days prior to their first experimental trial before each study. Anthropometric measures of height, weight, body fat percentage were made, as well as familiarisation of the breath sampling (gastric emptying breath sampling and expired air) testing procedures. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer, and body mass were measured to the nearest 0.01 kg

using electronic scales (GFK 150; Adam Equipment Co. Ltd., Milton Keynes). Body fat percentage was approximated using bioelectrical impedance analysis (Omron BF306; Kyoto, Japan). Blood pressure was measured using a digital sphygmomanometer (Omron M2, Kyoto, Japan). Following this, all participants completed a $\dot{V}O_{2peak}$ test.

For the studies reported in Chapters 3 and 4 involving brisk walking exercise, participants completed a $\dot{V}O_{2peak}$ test on a motorised treadmill. Initially, the treadmill speed was adjusted until a suitable brisk walking pace was determined. Participants were advised that brisk walking is defined as an exercise intensity yielding a mild shortening of breath yet still enabling to converse. Participants maintained this speed for 5 min. The speed of the treadmill was then increased to 8–12 km/h and the gradient increased by 2.5% every 3 min until volitional exhaustion. For the studies in chapters 5 and 6 involving cycling exercise, participants completed a $\dot{V}O_{2peak}$ test on a cycle ergometer (Corival cpet, Lode).

The protocol initially commenced with a 5 min warm-up with workload set on 0 watts, followed by increments of 1 watt every 2 seconds until volitional exhaustion. In all studies, expired air was continuously analysed using a breath by breath gas analyser (Chapters 3 and 4, Metalyzer 3b, Cortex, Leipzig, Germany; Chapters 5 and 6, Oxycon Pro, CareFusion, Leipzig, Germany). $\dot{V}O_{2peak}$ was calculated by averaging the highest oxygen volume consumed over the final 1 min period. Heart rate was measured continuously using a heart rate monitor (Polar H7, Kempele, Finland) and participants rating of perceived exertion (RPE) (Borg, 1982) was recorded every 3 min.

2.3. Pre-trial standardisation

In the 24 h preceding each experimental trial, participants were asked to refrain from alcohol and caffeine ingestion as well as the performance of the strenuous physical activity. Participants were asked to record their physical activity and food intake in the 24 h before the

start of their first experimental trial. Participants were then asked to replicate their activity and diet the day preceding their subsequent trials. All trials were separated by at least 7 days to provide enough time to allow ^{13}C levels in breath to washout, avoid carry-over effects between trials and for adequate recovery for intravenous (IV) cannulation. Participants were also asked to attend the laboratory in a fasted state, except for drinking 500 mL of water approximately 90 min prior to arrival at the laboratory. This was to ensure euhydration upon arrival and a consistent level of hydration status.

2.4. Experimental trials

2.4.1 Randomisation

All studies within the current thesis were conducted in a randomised crossover fashion. Randomisation of trials for all studies was conducted using the randomisation tool on Microsoft Excel (Kim & Shin, 2014). This was achieved by inputting the total of participants that was required to be recruited into the studies (total of 12 participants). The order of trials was randomised for each participant number to reduce the likelihood of order effects or bias from the investigator (Schulz and Grimes, 2002). Data was subsequently analysed to determine the success of randomisation within each experimental chapter (results of which are presented in each experimental chapter). As subjects were recruited, they were assigned a participant number in numerical order and conducted the order of trials that was generated. Due to the nature of the studies (fasted versus non-fasted exercise and food preparation) blinding of trials for both investigator and participant was not possible. Participants did not know which condition they were undertaking until the moment of the 'breakfast' meal period (except for the final trial by deduction).

2.4.2 Food choice

During all experimental trials, the choice of breakfast was cereal, and lunch was soup. All participants consumed the same amount of food during the trials. This approach was used to ensure consistency between participants within experimental chapters. Cereal was chosen instead of other breakfast food items due to the popularity and recognisable role of cereal supporting a balanced diet in the UK (Mckevith and Jarzebowska, 2010; Mckevith, 2004). The actual amount provided for the breakfast meal was determined by the manufacturer's recommendation of an average serving. The choice of soup for a lunch meal within all chapters as supposed to another lunch option was due to the semi-solid nature of this food, this allows gastric emptying rate to be measured using the ^{13}C breath test described below. The amount and type of food was changed from chapter 4 and details for this can be found in the experimental chapters.

2.4.3. Gastric emptying measurement and analysis

The assessment of GER was achieved using the ^{13}C breath test method. This method was deemed appropriate due to its non-invasiveness, high-validity and reliability of the repeated measurement of gastric emptying (Braden et al., 2007). The test meal provided for the measurement of GER within all studies was a semi-solid meal (soup) which contained 100 mg of ^{13}C-sodium acetate (Cambridge Isotope Laboratories, Inc). The ^{13}C sodium acetate breath test method is reliable with liquids and semi-solid test meals to measure gastric emptying (Braden et al., 1995). A basal end-expiratory breath sample was collected pre-meal ingestion then at 15 min intervals post-meal ingestion for a total of 2 h following food ingestion. On each occasion, breath samples were collected into a 100 mL foil bag by exhalation through a one-way valve mouthpiece (Wagner Analyzen-Technik, Bremen, Germany). Bags were then sealed

with a plastic stopper and stored for later analysis. Breath samples were analysed for the ratio of $^{13}CO_2$:$^{12}CO_2$ by non-dispersive infra-red spectroscopy (IRIS Dynamic, Kibion, Germany). The difference in the ratio of $^{13}CO_2$:$^{12}CO_2$ from baseline breath to post-ingestion breath samples are expressed as delta over baseline (DOB). Half-emptying time ($T_{½}$) and time of maximum emptying rate (T_{lag}) were calculated utilising the manufacturers integrated software evaluation incorporating equations of a previously described formula (Ghoos et al. 1993).

2.4.4. Substrate utilisation

Expired air was collected using a breath-by-breath gas analyser Oxycon Pro (CareFusion, Leipzig, Germany) or Metalyzer 3b (Cortex, Leipzig, Germany) at regular intervals throughout all trials. Samples were collected for 10 min at each measurement point during resting phases, and continuously throughout exercise bouts, with the last 5 min of each 15 min segment used to calculate substrate utilisation. The average maximum oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) measurements from the last 5 min of expired air collection were used to calculate fat and carbohydrate oxidation rates using stoichiometric equations (Péronnet and Massicotte, 1991).

2.4.5. Appetite assessment

Appetite was assessed using 100 mm visual analogue scales (VAS) from Flint, Raben, Blundell & Astrup, (2000). Ratings of hunger, fullness, prospective food consumption as well as ratings of food satisfaction, bloated were collected. The VAS scale included questions of "how hungry do you feel?", "how full do you feel?", "how much do you think you can eat?", "how satisfied do you feel? "and "how bloated do you feel?". The horizontal lines were linked with "I am not hungry at all - I have never been more hungry", "not at all full - totally full", "nothing at all - a lot", "not at all bloated - very bloated", "not at all nauseous - very nauseous", and "not satisfied at all – very satisfied", respectively (Flint et al., 2000).

2.4.6. Blood sampling and analysis

Venous blood samples were collected in the studies reported in chapters 3, 5 and 6 within this thesis. An intravenous cannula (BD Venflon™, 20G, Becton Dickinson Infusion Therapy, Helsingborg, Sweden) was inserted into an antecubital vein which remained in place for the duration of the trial. The cannula was kept patent with the infusion of isotonic saline after each sample collection. Blood samples were collected at baseline, post breakfast period, pre-exercise, immediately post-exercise, pre-semi-solid meal ingestion, then every 30 min post- semi-solid meal ingestion. To prevent the degradation of acylated ghrelin and active GLP-1, 50 µL of Pefabloc (Roche Diagnostics Limited, Burgess Hill, UK) and 50 µL of DPP-IV (Merck Millipore Ltd., Feltham, UK) was immediately added to blood samples and were kept on ice until all samples were collected. Blood samples were centrifuged at 1500× *g* for 15 min at 4˚C and the serum aliquoted and stored at −80˚C until analysis. Serum glucose, NEFA, triglycerides and total cholesterol concentrations were determined in duplicate using a clinical chemistry analyser (Randox Daytona, Crumlin, UK). Circulating concentrations of acylated ghrelin, active GLP-1 (both GLP-1^{7-36} and GLP-1^{7-37}), total PYY, PP, and insulin were determined in duplicate using multiplex analysis (Luminex 200, Luminex Corporation, Austin, TX, USA) with kits purchased from Merck-Millipore (HMHMAG-34K, Milliplex MAP, Merck Millipore Ltd., Feltham, UK). Intra-assay and inter-assay coefficient of variation (CV) for these analyses are presented in Table 1.

Table 1: Mean intra-assay coefficient of variation (CV) and mean inter-assay CVs for blood serum concentrations of metabolites and gut hormones within experimental studies collecting serum samples.

Intra-assay CVs (%)			
	Chapter 3	Chapter 5	Chapter 6
Ghrelin	8.7	5.2	4.9
GLP-1	11.2	8.9	8.9
Insulin	11.3	6.2	7.0
PP	10.6	8.2	8.2
PYY	7.8	* Insufficient analysis *	7.4
Glucose	1.1	1.1	1.0
Triglycerides	1.4	1.2	1.1
NEFA	3.6	3.8	4.2
Cholesterol	1.1	1.0	1.1
Inter-assay CVs (%)			
Ghrelin	12.5	19.9	18.1
GLP-1	14.7	16.5	17.0
Insulin	17.1	10.9	15.2
PP	17.9	14.9	14.9
PYY	17.6	* Insufficient analysis *	15.9
Glucose	8.3	11.3	15.8
Triglycerides	14.6	19.6	14.7
NEFA	17.4	16.6	14.2
Cholesterol	16.6	17.3	15.2

2.4.7. Salivary melatonin sampling and analysis

Melatonin concentrations were assessed in studies reported in chapters 4, 5 and 6 by the collection of a saliva sample, which was collected at the beginning of all trials by the passive drool method. This method required participants to pool saliva in his mouth and then drool (rather than spit) through a collection aid into the collection tube (5016.02-SAL, Salimetrics Europe Ltd, Newmarket, Suffolk, UK). Saliva samples were immediately stored at −80°C until analysis. On the day of analysis, saliva samples were thawed, vortexed, and then micro-centrifuged at 1500 × g for 15 min. Melatonin concentrations were determined in duplicate using ELISA (Kit assay #1-3402, Salimetrics, State College, PA, USA). Saliva sampling is deemed a reliable method to measure melatonin levels (Mirick & Davis, 2008). Mean intra-assay CV for melatonin samples was 3%, 4%, 5% and mean inter-assay CVs 10%, 11% and 14% for experimental chapters 3, 5 and 6, respectively.

3.0. THE EFFECT OF BRISK WALKING IN THE FASTED VERSUS FED STATE ON METABOLIC RESPONSES, GASTROINTESTINAL FUNCTION, AND APPETITE IN HEALTHY MEN[1]

[1]The data within the following study was published in "McIver VJ, Mattin L, Evans GH, Yau AMW. 2018. The effect of brisk walking in the fasted versus fed state on metabolic responses, gastrointestinal function, and appetite in healthy men. Int J Obes (Lond). 43(9), pp.1691-1700." https://www.nature.com/articles/s41366-018-0215-x

Preliminary data was also presented as an oral communication at the 4th UK Congress of Obesity conference, University of South Wales, Sept 7-8th 2017.

3.1. Introduction

Fasted exercise has become increasingly popular over recent years, with the belief that fasted exercise is optimal for weight management (Bachman et al., 2016). This is due to the evidence of fasted exercise utilising more fat compared to non-fasted exercise (Bachman et al., 2016). Previous studies have reported fasted exercise increases ghrelin levels (Cheng et al., 2009) while reducing PYY levels (Cheng et al., 2009), appetite (Cheng et al., 2009; Deighton et al., 2013) and 24 h EI (Bachman et al., 2016), and providing beneficial metabolic adaptations (Van Proeyen et al., 2011). However, some studies have found no differences between fasted or non-fasted exercise for GLP-$1^{(7–36)}$ and insulin responses (Gonzalez et al., 2013), as well as for improving metabolic responses and body composition (Gillen et al., 2013; Schoenfeld et al., 2014). Some of the aforementioned studies also used a high-fat (70%) meal (Cheng et al., 2009), which is not representative of a typical breakfast, compared meal-exercise sequence rather than omission of breakfast per se (Borer et al., 2005), or have not measured appetite hormone responses (Schoenfeld et al., 2014; Van Proeyen et al., 2011). Many studies have also employed the method of participants exercising following an overnight fast with no comparative postprandial condition (Broom et al., 2007, 2009; King et al., 2010, 2011; Vatansever-Ozen et al., 2011; Kawano et al., 2013; Tiryaki-Sonmez et al., 2013; Douglas et al., 2015).

The majority of studies that have measured appetite hormones in relation to fasted versus non fasted exercise, typically only measure one or two hormones, with ghrelin, GLP-1, and PYY being the most common (Broom et al., 2007, 2009; King et al., 2010, 2011; Vatansever-Ozen et al., 2011; Deighton et al., 2013; Kawano et al., 2013; Tiryaki-Sonmez et al., 2013; Douglas et al., 2015). In addition, the difference in exercise modes and study designs implemented, make it difficult to make comparisons. Therefore, measuring a wider number of

appetite hormones within the same study along with appetite, following an acute bout of fasted versus fed exercise is required. This can provide a greater insight of whether fasted exercise may be a useful strategy for weight loss.

The effect of fasted exercise on GER may be an important mechanistic consideration for subsequent food and EI. The regulation of appetite and GI motility appears to be intrinsically linked since the rate of gastric emptying determines the time of gastric distention, which is known to be a satiety signal (Horner et al., 2011; Horner et al., 2015). Therefore, GER may also play a role in metabolic health as the delivery and absorption of nutrients in the small intestine is largely dependent on this process. Consequently, the influence of fasted exercise on GI function is also of interest in postprandial metabolic responses. A previous study has shown postprandial glucose response to be less following fasted exercise (Gonzalez et al., 2013), which could be due in part to differences in GER. No studies have, yet, investigated fasted exercise on GER and the consequence this may have on metabolic responses.

Furthermore, the studies discussed above have typically recruited trained populations, performing a high-intensity exercise that requires exercise equipment, which may not be accessible to all. These factors make it difficult to generalise the results to the wider population. Brisk walking is the most popular modality of physical activity undertaken on a general population level (Public Health England, 2017), due to it being easily accessible and with no requirement for specialised equipment. Investigating whether fasted brisk walking may have more favourable benefits on appetite regulation compared with non-fasted walking is of interest. Therefore, the aim of this study was to assess the effect of brisk walking in the fasted versus fed state on GER and associated metabolic and appetite hormone responses. It was hypothesised (a) that GER is slower following fed exercise (b) that fasted exercise will result in a greater metabolic response without any compensatory effects in appetite following the

lunch meal and (c) that these variations correlate with respective responses in appetite hormones.

3.2. Method

3.2.1. Participants

Twelve recreationally active men volunteered to participate in this study. A sample size estimate was performed based on gastric emptying data from Achour et al., (2001). The effect size was 0.7 and considered to be medium, the sample size required with this effect size was approximately N = 6. Fat oxidation data from Iwayama et al., (2015) revealed an effect size of 0.8, which is large, requiring a sample size of N = 10. Ghrelin data from King et al., (2011) revealed a large effect size of 0.8, with a sample size of N = 12 required. Thus, the sample size of 12 was decided to be adequate for the main objective of this study. Participant characteristics are presented in Table 2.

Table 2: Participants characteristics.

	Participants (*n = 12*)
Age (years)	26 ± 5
Height (cm)	179 ± 6
Body mass (kg)	86 ± 14
BMI ($kg \cdot m^{-2}$)	27 ± 4
Body fat (kg)	20 ± 11
Fat free mass (FFM) (kg)	64 ± 6
Body fat (%)	20 ± 3
Fat free mass (FFM) (%)	64 ± 6
$\dot{V}O_{2peak}$ (ml/kg/min)	39 ± 6
Physical activity levels per week (min)	153 ± 49
Systolic Blood Pressure (mmHg)	129 ± 13
Diastolic Blood Pressure (mmHg)	84 ± 15

Values are presented at mean ± SD

3.2.2. Experimental trials

Participants completed two experimental trials in a randomised crossover fashion. Each trial commenced in the morning between 08:00 and 09:00 h and trials were separated by at least 7 days. Participants were required to fast from 20:00 h the night before experimental trials except for plain water consumption. Baseline assessments of appetite, substrate utilisation and a blood sample were collected using procedures outlined in general methods.

Following baseline measurements, participants ingested the test breakfast (FED) or remained fasted (FASTED) within a 15 min period. The breakfast consisted of 30 g of breakfast cereal (Special K, Kellogg's) with 125 mL of semi-skimmed milk. This amount was chosen based on the manufacturer's recommendation of an average serving and provided 733 kJ (175 kcal), 2.75 g fat, 30 g carbohydrate and 7.2 g protein. Post breakfast ratings of appetite and substrate utilisation were measured at the end of the 15 min breakfast period. Participants then

rested in a semi-supine position on a bed for 1 h before commencement of the exercise protocol. During this 1 h rest period, further measures of appetite were taken every 15 min and substrate utilisation every 30 min.

The exercise protocol involved 45 min of brisk walking on a level motorised treadmill at a speed determined in the preliminary trial as described in the general methods. The speed range was between 5.7–6.6 km/h, average speed was 6.1 ± 0.4 $km \cdot h^{-1}$ and the relative exercise intensity was 50 ± 0.8% $\dot{V}O_{2peak}$. Heart rate and RPE were measured every 15 min throughout the exercise, with expired air analysed continuously. Participants recovered for 30 min before they ingested a standardised lunch meal. The meal was 400 g (one can) of chicken and sweetcorn soup (1013 kJ (242 kcal)), containing 11.8 g fat, 25.1 g carbohydrate, 8.2 g protein. Subjective feelings of appetite and substrate utilisation were measured every 15 min post-exercise for a total period of 2 h. Further blood samples were also collected post breakfast, pre-exercise, immediately post-exercise, pre-soup ingestion, then every 30 min post-soup ingestion. A schematic diagram of the experimental protocol is presented in Figure 2.

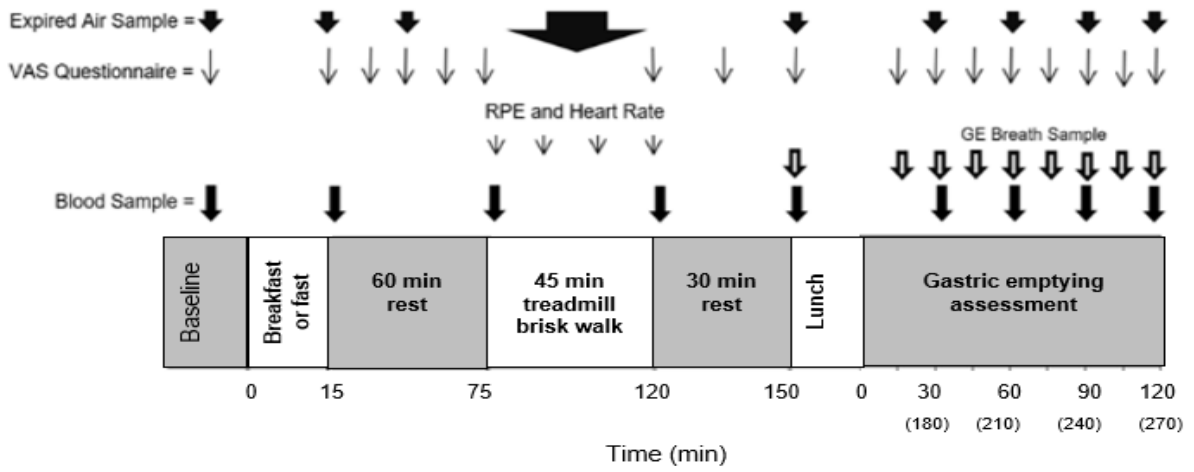

Figure 2: Schematic diagram of the experimental trial protocol. RPE, rating of perceived exertion. GE, gastric emptying.

3.2.3. Statistical Analysis

A two-way (trial x time) repeated measures analysis of variance (ANOVA) was used to assess differences between serum blood measures, gastric emptying delta over baseline (DOB), substrate oxidation and VAS ratings. Sphericity for repeated measures was assessed, and where appropriate, Greenhouse–Geisser corrections were applied for epsilon < 0.75, and the Huynh–Feldt correction adopted for less-severe asphericity. Significant F tests were followed by dependent Student's t-tests or one-way repeated ANOVA and Bonferroni adjusted pairwise comparisons as appropriate. Gastric emptying $T_{½}$ and T_{lag} data were analysed using dependent Student's t-test. Incremental area under the curve (iAUC) was calculated using the Time Series Response Analyser (TSRA) created by Narang et al., (2020). A one-way repeated measures ANOVA was also implemented to assess differences in incremental area under the curve (iAUC) for serum blood measures, gastric emptying DOB, substrate oxidation and VAS ratings. A Pearson Correlation was calculated to examine the relationship between BMI and body fat percentage against gastric emptying half time ($T_{1/2}$ and lag phase (T_{lag}). All analyses were carried out using IBM SPSS statistics (v22.0 for Windows; SPSS, Chicago, IL). The level of significance was set at $P < 0.05$. Descriptive data are expressed as mean ± standard deviation (SD).

3.3. Results

3.3.1. Trial order analysis

Analysis demonstrated that there were no significant differences in responses according to the order of trials that the participants completed (all P>0.05) (Table 3).

Table 3: Trial order analysis, mean ± SD for gastric emptying half time (T1/2), and incremental area under the curve (iAUC) for subjective feeling of hunger, and glucose concentrations.

Variable	Trial 1	Trial 2	P value
Gastric emptying $T_{1/2}$ (min)	89 ± 21	88 ± 23	0.862
Subjective feeling of hunger (mm^{-1} x 270 min)	1977 ± 1656	1794 ± 2284	0.809
Glucose concentrations ($mmol/L^{-1}$ x 270 min)	97 ± 65	105 ± 69	0.766

3.3.2. Gastric emptying rate

No trial × time interaction effect (P = 0.341) or main effect of trial (P = 0.332) was detected for DOB, although, a main effect for time was found (P < 0.001) (Figure 3a) iAUC for gastric emptying DOB was not different between trials (P = 0.328) (Figure 3b). No differences between trials (FASTED v. FED) were observed for gastric emptying T_{lag} (55 ± 15 vs. 54 ± 14 min; P = 0.704), and $T_{½}$ (89 ± 22 vs. 89 ± 24 min; P = 0.868) (Figure 4a & 4b).

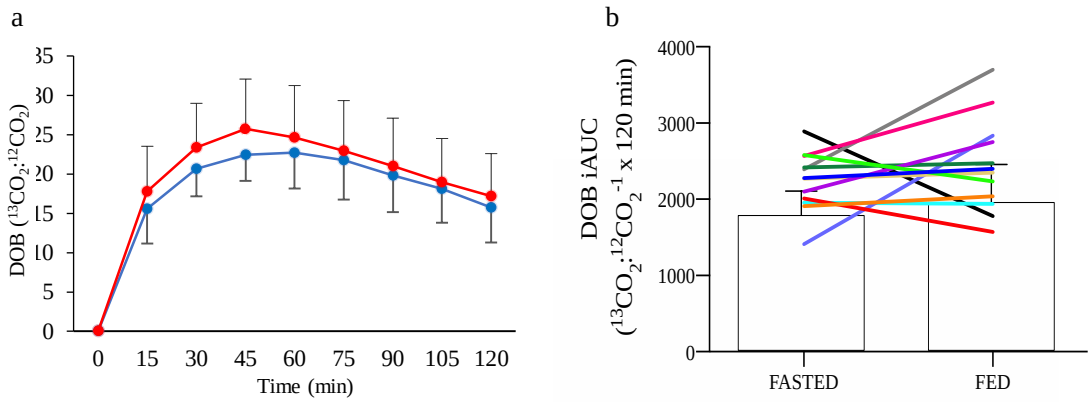

Figure 3: Gastric emptying assessment, a) Delta over baseline (DOB), b) incremental area under the curve (iAUC) for DOB of a standardised lunch (400 g chicken and sweetcorn soup) following 45 min of brisk walking either fasted (FASTED) or after breakfast consumption (FED). Values are mean ± SD; n=12.

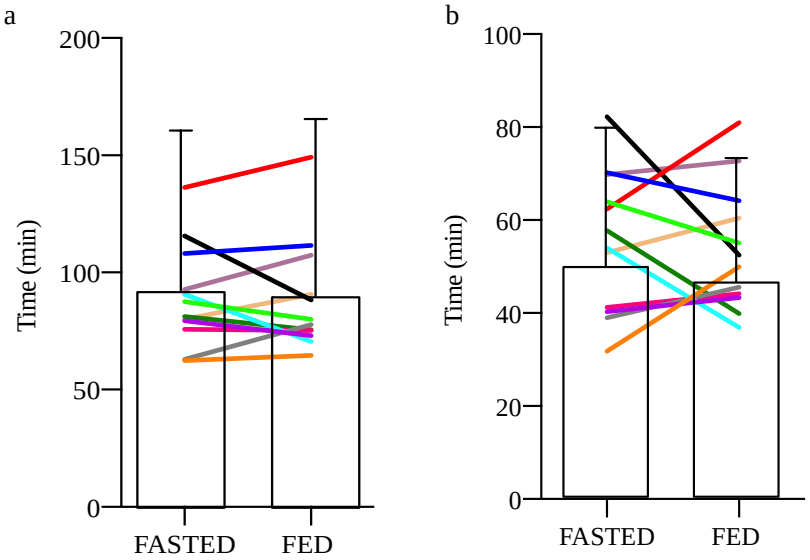

Figure 4: Gastric emptying a) half time (T½) and b) time of maximal emptying rate (Tlag) of a standardised lunch (400 g chicken and sweetcorn soup) following 45 min of brisk walking either fasted (FASTED) or after breakfast consumption (FED). Values are mean ± SD; n=12

3.3.3. Gut hormones

No main effect of trial, main effect of time or trial × time interaction effect was seen for ghrelin concentrations (P = 0.192, P = 0.134, P = 0.110, respectively; Figure 5a) or PYY concentrations (P = 0.929, P = 0.382, P = 0.839, respectively; Figure 5b). For GLP-1, there was a main effect of trial (P = 0.017) but no main effect of time (P = 0.351) or trial × time interaction effect (P = 0.104) (Figure 5c).

A main effect of trial (P = 0.019), a main effect of time (P = 0.008) and a trial × time interaction effect was observed for PP (P = 0.012) (Figure 5d). PP concentrations were higher during FED compared with FASTED pre-exercise at 75 min (23.42 ± 17.57 vs. 10.99 ± 2.76 pmol/l; P = 0.036), post exercise at 120 min (95.11 ± 99.70 vs. 29.37 ± 42.73 pmol/l; P = 0.016), 30 min post exercise at 150 min (30.96 ± 24.41 vs. 14.60 ± 13.82 pmol/l; P = 0.017), and 30 min post soup consumption at 180 min (67.35 ± 44.18 vs. 42.96 ± 27.11 pmol/l; P = 0.010).

No main effect of trial (P = 0.529) but a main effect of time (P < 0.001) and a trial × time interaction effect (P < 0.001) was seen for insulin concentrations. Insulin concentrations were higher during FED compared with FASTED immediately post breakfast at 15 min (139.56 ± 86.54 vs. 81.59 ± 55.91 pmol/l; P = 0.010) and pre-exercise at 75 min (119.11 ± 65.28 vs. 74.18 ± 58.56 pmol/l; P = 0.001). Insulin concentrations increased in the FASTED trial greater than the FED trial 1.5 h post soup consumption at 240 min (86.04 ± 48.18 vs. 69.09 ± 49.29 pmol/l; P = 0.006) (Figure 5e).

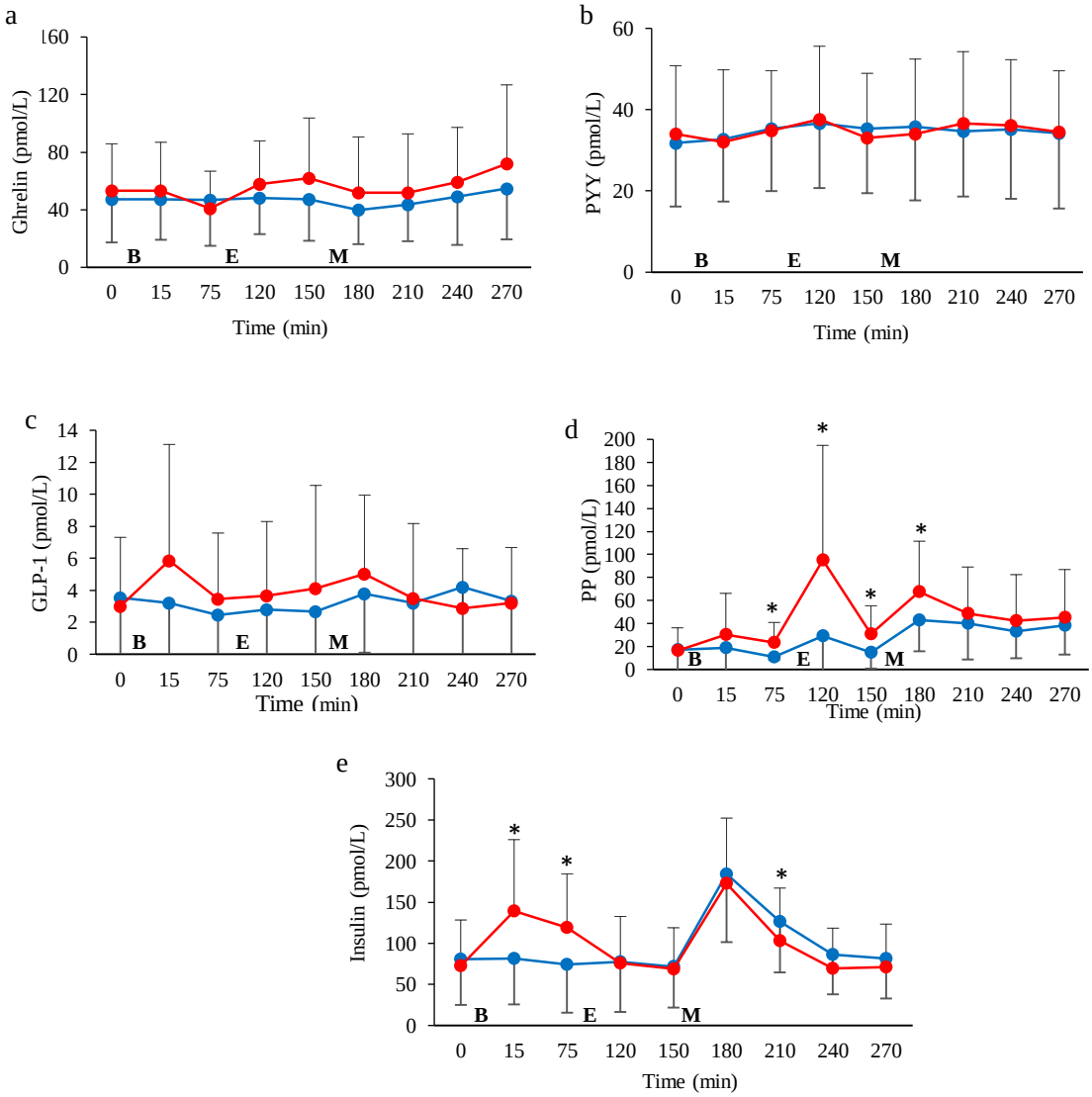

Figure 4: Hormonal responses during both trials. Serum concentrations of (a) Ghrelin (n=12), (b) Peptide tyrosin tyrosin (PYY) (n=10), (c) Glucagon like peptide-1 (GLP-1) (n=10), (d) Pancreatic polypeptide (PP) (n=11), and (e) Insulin (n=12). Values represent mean ± SD. The blue line corresponds to FASTED and red line = FED. *P<0.05 versus corresponding time point in other trial. '**B**' indicates breakfast period, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, '**E**' indicates exercise period, where participants completed a 45 min brisk walk and '**M**' indicates standardised lunch meal where 400 g chicken and sweetcorn soup was ingested.

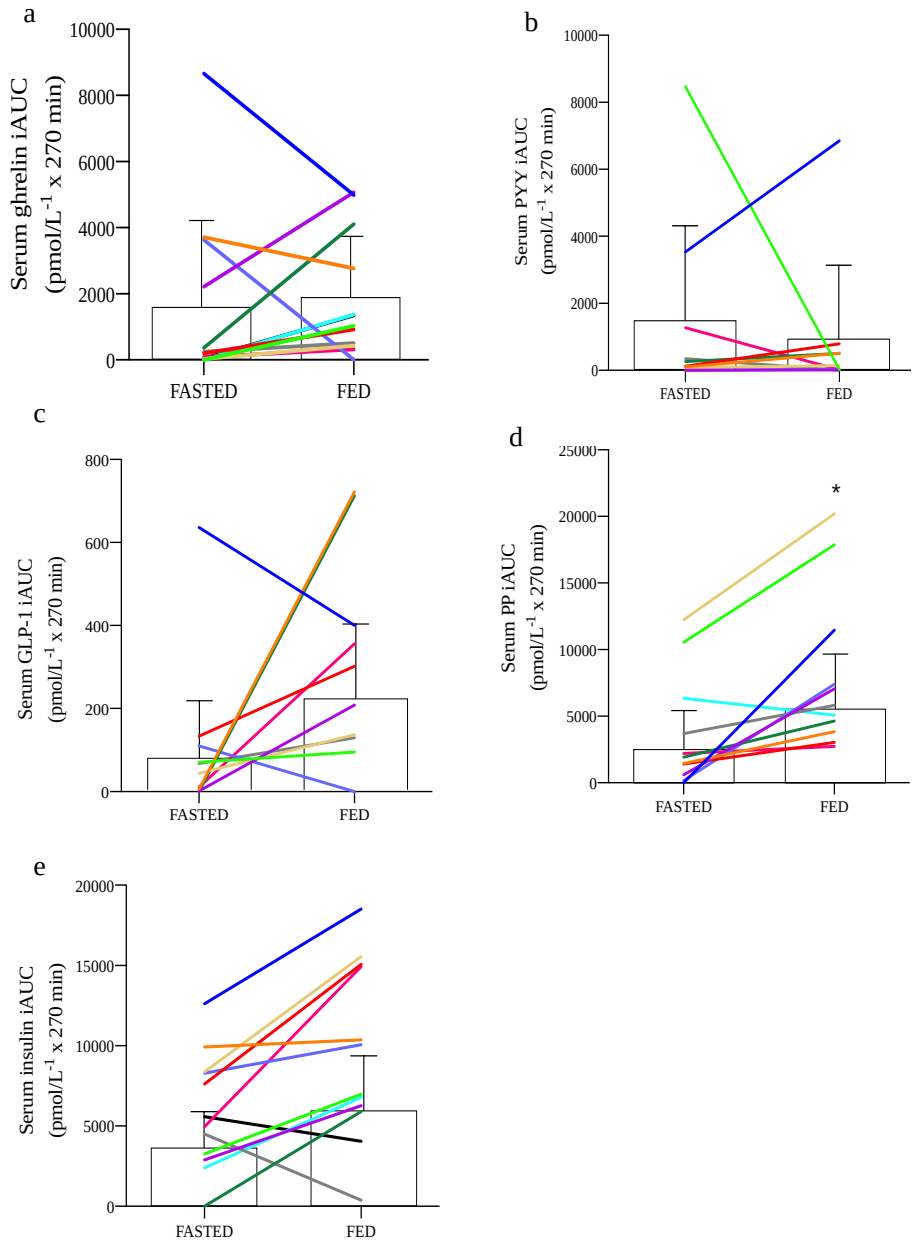

Figure 5: Hormonal responses during both trials, FASTED and FED. Incremental area under curve (iAUC) serum concentrations of (a) Ghrelin (n=12), (b) Peptide tyrosin tyrosin (PYY) (n=10), (c) Glucagon like peptide-1 (GLP-1) (n=10), (d) Pancreatic polypeptide (PP) (n=11), and (e) Insulin (n=12).Values represent mean ± SD. *P<0.05 versus corresponding trial.

3.3.4. Metabolic markers

No main effects of trial or trial x time interaction effects were seen for blood glucose concentration ($P = 0.128$ and $P = 0.217$; Figure 7a) and cholesterol concentration ($P = 0.930$ and $P = 0.383$; Figure 7b). No main effect of trial ($P = 0.729$) or trial x time interaction effect ($P = 0.056$) was seen for triglycerides concentration (Figure 7c). Main effects of time were seen for blood glucose, triglycerides and cholesterol (all $P < 0.001$).

A main effect of trial ($P = 0.037$), time ($P < 0.001$) and trial x time interaction effect ($P < 0.001$) was seen for NEFA concentration (Figure 7d). NEFA concentrations in the FASTED trial were greater post breakfast period (pre-exercise) at 75 min (0.39 ± 0.26 vs. 0.09 ± 0.06 mmol/L; $P = 0.003$), immediately post exercise at 120 min (0.90 ± 0.48 vs. 0.54 ± 0.32 mmol/L; $P = 0.004$), and pre-lunch ingestion at 150 min (0.56 ± 0.22 vs. 0.42 ± 0.19 mmol/L; $P = 0.009$). However, NEFA concentrations were higher in the FED trial compared with the FASTED at 1.5 h post soup consumption (0.32 ± 0.27 vs. 0.16 ± 0.11 mmol/L; $P = 0.018$).

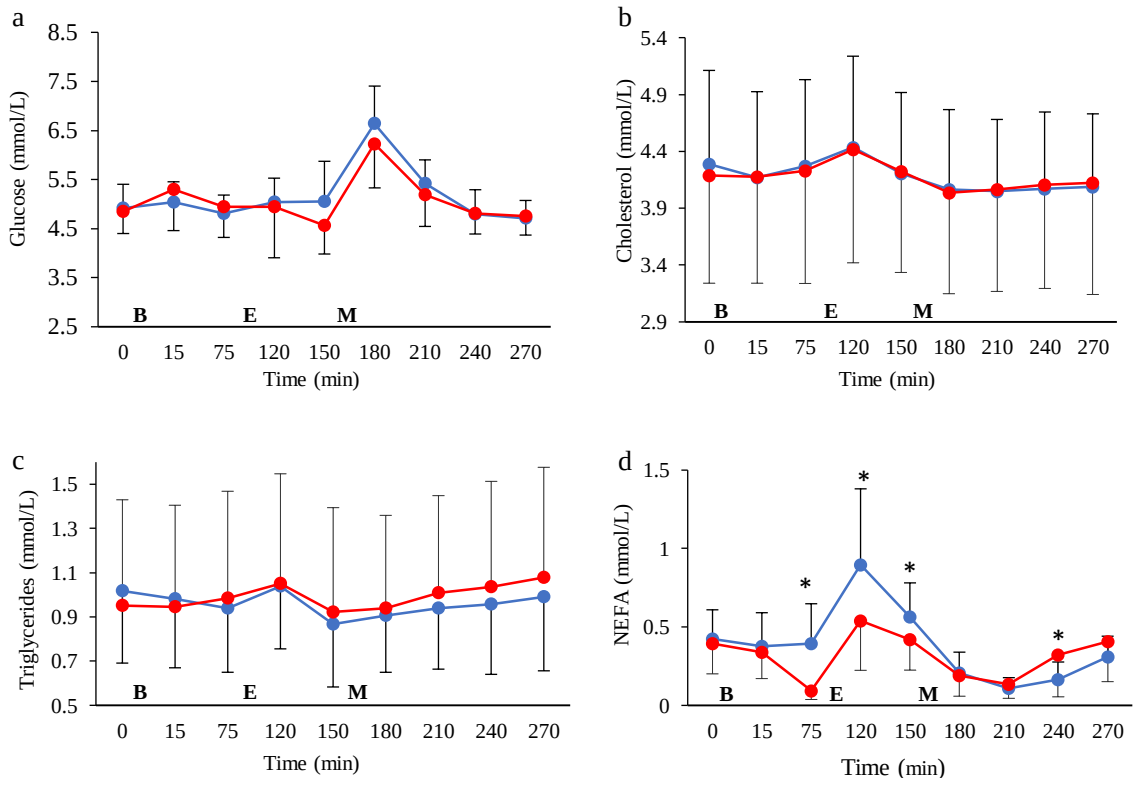

Figure 6: Metabolic responses during trials. Serum concentrations of (a) Glucose, (b) Cholesterol, (c) Triglycerides and (d) Non-esterified fatty acids (NEFA). Values represent mean ± SD; n=12. *P<0.05 versus corresponding time point in other trial. The blue line corresponds to FASTED and red line = FED. '**B**' indicates breakfast period, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, '**E**' indicates exercise period, where participants completed a 45 min brisk walk and '**M**' indicates standardised lunch meal where 400 g chicken and sweetcorn soup was ingested.

Serum glucose, cholesterol, triglycerides iAUC showed no significant differences between trials (P = 0.594; P = 0.712; P = 0.086). However, iAUC for NEFA concentrations were significantly greater in the FASTED trial compared to FED (24.1 ± 18. 9 vs. 10.1 ± 14.5 mmol/L; P = 0.021) (Figure 8).

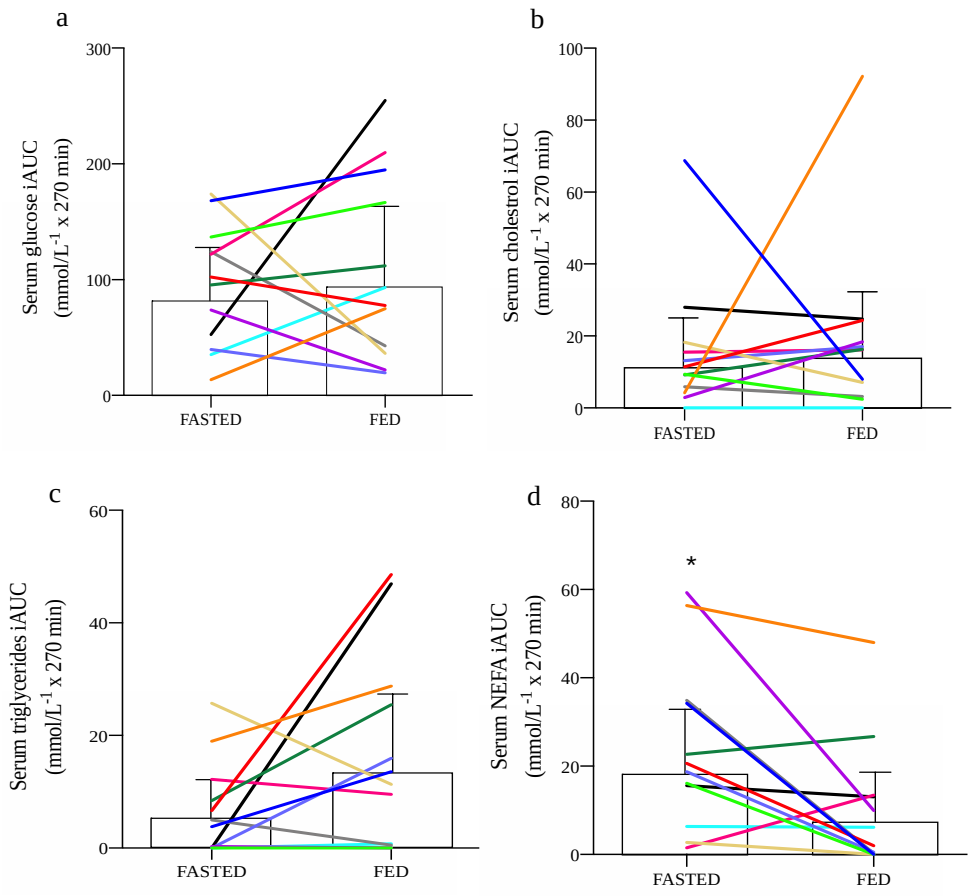

Figure 7: Metabolic responses during trials, FASTED and FED. Incremental area under the curve (iAUC) serum concentrations of (a) Glucose, (b) Cholesterol, (c) Triglycerides and (d) Non-esterified fatty acids (NEFA). Values represent mean ± SD; n=12. *P<0.05 versus corresponding time point in other trial.

3.3.5. Appetite

A main effect of trial (P = 0.006), time (P = 0.006) and trial x time interaction effect (P < 0.001) was observed for hunger. Hunger ratings were lower post breakfast consumption in FED compared with FASTED at 15 min (40 ± 27 vs. 73 ± 22 mm, P = 0.004), 30 min (44 ± 25 vs. 78 ± 17 mm, P < 0.001), 45 min (53 ± 19 vs. 84 ± 17 mm, P < 0.001), 60 min (54 ± 20 vs. 83 ± 18 mm, P < 0.001) and 75 min (60 ± 17 vs. 81 ± 20 mm, P = 0.001). This remained lower immediately post exercise (68 ± 15 vs. 79 ± 13 mm, P = 0.024), however, no further differences were found following this (Figure 9a).

A main effect of trial ($P = 0.008$), time ($P < 0.001$) and trial x time interaction effect ($P < 0.001$) was observed for fullness. Fullness was higher post breakfast consumption at 15 min (45 ± 24 vs. 14 ± 18 mm, $P = 0.008$), 30 min (43 ± 19 vs. 9 ± 7 mm, $P < 0.001$), 45 min (31 ± 17 vs. 8 ± 7 mm, $P < 0.001$) and 60 min (28 ± 16 vs. 10 ± 10 mm, $P = 0.007$) in FED compared with FASTED (Figure 9b).

A main effect of trial ($P < 0.001$), time ($P < 0.001$) and trial x time interaction effect ($P < 0.001$) was observed for prospective food consumption. A higher prospective food consumption was seen post breakfast period at 15 min (76 ± 24 vs. 48 ± 25 mm, $P = 0.004$), 30 min (80 ± 23 vs. 50 ± 23 mm, $P < 0.001$), 45 min (81 ± 21 vs. 57 ± 21 mm, $P < 0.001$), 60 min (80 ± 24 vs. 62 ± 23 mm, $P = 0.009$), 75 min (81 ± 20 vs. 62 ± 21 mm, $P < 0.001$), post exercise prior to lunch at 150 min (83 ± 21 vs. 75 ± 15 mm, $P = 0.014$) and 45 min post lunch ingestion (63 ± 21 vs. 54 ± 26 mm, $P = 0.009$) in FASTED compared with FED (Figure 9c).

No main effect of trial ($P = 0.408$) or trial x time interaction effect ($P = 0.149$) was observed for food satisfaction. There was a main effect of time ($P = 0.011$), however no differences between trials were found (Figure 9d).

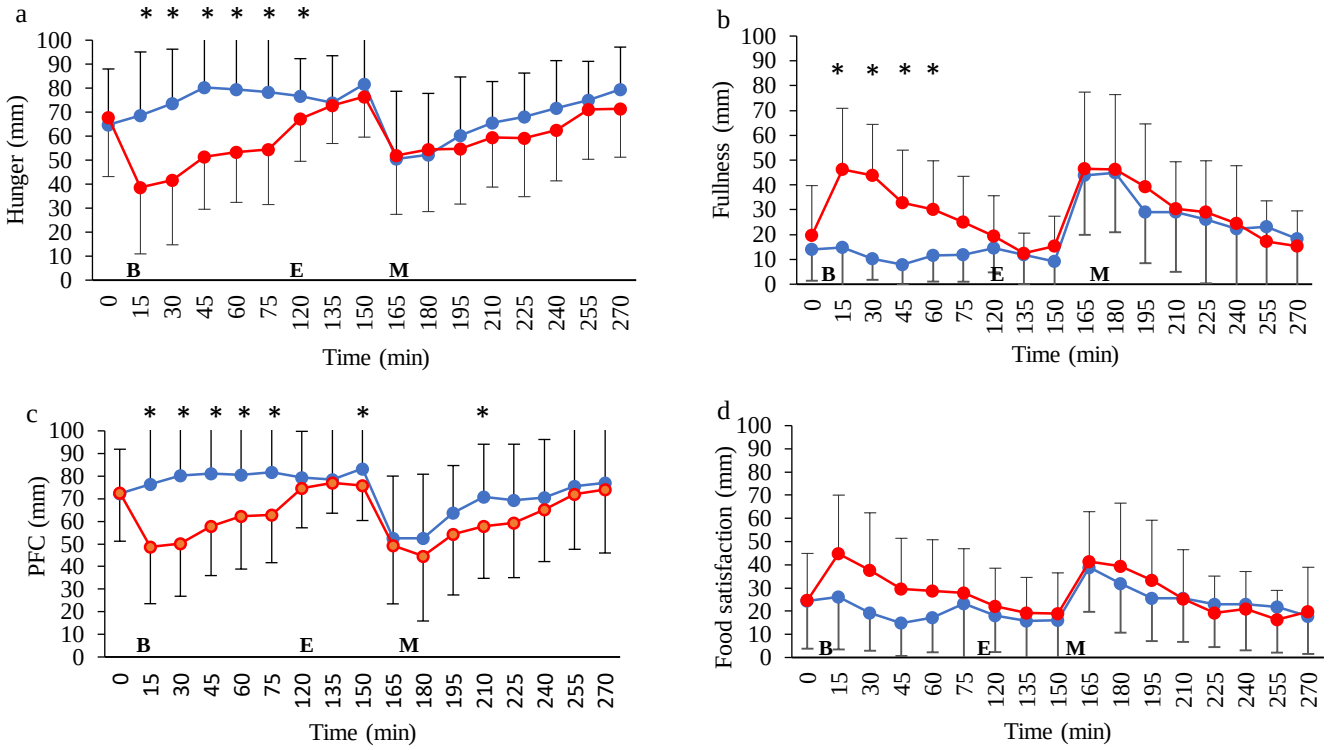

Figure 8: Appetite scores during trials, assessed by 100 mm visual analogue scale (VAS); (a) Hunger, (b) Fullness, (c) Prospective food consumption (PFC), (d) Food satisfaction. Values represent mean ± SD, n=12. The blue line corresponds to FASTED and red line = FED. *P<0.05 versus corresponding time point in other trial. '**B**' indicates breakfast period, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, '**E**' indicates exercise period, where participants completed a 45 min brisk walk and '**M**' indicates standardised lunch meal where 400 g chicken and sweetcorn soup was ingested.

Hunger and prospective food consumption iAUC ratings were higher in the FASTED trial compared to FED (2610 ± 1501 vs. 1160 ± 2138 mm; P = 0.035; 2085± 1797 vs. 965 mm; P = 0.049, respectively). In contrast iAUC fullness was greater in FED trial compared to FASTED (4231 ± 3234 vs. 2183 ± 1847 mm; P = 0.036; Figure 10c). Food satisfaction iAUC was not significantly different between trials (P = 0.249) (Figure 10d).

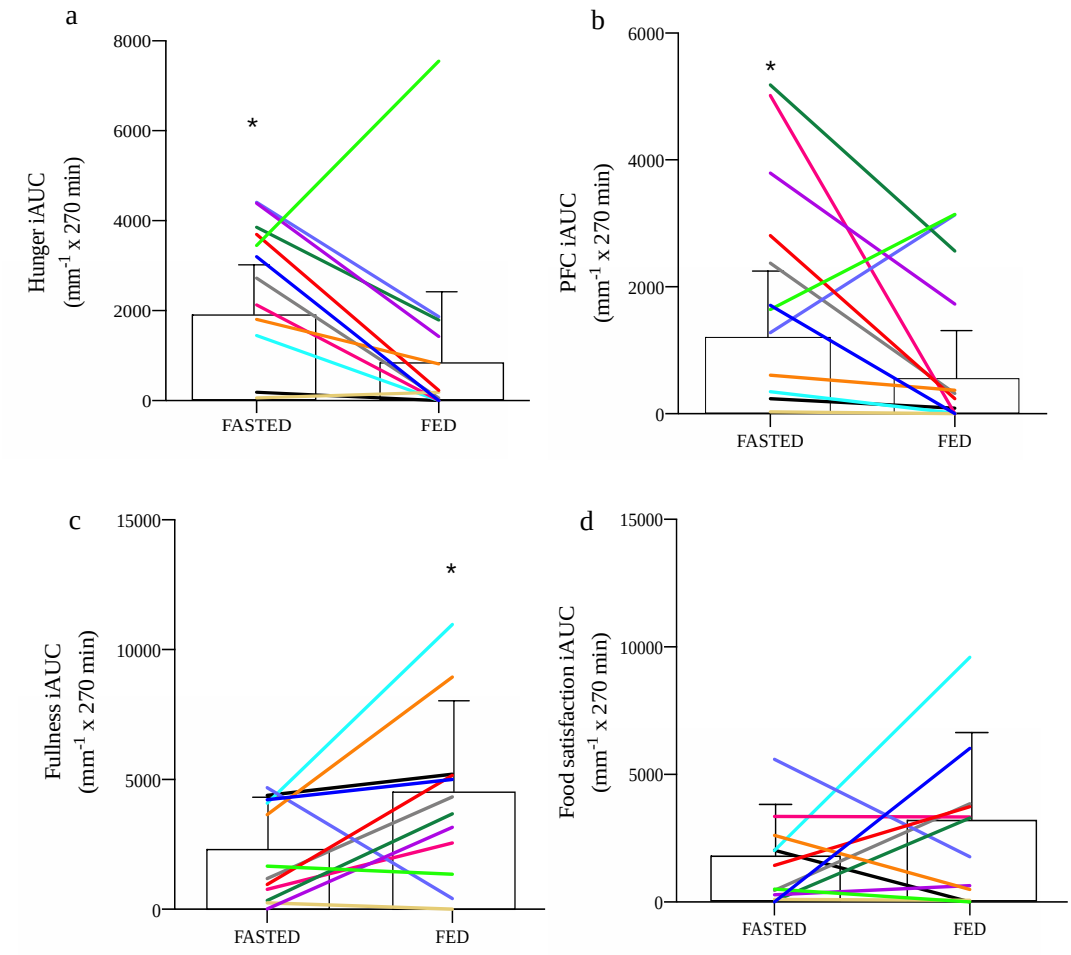

Figure 9: Incremental area under curve (iAUC) appetite scores during trials, assessed by 100 mm visual analogue scale (VAS); (a) Hunger, (b) Fullness, (c) Prospective food consumption (PFC), (d) Food satisfaction. Values represent mean ± SD n=12. *P<0.05 versus corresponding time point in other trial.

3.3.6. Substrate oxidation

No trial × time interaction effect (P = 0.096) or main effect of the trial (P = 0.374) was observed for fat oxidation, although, a main effect of time was present (P<0.001) (Figure 10). Carbohydrate oxidation also had no main effect of the trial (P = 0.193), although, a main effect of time (P < 0.001) and trial x time interaction effect (P = < 0.001) was observed. Carbohydrate oxidation was higher at 30 min (1.16 ± 0.28 vs. 0.87 ± 0.35 g/min; P = 0.023) and 45 min (1.03 ± 0.29 vs. 0.74 ± 0.30 g/min; P = 0.015) of exercise in FED compared with FASTED (Figure 11c). iAUC for fat oxidation was not significantly different between trials (P = 0.748), although

iAUC for carbohydrate oxidation was greater during the FED trial compared to FASTED (54.6 ± 30 vs. 35.1 ± 26.3 g/min; P = 0.049) (Figure 11d).

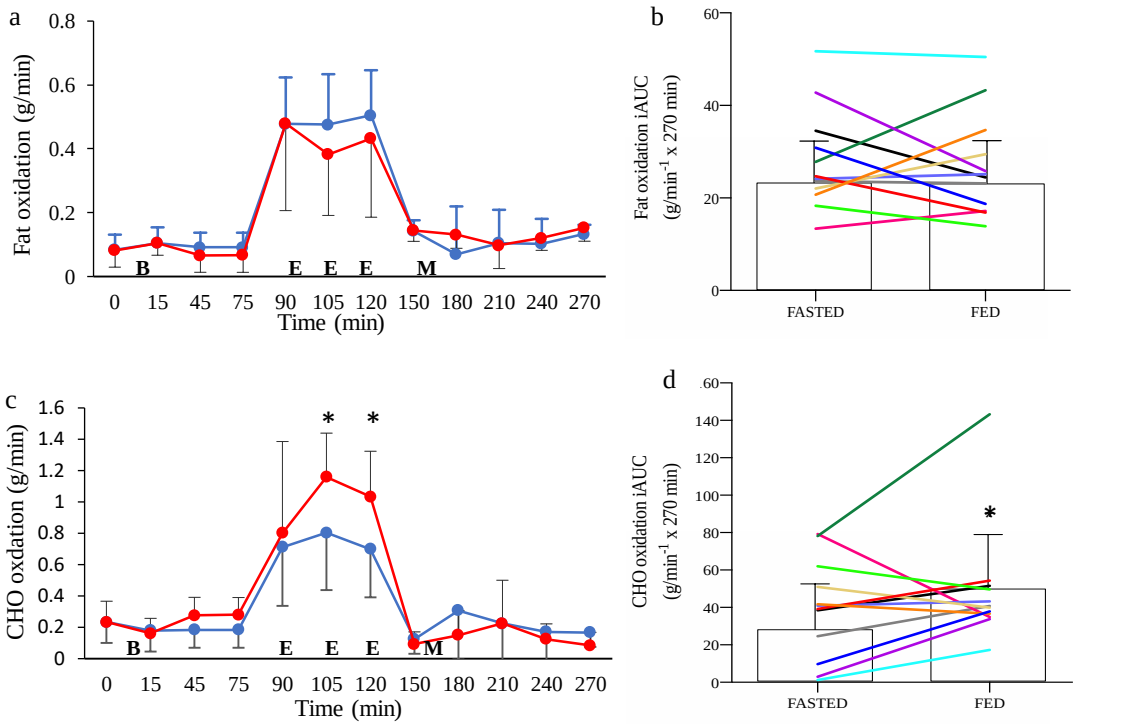

Figure 10: Substrate utilisation throughout both trials. (a) Fat oxidation and (b) iAUC fat oxidation, (c) Carbohydrate oxidation (CHO) and (d) iAUC CHO oxidation. Values represent mean ± SD; n=12. The blue line corresponds to FASTED and red line = FED. *P<0.05 represents difference to other trial. '**B**' indicates breakfast period, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, '**E**' indicates Exercise period, where participants completed a 45 min brisk walk and '**M**' indicates standardised lunch meal where 400 g chicken and sweetcorn soup was ingested.

3.3.7. Physiological measurements during the exercise bout

No significant differences between HR, RPE and VO_2 measurements were seen across or between trials (all P>0.05) (Table 4).

Table 4: Physiological responses during the brisk walking exercise within both trials.

	FASTED				**FED**			
Time (min)	0	15	30	45	0	15	30	45
RPE	6 ± 0	9 ± 1	11 ± 1	12 ±1	6 ± 0	9 ± 1	11 ± 1	12 ± 1
Heart rate (bpm)	78 ± 7	123 ± 1	134 ± 2	137 ± 1	74 ± 1	121 ± 2	129 ± 5	137 ± 2
VO_2 (L/min)	4 ± 1	19 ± 1	19 ±1	19 ± 1	4 ± 0.4	18 ± 1	20 ± 1	19 ± 1
VO_2 (%)	10 ± 1	50 ±6	49 ± 6	49 ± 6	11 ± 2	48 ± 7	51 ± 8	49 ± 7

Rating of perceived exertion (RPE), heart rate (HR) and oxygen consumption (VO_2)

3.3.8. Correlations between gastric emptying and BMI & body fat percentage

No correlation was observed between BMI and GE T½ for the FASTED or FED trial (r = -.02, P = .941, n = 12; r = .04, P = .899, n = 12, respectively). No correlation was observed between BMI and GE T_{lag} for the FASTED trial (r = - .10, P = .754, n = 12 or the FED trial (r = .04, P = .879, n = 12) (Figure 12a and 12b). No correlation between BF % and GE T½ in the FASTED and FED trial (r = .13, P = .693, n = 12; r = .30, P = .342, n = 12, respectively), as well as BF % and GE T_{lag} in the FED trial (r = .29, P = .348, n = 12). No correlation was observed between body fat % and GE T_{lag} for the FASTED trial (r = -.08, P = .758, n = 12) (Figure 12c & 12d).

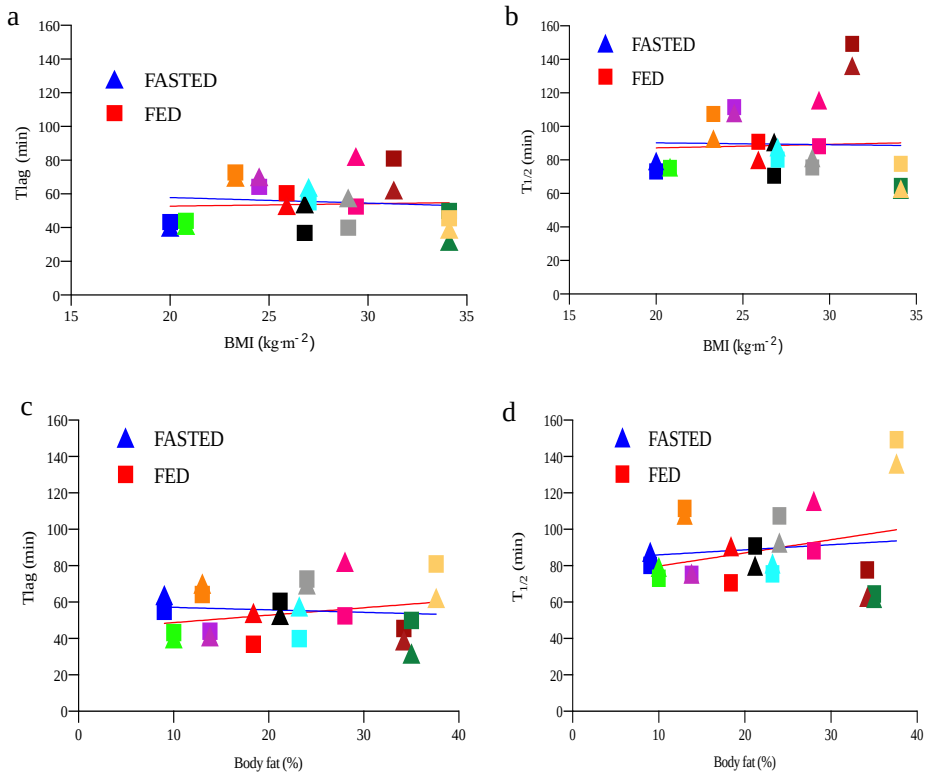

Figure 11: Linear regression to illustrate relationships between (a) BMI and Tlag (b) BMI and T1/2 (c) body fat (BF) percentage and Tlag and (d) BF percentage and T1/2 (n=12). Shape represents, Triangle = FASTED, and Square = FED.

3.4. Discussion

Fasted brisk walking did not result in any difference in GER of a subsequent meal compared with brisk walking in the postprandial state. Minimal differences in perception of appetite post-exercise were observed. Fasted exercise had limited effects on metabolic and gut hormone responses compared with exercise performed after breakfast ingestion. These findings suggest that fasted low-intensity exercise may not elicit a compensatory effect of energy intake in the immediate hours following exercise.

This is the first study that has assessed GER between fasted vs. fed exercise. A difference in GER between trials may not have been observed in this present study owing to a relatively low meal volume and energy content. Alternatively, no difference may have been seen owing to the high variation in participants BMI and body fat percentages. Studies investigating the influence of BMI on GER are inconsistent, with some studies reporting no differences in GER according to BMI (Wright et al., 1983; Hellmig et al., 2006; Buchholz et al., 2013), in contrast to others who have found differences (Bluemel et al., 2017; Lavigne et al., 1978). It was suspected that the participants with a higher body fat in the present study may have had a slower gastric emptying half time in both trials, and slower lag phase during the FED trial. However, no correlation between body fat and GE half time for both FASTED and FED trials, and between body fat and lag phase for the FED trial was seen. The large, variation in body composition within such a small sample (n=12) may account for the no significant correlations observed. Additionally, a post-hoc power analysis was performed to identify the sample specific parameters. GER and glucose concentration data advocated that 15 and 13, respectively, is required to observe any meaningful differences. The current study recruited 12 subjects and thus it is important to consider that the lack of differences could be due to type II error.

Concentrations of the satiety hormone PYY has also been shown to increase with an acute bout of aerobic exercise (Broom et al., 2009; King et al., 2011). However, the present study showed minimal differences in PYY concentrations over time and between trials, especially post-exercise, which coincides with the subsequent hunger and satiety VAS ratings. These results may be owing to the relatively small calorie content of the breakfast provided within the present study (724 kJ (173 kcal)) in comparison with those provided in most other investigations (typically > 1674 kJ (400 kcal)). In addition, total PYY was measured in the present study. The measurement of the active form $PYY^{3–36}$ would have been more desirable.

The findings of PP agree with previous literature regarding elevated levels following food consumption. PP levels remained higher in the FED trial post-exercise, which is consistent with literature reporting elevated PP levels following acute exercise performed in the fed state with no effect on ghrelin levels (Martins et al., 2007). High concentrations of PP are typically associated with decreased perceptions of hunger (Kawano et al., 2013; Koska et al., 2004). This is reflected within the findings of the present study until post-exercise where the hunger scores dissociate from PP as hunger did not change post-exercise. A possible explanation for this is that hunger ratings may be influenced more by GLP-1, PYY and GHR concentrations. The combined lack of differences in these hormones and hunger post-exercise could suggest that regardless of an increased energy expenditure being incurred from the exercise, there will likely be no compensatory increase in energy intake post-exercise to account for the omission of energy intake prior to the exercise. This may, therefore, create a small short-term negative energy balance and if sustained in the long-term, the cumulative effects may have an important role in weight maintenance. However, it is important to consider that although appetite is anticipated to reflect energy intake, the two do not always correlate, and a delayed response in energy intake may still be possible, although some studies have not reported this finding

(Clayton et al., 2015; Levitsky & Pacanowski, 2013). Further research on both the shorter-term effects of an acute bout of exercise and the cumulative effects of fasted exercise over a period is required.

Fasted brisk walking did not alter glycaemic, triglyceride or cholesterol responses to subsequent meal ingestion. However, concentrations of NEFA were greater in the FASTED trial pre- and post-exercise, then lower post subsequent meal ingestion. Increased NEFA prior to the lunch meal indicates greater fat mobilisation for metabolism and is consistent with the knowledge that fat oxidation is increased with fasting, although the results of the present study only show a tendency of increased fat oxidation during fasted exercise. This may have been due to the relatively low intensity of exercise performed in this study. However, carbohydrate was the preferred substrate for exercise in this trial rather than fat stores owing to the provision of exogenous carbohydrate from the breakfast consumption, which also corresponds to the elevated insulin concentrations observed from the breakfast consumption.

In conclusion, these findings demonstrate that GER, appetite and appetite regulatory hormones are not affected by an acute bout of low-intensity exercise in the fasted state compared with the fed state. The indication that no compensatory increase in energy intake will occur post-exercise potentially holds positive implications for fasted brisk walking in the long-term control of body mass. Further work is required to confirm these conclusions, it would be valuable to investigate the effect of fasted exercise at different times of the day, to explore if appetite regulation is affected when conducting fasted exercise outside of the traditional over-night fast. Including 24 h energy intake post-trials would also provide a greater indication on whether compensatory effects occurred once participants left the laboratory.

4.0. Diurnal influences of fasted and non-fasted brisk walking on gastric emptying rate, metabolic responses, and appetite in healthy males[2]

[2]The data from this study contained within the thesis chapter has been published in "McIver VJ., Mattin LR., Evans GH., Yau AMW. (2019). Diurnal influences of fasted and non-fasted brisk walking on gastric emptying rate, metabolic responses, and appetite in healthy males. Appetite. 143, pp. 104411 epub."

Some preliminary data was presented as a poster communication at Europhysiology 2018, QEII Centre, London, UK, 14-16th September 2018.

4.1. Introduction

A growing interest in nutrition and the circadian system has produced many insights within recent years, with diurnal rhythm, metabolism, and nutrition suggested to be intimately linked (Johnston, 2014; Wehrens et al., 2017). Diurnal variations can influence the metabolic pathways, for example, glucose concentrations are higher in the morning before the onset of activity compared to evening (Johnston, 2014; Qian and Scheer, 2016). External factors such as food intake and exercise are known to influence clock genes (Morris, Yang and Scheer, 2012). Clock genes have been established in various organs and tissues, regulating the timing of physiological processes, specifically those involved in the digestion of food, nutrient uptake, and nutrient metabolism (Ruddick-Collins et al., 2018). There has been a considerable amount of interest in the role of clock genes in regulating biochemical pathways and metabolic processes (Marcheva et al., 2014; Sahar and Sassone-Corsi, 2012). However, less attention has been given on examining how the circadian system affects eating patterns combined with exercise, and how this may affect GER and appetite regulation.

Diurnal variations are evident in GI absorption rate and GER, by acting to control food intake differentially at different times of the day. Previous studies have observed slower emptying of the stomach in the evening (Goo et al., 1987; Grammaticos et al., 2015; Orr et al., 2004). Thermic effect of food has been shown to follow a time of day variance, with elevated levels in the morning, which may contribute to the diurnal variation observed for GER (Morris, Garcia, et al., 2015; Morris, Yang, et al., 2015a). However, although the aforementioned studies are informative there are some notable limitations, with very low sample size recruited or no exercise performed (Goo et al., 1987; Grammaticos et al., 2015) and mice studies which may not translate to human physiology (Kentish et al., 2014). Animal models have played a critical role in human diseases; mice studies in particular are often chosen due to their

resemblance to humans in terms of genetics, anatomy and physiology (Barré-Sinoussi & Montagutelli, 2015; Vandamme, 2014), as well as their ease of manipulation and human liability. However, animal studies investigating diurnal variation and nutrition may not be readily translatable to humans due to differences in underlying physiology (Johnston et al., 2016). For example, mice are nocturnal creatures, and thus mice experiments may be methodologically inadequate for humans (e.g. controlled environment, forced activity, time sleep deprivation) (Nunez et al., 2018). Therefore, it is still unknown how circadian variations in GER may differentially influence postprandial metabolism and appetite regulation, particularly, the variation of fasted versus fed exercise on GI function and subsequent appetite.

The aim of this study was to assess the effect of brisk walking in the fasted and non-fasted state on metabolic responses, appetite and GER of a subsequent meal at two different times of the day. It was hypothesized that (a) GER is slower during evening trials in comparison to morning trials (b) evening and morning trials result in differences in appetite and metabolic responses post-exercise (c) fat oxidation is higher during fasted exercise, and carbohydrate oxidation is higher during non-fasted exercise, regardless of time of day and (d) there are no compensatory effects for appetite post-exercise.

4.2. Methods

4.2.1. Participants

Twelve recreationally active men volunteered to participate in this study. A sample size estimation was performed based on gastric emptying data from Goos et al., (1978). This data set was used to reflect a more accurate comparison for the study design in chapter 4 onwards. The effect size was 0.6 and considered to be medium, with a sample size of N = 8 required. Total energy intake data from Bachman et al., (2016) and fat oxidation data from Iwayama et al., (2015) revealed an effect size of 0.8 for both variables and is considered to be large. The sample size required was N = 12 and N = 10, respectively. Therefore, the sample size of 12 was decided to be an adequate for the main objectives of this study. Participant characteristics are shown in Table 5.

Table 5: Participant Characteristics.

	Participants (*n = 12*)
Age (years)	25 ± 3
Height (cm)	178 ± 6
Body mass (kg)	83 ± 12
BMI ($kg \cdot m^{-2}$)	26 ± 3
Body fat (kg)	17 ± 7
Fat free mass (FFM) (kg)	66 ± 5
Body fat (%)	21 ± 5
FFM (%)	79 ± 5
$\dot{V}O_{2peak}$ (ml/kg/min)	39 ± 4
PA levels per week (min)	162 ± 39
Systolic BP (mmHg)	123 ± 11
Diastolic BP (mmHg)	82 ± 13

Values expressed as mean ± SD.

4.2.2. Experimental trials

Participants completed four experimental trials in a randomised crossover fashion. Two-morning trials (08:00 - 13:00 h) fasted (AM-FASTED) and non-fasted (AM-FED) and two evening trials (15:00 - 20:00 h) fasted (PM-FASTED) and non-fasted (PM-FED).

On the morning trials, participants were required to fast from 00:00 the evening before, and on the evening trials, participants were required to have breakfast and then fast from 07:00 except for plain water consumption. Baseline measurements of appetite, expired air and a blood sample were collected.

Following baseline measurements, participants ingested the test ‘breakfast’ in FED within a 15 min period or remained fasted in FASTED. The test ‘breakfast’ consisted of 30 g of breakfast cereal with 125 mL of semi-skimmed milk, and a croissant, which provided in total 1,438 kJ (341 kcal) and contained 10.2 g fat, 48 g carbohydrate and 11.2 g protein. The nutritional profile of this breakfast meal was increased from 175 kcal to 341 kcal to correspond

with recommended guidelines of a breakfast serving being approximately 300-400 kcals (UK Health Forum, 2016; Public Health England, 2018). Participants consumed all of the breakfast within the 15 min window. Post-breakfast ratings of appetite and substrate utilisation were measured at the end of the 15 min breakfast period. Participants then rested for 1 h before commencement of the exercise protocol. During this 1 h rest period, further measures of appetite were taken every 15 min and substrate utilisation every 30 min. The exercise protocol involved 45 min of brisk walking on a level motorised treadmill at the speed determined in the preliminary trial (speed range of 5.9–7.0 $km \cdot h^{-1}$ and average speed of 6.5 ± 0.6 $km \cdot h^{-1}$). The relative exercise intensity was 55 ± 0.8% $\dot{V}O_{2peak}$. Heart rate and RPE were measured every 15 min throughout the exercise, with expired air measured continuously. After completion of the exercise bout, participants recovered for 30 min (showered if desired) before they ingested a standardised meal. The meal was 800 g (2 cans) of vegetable soup (1584 kJ (376 kcal)), containing 6.8 g fat, 66.4 g carbohydrate, 8.8 g protein. Following on from chapter 3, the lunch meal was changed to vegetable soup as well as increasing the volume from 400 g (242 kcal) to 800 g (376 kcal). The change in the lunch meal allowed a wider audience to participant (e.g. non-meat eaters), and increasing the volume of calories brings the amount closer to the recommended calories of a lunch serving in the UK than in chapter 3 (Public Health England, 2018). Additionally, increasing the volume and calorie content of the test meal was anticipated to provide a greater stimulus for the delay in gastric emptying so that any differences in emptying rate may be more detectable. Subjective feelings of appetite and substrate utilisation were measured every 15 min post-ingestion for a total period of 2 h. 24 h energy intake post-trial was recorded via a weighed food diary by the participants and later analysed using Nutritics dietary analysis. A schematic diagram of the experimental protocol is presented in Figure 13.

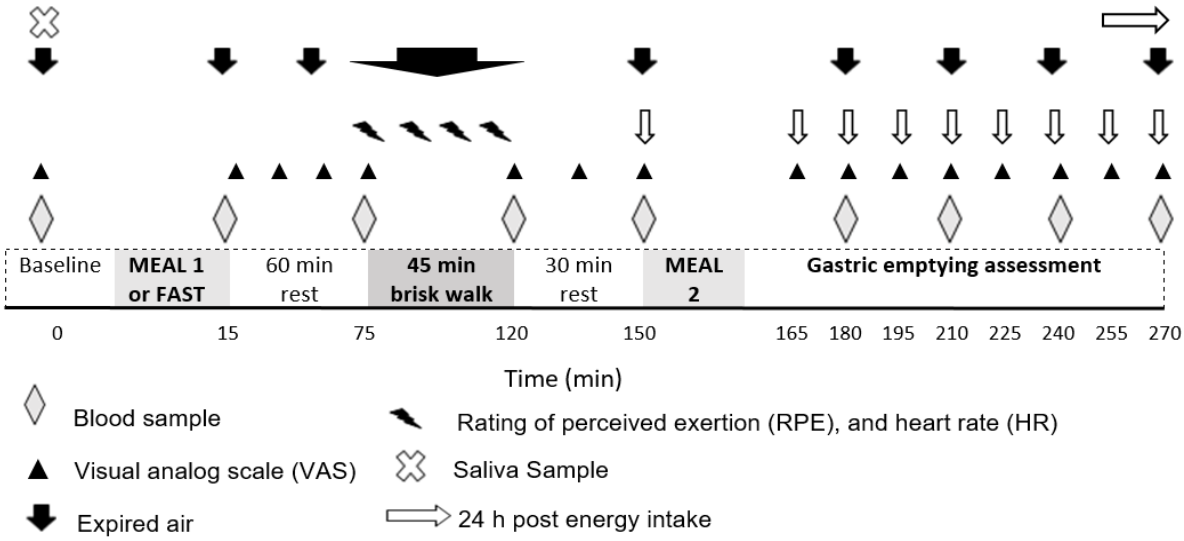

Figure 12: Schematic diagram of the experimental trial protocol.

4.2.3. Blood Sampling

Blood glucose concentration was measured via a capillary blood sample from the tip of the finger, with the participant in a seated position. Capillary blood samples were taken at baseline, post breakfast period, pre-exercise, immediately post-exercise, pre-soup ingestion, then every 30 min post soup ingestion. A 23-gauge single-use sterile lancet (Unistik-3, Owen Mumford, Oxford, UK) was used to create a small incision (approx. 3mm puncture) on the fingertip. From this incision, a free-flowing capillary blood sample was collected in microcuvettes (Hemocue Glucose 201+ Microcuvettes, Ângelholm, Sweden) containing anticoagulants EDTA and lithium heparin. Whole blood was collected and analysed immediately using a desktop glucose analyser (Hemocue Glucose 201+ analyser, Ângelholm, Sweden).

4.2.4. Statistical Analysis

A three-way (trial x time of day x time across trial) repeated-measures analysis of variance (ANOVA) to assess trial (fasted vs. fed) x time of day (morning vs. evening) x time across trial differences for blood glucose concentration, gastric emptying DOB, substrate oxidation, and VAS ratings. Incremental area under the curve (iAUC) was calculated using the Time Series Response Analyser (TSRA) created by Narang et al., (2020). A two-way repeated-measures ANOVA was used to assess iAUC for trial x time of day differences for blood glucose concentration, gastric emptying DOB, substrate oxidation, and VAS ratings. A two-way repeated measures ANOVA was also used to assess trial x time of day differences for gastric emptying $T_{½}$ and T_{lag} data, melatonin concentration and 24-hour energy intake. Sphericity for repeated measures was assessed, and where appropriate, Greenhouse–Geisser corrections were applied for epsilon <0.75, and the Huynh–Feldt correction adopted for less severe asphericity. Significant F-tests were followed by dependent Student's t-Tests or one-way repeated measures ANOVA and Bonferroni adjusted pairwise comparisons as appropriate. One-way repeated-measures ANOVA was also implemented to ensure no differences in body mass and mid-point of sleep were observed between trials. A one-way repeated measures ANOVA was also implemented to assess differences in incremental area under the curve (iAUC) for glucose, gastric emptying DOB, substrate oxidation and VAS ratings. A Pearson Correlation was implemented to examine the relationship between BMI and body fat percentage against gastric emptying (GE) half time ($T_{1/2}$) and GE lag phase (T_{lag}). All analyses were carried out using IBM SPSS statistics (v25.0 for Windows; SPSS, Chicago, IL). The level of significance was set at $P<0.05$. Descriptive data are expressed as mean ± standard deviation (SD).

4.3. Results

4.3.1. Trial order analysis

Analysis demonstrated that there were no significant differences in responses according to the order of trials that the participants completed (all P>0.05) (Table 6).

Table 6: Trial order analysis, mean ± SD for gastric emptying half time ($T_{1/2}$), and incremental area under the curve (iAUC) for subjective feeling of hunger, and glucose concentrations

Variable	Trial 1	Trial 2	Trial 3	Trial 4	P value
Gastric emptying $T_{1/2}$ (min)	101 ± 21	130 ± 51	107 ± 25	117 ± 51	0.108
Subjective feeling of hunger (mm^{-1} x 270 min)	1692 ± 2181	2140 ± 3283	1911 ± 4324	1543 ± 3574	0.902
Glucose concentrations ($mmol/L^{-1}$ x 270 min)	110 ± 84	188 ± 108	183 ± 161	193 ± 99	0.226

4.3.2. Gastric emptying rate

Three factor ANOVA demonstrated no trial x time of day x time interaction (P= 0.088), time of day effect (P= 0.073) or main trial effect (P= 0.209) for DOB, although, a main effect for time was found (P< 0.001; Figure 14a). No main trial, time of day, or trial x time of day interaction effect was observed for iAUC gastric emptying DOB (P = 0.226; P = 0.075; P = 0.177, respectively) (Figure 14b).

No main effect of time of day (P= 0.128), trial (P= 0.111) or trial x time of day interaction effect (P= 0.430) for $T_{½}$ were found (Figure 15a). A main effect of time of day (P= 0.021) and an interaction trial x trial (P= 0.023) was observed but no main effect of trial for T_{lag} (P= 0.256). T_{lag} was slower in PM-FASTED compared to AM-FASTED, AM-FED and PM-FED (75 ± 18 vs. 63 ± 14 min, P= 0.001 vs. 65 ± 10 min, P= 0.028 and vs. 67 ± 16 min, P= 0.007, respectively) (Figure 15b).

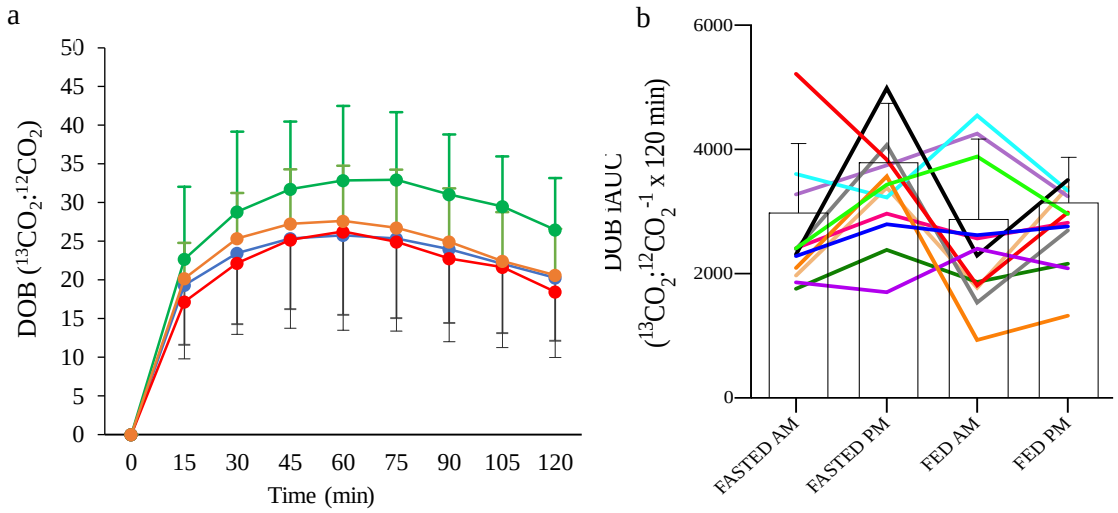

Figure 13: Gastric emptying assessment, for all four trials AM-FASTED, AM-FED, PM-FASTED, and PM-FED a) Gastric emptying delta over baseline (DOB) and b) iAUC for DOB of MEAL 2 (800 g vegetable soup) following 45 min of brisk walking either fasted (FASTED) or after breakfast consumption (FED). Values are mean ± SD; n=12. The blue line corresponds to AM-FASTED, red line = AM-FED, green line = PM-FASTED, orange = PM-FED.

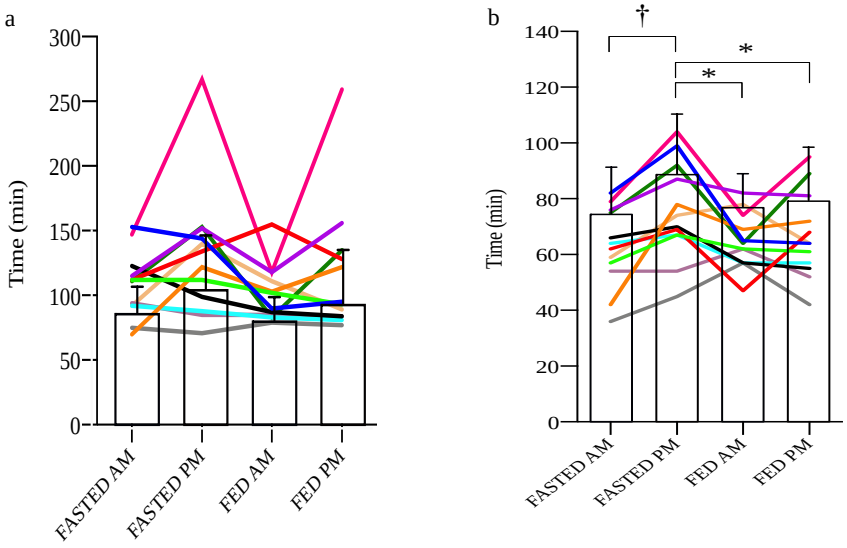

Figure 14: Gastric emptying assessment, AM-FASTED, AM-FED, PM-FASTED, and PM-FED a) Gastric emptying half time ($T_{½}$) and b) time of maximal emptying rate (T_{lag}) for all four trials of MEAL 2 (800 g vegetable soup). * indicates significance ($P < 0.05$) difference between the trials indicated by the brackets. † indicates diurnal significance between morning and evening trials (i.e. AM vs. PM). Values represent mean ± SD; n = 12.

4.3.3. Blood glucose concentrations

A main effect of trial ($P = 0.007$) and time ($P < 0.001$) was observed for glucose concentration, although no main effect for time of day ($P = 0.854$), or trial x time of day x time main interaction effect ($P = 0.058$) was observed (Figure 16a). A significant time of day effect for iAUC glucose concentrations was observed ($P = 0.020$), however, no main trial ($P = 0.109$), or trial x time of day interaction effect ($P = 0.738$) was observed (Figure 16b).

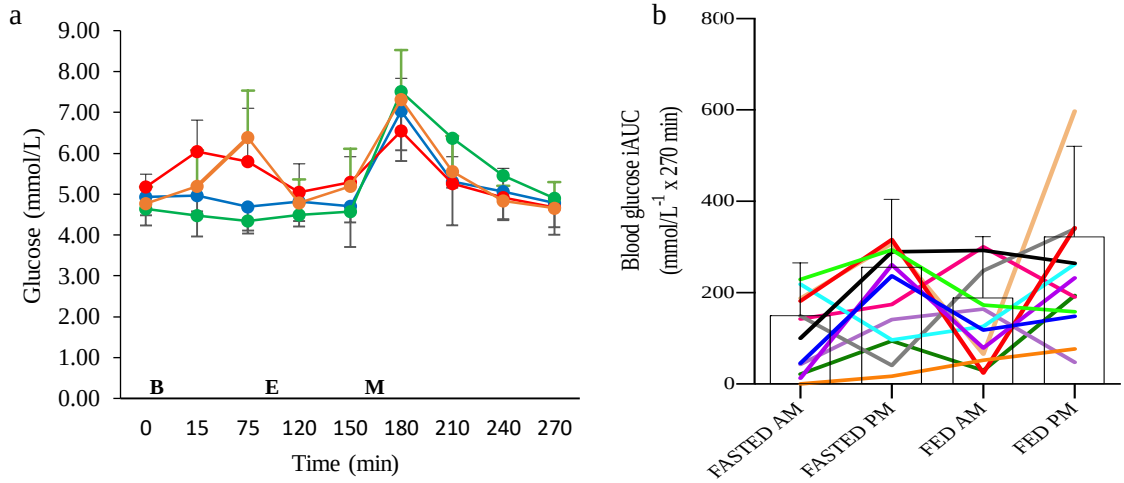

Figure 15: Blood glucose responses. a) Blood glucose concentrations during trials, AM-FASTED, AM-FED, PM-FASTED, and PM-FED. b) iAUC blood glucose responses for all trials. Values represent mean ± SD; n = 12. The blue line corresponds to AM-FASTED, red line = AM-FED, green line = PM-FASTED, orange = PM-FED. '**B**' indicates breakfast period, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, '**E**' indicates Exercise period, where participants completed a 45 min brisk walk and '**M**' indicates standardised lunch meal where 800 g vegetable soup was ingested.

4.3.4. Appetite

A main effect of trial (FASTED vs. FED; $P < 0.001$), time of day ($P = 0.003$) and time ($P < 0.001$) was observed for hunger, although no trial x time of day x time interaction effect was observed ($P = 0.855$). A main effect of trial ($P < 0.001$) and time ($P < 0.001$) was observed for fullness, although no main effect for time of day ($P = 0.057$), or trial x time of day x time interaction ($P = 0.074$) effect was observed. A main effect of trial ($P = 0.008$), time of day

($P < 0.001$) and time ($P < 0.001$) was observed for prospective food consumption (PFC) although no trial x time of day x time interaction effect was observed ($P = 0.577$). A main effect of trial ($P = 0.003$) and time ($P < 0.001$) was observed for food satisfaction, however, no main effect for time of day ($P = 0.078$), or trial x time of day x time interaction effect ($P = 0.679$) was observed (Figure 17).

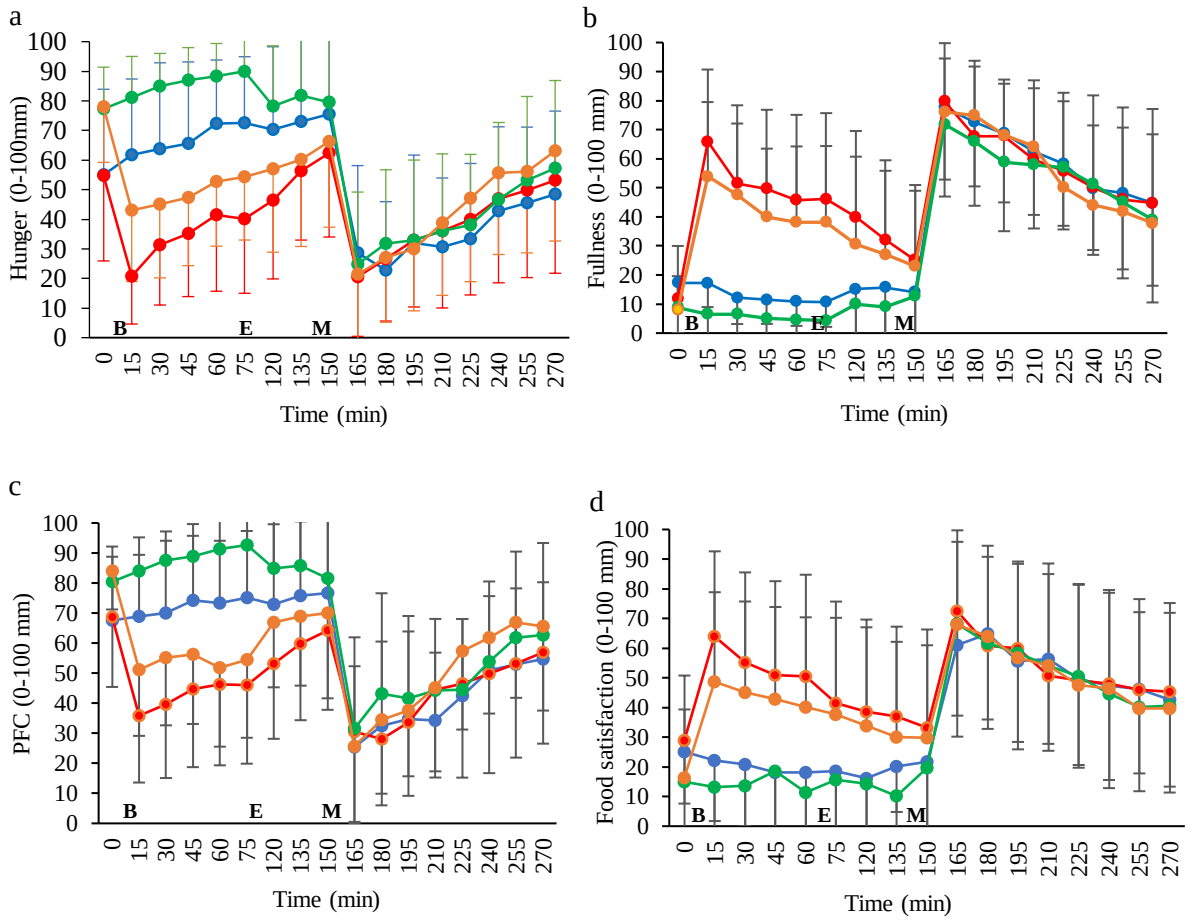

Figure 16: Appetite ratings during trials, AM-FASTED, AM-FED, PM-FASTED, and PM FED. Appetite was assessed by 100 mm visual analogue scale (VAS); (a) Hunger, (b) Fullness, (c) Prospective food consumption (PFC) and (d) Food satisfaction. Values represent mean ± SD; n = 12. The blue line corresponds to AM-FASTED, red line = AM-FED, green line = PM-FASTED, orange = PM-FED. '**B**' indicates breakfast period, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, '**E**' indicates Exercise period, where participants completed a 45 min brisk walk and '**M**' indicates standardised lunch meal where 800 g vegetable soup was ingested.

A significant main effect for trial was observed for iAUC for hunger ratings (P=0.019), however, no time of day (P = 0.129), or trial x time of day interaction effect (P = 0.223) was observed. Similar to this, iAUC for fullness ratings, PFC and food satisfaction showed significant main effects for trial (all P = 0.001). However, no significant differences were observed for time of day or trial x time of day interaction effect (P>0.05) (Figure 18).

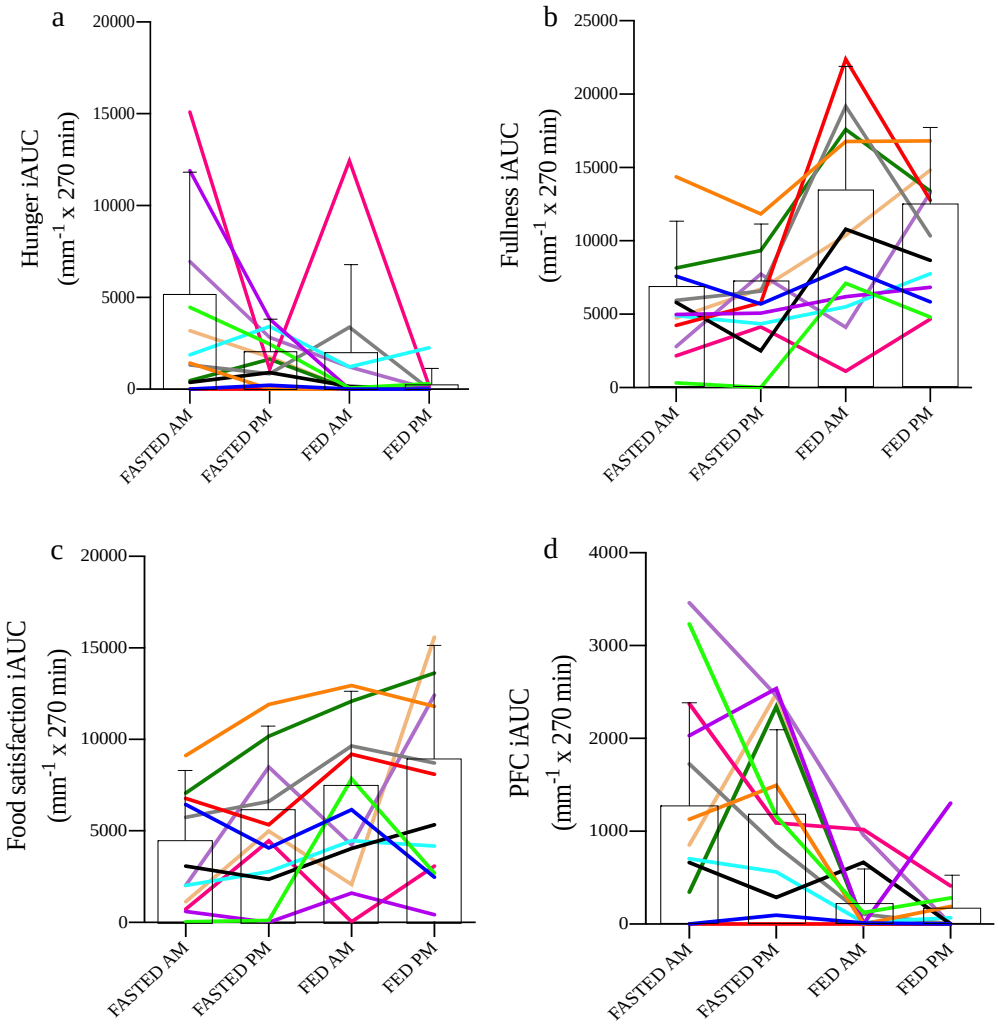

Figure 17: iAUC appetite scores during trials, assessed by 100 mm visual analogue scale (VAS); (a) Hunger, (b) Fullness, (c) Prospective food consumption (PFC), and (d) Food satisfaction. Values represent mean ± SD n=12.

4.3.5. Substrate oxidation

A main effect of trial ($P < 0.001$) and time ($P < 0.001$) was observed for fat oxidation, however, no main effect for time of day ($P = 0.469$) or trial x time of day × time interaction effect was observed ($P = 0.740$; Figure 19a). A main effect for trial ($P < 0.001$), and time ($P < 0.001$) was observed for CHO oxidation, however, no main effect for time of day ($P = 0.296$) or trial x time of day × time interaction effect was observed ($P = 0.366$; Figure 19b).

A significant main trial effect was observed with iAUC for fat oxidation and CHO oxidation ($P < 0.001$; $P < 0.001$, respectively). However, no time-of-day effect or trial x time of day interaction effect was observed for both fat oxidation ($P = 0.951$; $P = 0.671$, respectively) and CHO oxidation ($P = 0.941$; $P = 0.416$, respectively; Figure 19).

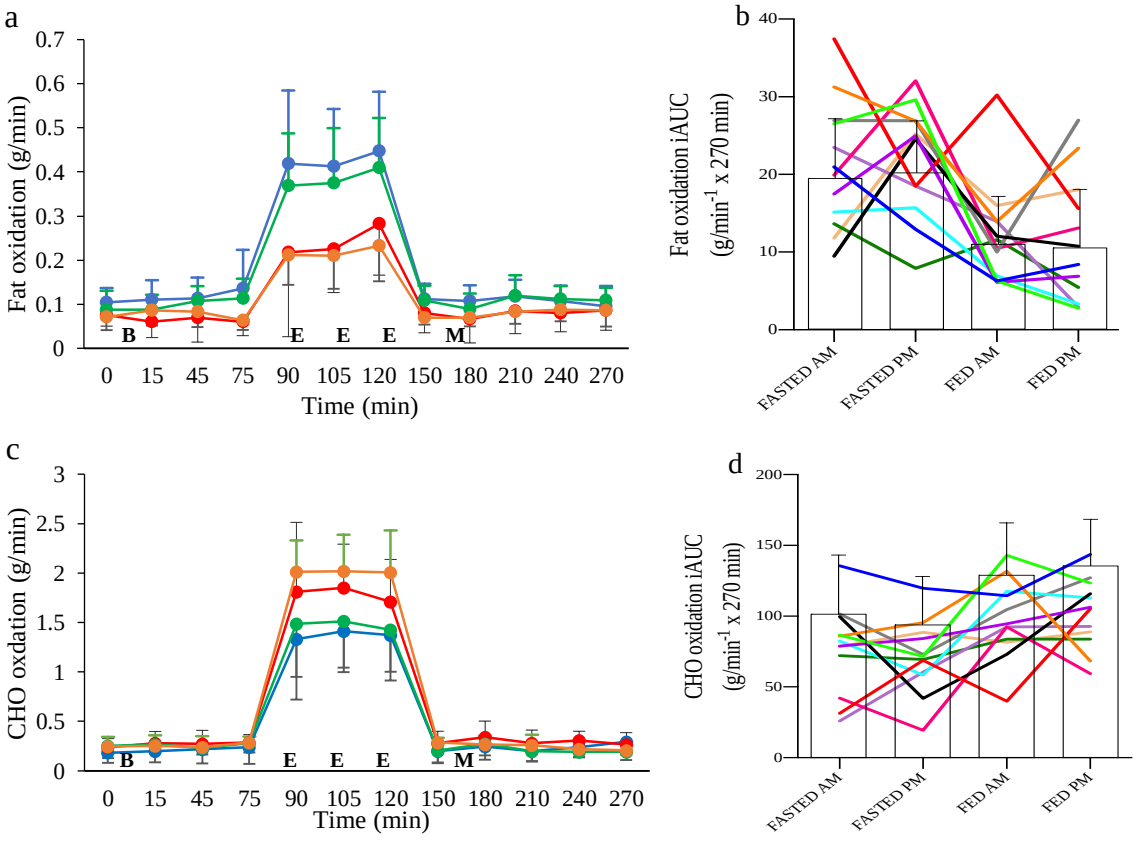

Figure 18: Substrate utilisation. a) Fat oxidation, b) iAUC fat oxidation, c) Carbohydrate (CHO) oxidation and d) iAUC CHO oxidation, during the trials, AM-FASTED, AM-FED, PM-FASTED, and PM-FED. Values represent mean ± SD; n = 12. The blue line corresponds to AM-FASTED, red line = AM-FED, green line = PM-FASTED, orange = PM-FED. '**B**' indicates breakfast period, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, '**E**' indicates Exercise period, where participants completed a 45 min brisk walk and '**M**' indicates standardised lunch meal where 800 g vegetable soup was ingested.

4.3.6. Physiological measurements during the exercise bout

No significant differences between HR, RPE and VO_2 measurements were seen across and between trials (all P>0.05) (Table 7).

Table 7: Illustrates the physiological responses during the brisk walking exercise within all four trials.

	FASTED-AM				**FED-AM**			
Time (min)	0	15	30	45	0	15	30	45
RPE	6 ± 0	11 ± 2	12 ± 2	12 ± 2	6 ± 0	11 ± 2	12 ± 2	12 ± 2
Heart rate (bpm)	77 ± 1	127 ± 2	132 ± 2	136 ± 2	77 ± 1	129 ± 2	133 ± 2	135 ± 2
VO_2 (L/min)	4 ± 6	22 ± 1	21 ±3	22 ± 1	5 ± 4	21 ± 3	23 ± 3	20 ± 4
VO_2 (%)	9 ± 1	54 ± 4	55 ± 6	55 ± 4	10 ± 2	55 ± 4	55 ± 3	54 ± 6
	FASTED-PM				**FED-PM**			
Time (min)	0	15	30	45	0	15	30	45
RPE	6 ± 0	11 ± 2	12 ± 3	12 ± 3	6 ± 0	11 ± 1	12 ± 1	12 ± 2
HR (bpm)	79 ± 1	129 ± 2	135 ± 2	137 ± 2	75 ± 1	130 ± 2	136 ± 2	138 ± 2
VO_2 (L/mn)	3 ± 3	19 ± 4	21 ± 3	20 ± 6	4 ± 8	20 ± 5	20 ± 4	19 ± 9
VO_2 (%)	8 ± 5	54 ± 7	55 ± 8	55 ± 8	9 ± 3	55 ± 7	55 ± 6	55 ± 8

Rating of perceived exertion (RPE), heart rate (HR) and oxygen consumption (VO_2)

4.3.7. 24 h energy intake

Two factor ANOVA demonstrated no main effect of time of day (P = 0.170), no main effect of trial (P = 0.564) and no interaction effect (P = 0.718) for 24-hour post-trial energy intake (Table 8).

Table 8: 24 h post-trial energy intake and macronutrient breakdown for participants (n = 12; mean ± SD).

	AM-FASTED	**AM-FED**	**PM-FASTED**	**PM-FED**
Energy intake (kcal)	2789 ± 520	2704 ± 655	2639 ± 668	2490 ± 749
Protein (g)	138 ± 33	145 ± 70	126 ± 54	127 ± 59
Carbohydrate (g)	332 ± 106	296 ± 71	237 ± 119	273 ± 83
Fat (g)	104 ± 43	110 ± 43	134 ± 80	101 ± 49

4.3.8. Salivary Melatonin

Two factor ANOVA demonstrated a main effect of time of day (P = 0.002), no main effect of trial (P = 0.345) and no interaction (P = 0.159) for salivary melatonin concentration. Salivary melatonin concentration was significantly different between morning and evening trials (AM-FASTED, AM-FED vs. PM-FASTED, PM-FED; P= 0.002, Table 9). No significant differences between trials (AM-FASTED vs. AM-FED vs. PM-FASTED vs. PM-FED) for the midpoint of sleep was detected (P= 0.159) (Table 9).

Table 9: Melatonin measurements for participants (mean ± SD).

	AM-FASTED	AM-FED	PM-FASTED	PM-FED
Melatonin (pg/mL)	20.5 ± 6.6 *	23.3 ± 13.4*	15.2 ± 12.3*	9.3 ± 5.1*
Midpoint of sleep	02:40 ± 0:2	02:25 ± 0:4	02:32 ± 0.5	02:42 ± 0.5

* indicates significant difference to corresponding trial (AM-FASTED vs. PM-FASTED).

4.3.9. Correlations between gastric emptying and BMI & body fat percentage (%)

A weak negative correlation was observed between BMI and GE $T_{½}$ for the AM-FASTED, AM-FED, PM-FASTED and PM-FED (r = -.456, P = .136, n = 12; r = -.275, P = .387, n = 12; r = -.331, P = .293, n = 12, respectively). No correlation between BMI and GE T_{lag} was observed for trials, AM-FASTED, PM-FASTED and PM-FED (r = -.331, P = .293, n = 12; r = -.013, P = .969, n = 12; r = -.002, P = .995, n = 12, respectively). A weak positive correlation for AM-FED (r = .35, P = .264, n = 12). Like BMI, no correlation was observed between body fat % and GE $T_{½}$ in all trials, AM-FASTED, AM-FED, PM-FASTED and PM-FED (r = -.49, P = .106, n = 12; r = -.399, P = .199, n = 12; r = -.239, P = .454, n = 12; r = -.159, P = .621, n = 12 respectively). No correlation between body fat % and GE T_{lag} for AM-FASTED, PM-FASTED and PM-FED (r = -.36, P = .271, n = 12; r = -.072, P = .825, n = 12; r = -.166, P = .606, n = 12, respectively). No correlation for AM-FED was observed (r = .41, n = 12, P = .186). No correlations made between gastric emptying, BMI and body fat percentage were statistically significant (Figure 20).

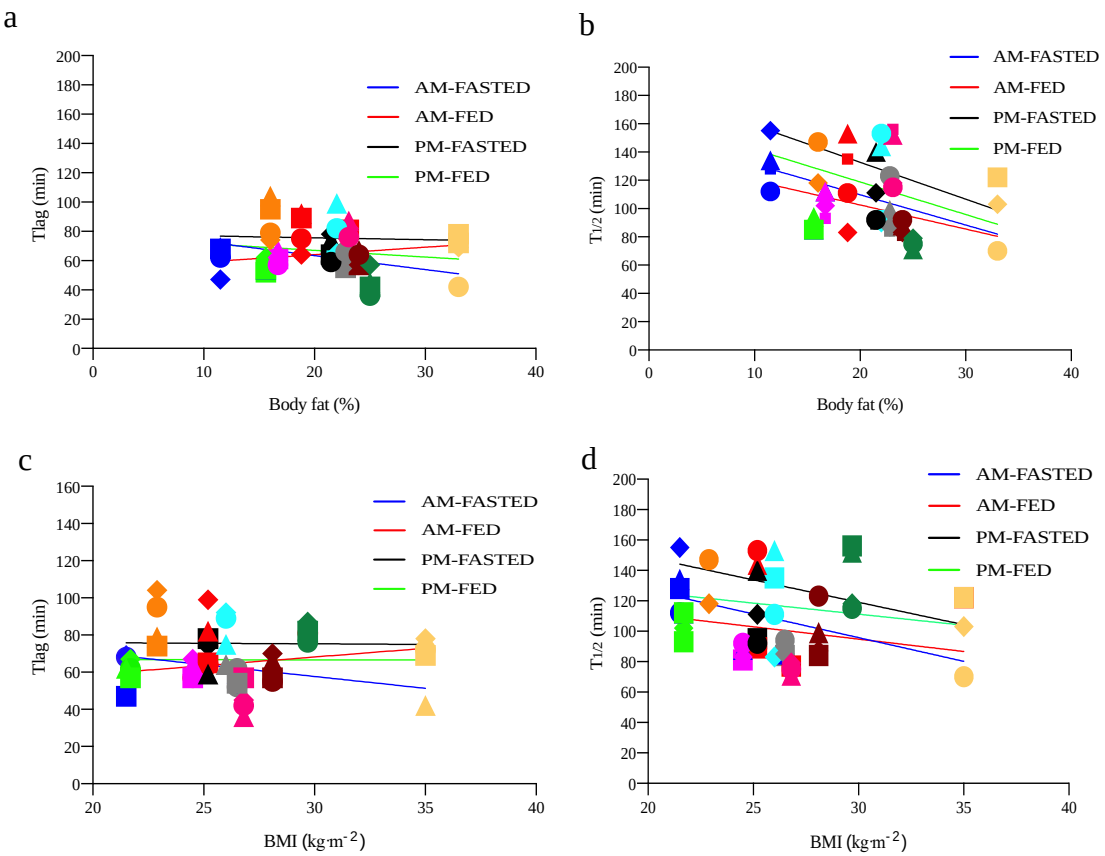

Figure 19: Linear regression to illustrate relationships (a) between body fat (BF) percentage with T_{lag} (b) BF percentageand $T_{1/2}$ (c) T_{lag} and BMI and (d) $T_{1/2}$ and BMI (n=12). Shape represents, Circle = AM-FASTED, Diamond = PM-FASTED, Triangle = AM-FED, Square = PM-FED.

4.4. Discussion

A meal in the evening following fasted exercise elicits a slower maximal GER in comparison to a meal following morning fasted and evening non-fasted exercise. Appetite does not follow a diurnal variation following fasted low-intensity exercise, and regardless of the time of day, fasted exercise favours fat oxidation which may help induce a negative energy balance without a subsequent compensatory response in energy intake.

This is the only study that has investigated the diurnal variation of GER response from a subsequent meal following fasted versus fed exercise. Previous studies that have investigated the effect of GER between morning and evening have found half time was significantly delayed in the evening (Goo et al., 1987; Grammaticos, Doumas, and Koliaskos, 2015; Orr et al., 2004). The present study observed a significantly slower maximal emptying rate only, which was not the case for half emptying time. This may be due to the meal context in the current study in comparison to others when measuring gastric emptying. Goo et al. (1987) found that in 16 healthy men, only gastric emptying half-times for the evening (20:00) meal were significantly longer for solids but not liquids when compared with morning (08:00) emptying half-times. The present study used a soup meal that contained a large liquid component, which may be an explanation for the lack of difference in half time as a greater delay of emptying with solid food compared with liquids is commonly observed (Hellström, Grybäck, & Jacobsson, 2006).

In addition to this, it is well known that variations in gastric emptying can have a major impact on the postprandial glycaemic profile, and incretin hormone secretion (Marathe, Rayner, Jones, & Horowitz, 2013; Trahair et al., 2014). Whether a delayed gastric emptying in the evening versus morning would be more beneficial for appetite regulatory parameters for weight management is unknown and requires further study. This may be of particular importance for some clinical populations, such as overweight and type 2 diabetes, with research

providing a number of strategies to optimise postprandial glycaemic control based on modulation of GER (Jones et al., 2001; Marathe et al., 2013; O'Keefe et al., 2011; Phillips et al., 2015). It is suggested that a slower rate of nutrient delivery to the small intestine would be desirable to compensate for the delay in insulin release and the resistance to its actions (Marathe et al., 2013). However, it is difficult to draw accurate comparisons due to no existing studies measuring gastric emptying rate at different times of the day in response to exercise.

Similar to gastric emptying, it is well recognised that fat, CHO, and glucose metabolism display time-of-day dependent rhythms, which align with daily rhythms in behaviours, such as sleep/wake, feeding/fasting, and activity cycles (Bailey et al., 2014; Kalsbeek et al., 2014). Previous evidence has observed higher fat oxidation rates in the evening in comparison to the morning (Darvakh et al., 2014), while in contrast, CHO and glucose metabolism are higher in the morning in comparison to the evening (Kessler et al., 2017; Qian & Scheer, 2016). However, these conclusions do not translate on to the present study findings, with no time-of-day effect observed in any of the metabolic measures. A possible explanation for the lack of time-of-day variance may be due to the exercise elicited within the current study (55% $\dot{V}O_{2peak}$). Previous studies that observed a time-of-day variance in fat/CHO oxidation conducted higher exercise intensities (Mohebbi, Azizi and Tabari 2011; Suk, Moon, Park, Park, & Shin, 2015). It is thought that during periods of increased physical activity, non-insulin mediated glucose utilisation increases, and the relative contribution of aerobic to anaerobic utilisation is dependent upon exercise intensity (Alberts, Johansson, & McArthur, 2006; Calvo et al., 2008; Melzer, 2011; Röhling, Herder, Stemper, & Müssig, 2016; Rose & Richter, 2005). Nevertheless, the results of this study correspond with existing literature showing energy metabolism is predominantly dependent on feeding behaviours, and regardless of the time of day, fasted exercise favoured fat oxidation while eating before exercise elicits a greater CHO

oxidation response (Achten & Jeukendrup, 2004; Bachmen, 2016; Iwayama et al., 2017). It would be interesting to examine the metabolic responses of energy balance on time-of-day following a mode of exercise that is better controllable and capable of eliciting a greater energy response.

The current study hypothesised that evening and morning trials would result in differences in appetite and metabolic responses post-exercise. However, appetite stabilised across all trials post-exercise, which corresponds with the substrate utilisation and glucose findings. This may be due to a suppression of appetite which has been reported during and briefly following moderate-to-high intensity bouts of running exercise (Broom et al., 2008; Vatansever-Ozen et al., 2011). The combined lack of differences in hunger and energy metabolism post-exercise, followed by no differences in 24 h post-energy intake, suggests that regardless of an increased energy expenditure being incurred from exercise there will likely be no compensatory increase in energy intake post-exercise to account for the omission of energy intake prior to exercise. This may, therefore, create a small short-term negative energy balance and if sustained in the long-term, the cumulative effects may have an important role in weight maintenance, which has been found in previous studies from fasted exercise. Previous studies have found that alternate-day fasting combined with endurance exercise was effective for weight loss for obese participants following 12-week training programme (Bhutani et al., 2013) and fasting before morning exercise decreased 24-hour energy intake (Bachman, Deitrick, & Hillman, 2016). Further research on both the shorter-term effects of an acute bout of exercise and the cumulative effects of frequent fasted exercise at various times of day over a period of time is required to fully understand if compensatory effects occur.

Healthy men were recruited within the general public that met the screening criteria. However, looking closely at the data there was a wide variation in body mass and body fat

within the sample size. Correlations were conducted within the current data set, focusing on BMI and body fat against GE half time and lag phase. No correlation was observed between body fat and BMI for all trials. It was thought that lag phase and half time appeared to be higher as participants body fat was lower, thus, a suggesting a slower gastric emptying in the lower body fat group individuals within the cohort. However, like chapter 3, no correlations between participant body composition and gastric emptying parameters could have been due to the variation of participants recruited. Previous studies have found that body fat can regulate appetite, appetite hormones and energy intake post-exercise (Dorling et al., 2018). This has been evident within appetite hormones, as studies have observed fasting concentrations of acylated ghrelin, PYY and GLP-1 are lower and postprandial changes are blunted when body fat rises (Considine et al., 1996; Coutinho et al., 2018; Karra & Batterham, 2010). Similar to this, increases in leptin and insulin concentrations for individuals with higher body fat have been observed (Bagdade et al., 1967; Considine et al., 1996). Given the existing literature on such differences, body composition of an individual should be taken into account with exercise studies investigating the effect on GI function, EI and appetite hormones in order to provide more accurate findings. It would be particularly interesting to investigate if gastric emptying rate was also affected by body composition and whether individuals at a wide range of adiposity levels respond differently to fasted exercise at various times of day.

In conclusion, these findings demonstrate that GER of a subsequent meal is slower in the evening following fasted exercise. In the postprandial stages, regardless of the time of day, appetite, blood glucose concentration, substrate utilisation, and 24 h post energy intake is not sensitive to an acute bout of low-intensity exercise in the fasted state compared to the fed state. The indication that no compensatory increase in energy intake will occur post-exercise potentially holds positive implications for fasted brisk walking in the long-term control of

weight management. Going forward, it would be valuable to measure appetite-regulatory hormones, to explore whether these are affected by the time of day of fasted exercise, and to control participant criteria for body composition is essential. This can provide a clearer understanding of these findings in relation to different population groups.

5.0. THE EFFECT OF FASTED VERSUS NON-FASTED CYCLING AT DIFFERENT TIMES OF THE DAY ON METABOLIC RESPONSES, GASTROINTESTINAL FUNCTION, AND APPETITE IN LEAN MEN

Some preliminary data was presented as a poster communication at Physiology 2019 Conference, Aberdeen, UK, 8-10th July 2019.

5.1. Introduction

It was shown in chapter 4 that GER is slower in response to a meal following fasted exercise in the evening. As discussed within the previous chapter, it would be valuable to measure gastrointestinal appetite regulatory hormones to explore whether appetite regulatory hormones also follow a diurnal variation. Additionally, implementing a mode of exercise, such as cycling, that is better controllable for intensity is advantageous. The use of cycling may potentially impose a greater physiological demand compared to brisk walking. Due to the capability of increasing intensity and greater recruitment of muscle mass in combination of oxidative capacity being utilised (Carter et al., 2000; Kriel et al., 2018; Millet et al., 2009).

It is well established that time of day plays a key part in the integration of metabolism, hormonal secretion, physical coordination, and sleep (Froy & Miskin, 2010; Patterson et al., 2015). Hormonal fluctuations are exhibited across the 24 h cycle (Gamble et al., 2014). Research over the years have demonstrated endocrine and circadian rhythms to be interconnected, and in order for the internal clock to maintain its internal balance, the internal clock interacts with external factors (Gnocchi & Bruscalupi, 2017; Patterson & Sears, 2017). Hormones such as insulin, leptin and ghrelin are key factors in metabolic regulation, with circadian factors now recognised as regulating their secretion and activity (Gnocchi & Bruscalupi, 2017).

Gut hormones such as GLP-1, PYY (satiety-inducing hormones) and ghrelin (hunger-inducing hormone) play a vital role in regulating appetite and gastric emptying rate, and thus, the glycaemic response to meals (Parr, Heilbronn, and Hawley, 2019). Hormonal secretion is under circadian regulation (Morris et al., 2015). For instance, the secretion of ghrelin peaks mid-evening (approx. 20:00), whilst at its lowest when wakening (Qian et al., 2018); this pattern can, partially, be due to the biological drive to eat at night (Parr, Heilbronn, and Hawley,

2019). Diets that provide greater food intake in the morning compared to the evening are likely to produce sustained weight loss (Garaulet et al., 2013; Jakubowicz et al., 2013). However, no studies have previously focused on the diurnal variation of appetite regulatory hormones in response to fasted versus non-fasted exercise.

The effect of fasted exercise on subjective appetite and appetite hormones has received significant attention within the literature, along with the increased attention on diurnal variation playing a potentially important role in metabolism and appetite regulation over recent years. Therefore, the aims of this study were to 1) assess the effects of fasted exercise versus non-fasted exercise on GER, appetite regulatory hormones, metabolic responses and appetite regulation in lean men. 2) to explore the diurnal variation of these outcomes in response to fasted exercise in lean individuals.

5.2. Methods

5.2.1. Participants

Twelve lean healthy men completed four experimental trials in a randomised order. Following WHO criteria, a lean individual was defined by having a BMI and body fat percentage between 18.5–24.9 $kg.m^{-2}$ and 12-17%, respectively. A sample size estimation was performed using gastric emptying data from Goos et al., (1978) and data from chapter 4, an effect size for both data sets was 0.6, which is considered medium. The sample size required was N = 8 and N = 10, respectively. Total energy intake and glucose data from Bachman et al., (2016) and fat oxidation data from Iwayama et al., (2015) found that an effect size for all three variables was 0.8, which is considered large. The sample size required for this effect size was N = 12, N = 11 and N = 10, respectively. Therefore, the sample size of 12 was decided to be an adequate for the main objective of this study. Participant characteristics are shown in Table 10.

Table 10: Participant characteristics.

	Participants (*n = 12*)
Age (years)	24 ± 3
Height (cm)	179 ± 5
Body mass (kg)	70 ± 7
BMI ($kg \cdot m^{-2}$)	22 ± 1
Body fat (kg)	11 ± 2
Fat free mass (FFM) (kg)	59 ± 6
Body fat (%)	12 ± 2
FFM (%)	87 ± 2
$\dot{V}O_{2peak}$ (ml/kg/min)	45 ± 7
PA levels per week (min)	174 ± 29
Systolic BP (mmHg)	121 ± 10
Diastolic BP (mmHg)	78 ± 9

All values expressed as mean ± SD

5.2.1. Experimental trials

The trials and study design of this chapter are the same as those within chapter 4 of this thesis, but the mode of exercise was changed to cycling. Participants completed four experimental trials in a randomised crossover fashion. These were two-morning trials (08:00 h - 13:00 h) fasted (AM-FASTED) and non-fasted (AM-FED) and two evening trials (15:00 h - 20:00 h) fasted (PM-FASTED) and non-fasted (PM-FED).

On the morning trials, participants were required to fast from 00:00 the evening before, and on the evening trials, participants were required to have breakfast and then fast from 07:00 with the exception of plain water consumption. Baseline assessments of appetite, substrate utilisation and a blood sample were collected using procedures outlined in general methods.

Following baseline measurements, participants ingested the test 'breakfast' in FED within a 15 min period or remained fasted in FASTED. The test 'breakfast' consisted of 30 g of breakfast cereal with 125 mL of semi-skimmed milk, and a croissant, which provided in total 1,438 kJ (341 kcal) and contained 10.2 g fat, 48 g carbohydrate and 11.2 g protein. Participants consumed all the breakfast within the 15 min window. Post-breakfast ratings of appetite, substrate utilisation and blood sample were measured at the end of the 15 min breakfast period. Participants then rested in a semi-supine position on a bed for 1 h before commencement of the exercise protocol. During this 1 h rest period, further measures of appetite were taken every 15 min and substrate utilisation every 30 min. The exercise protocol involved 60 min of cycling on a Lode Corival ergometer at a workload intensity of 60% $\dot{V}O_{2peak}$ pre-determined in the preliminary trial (average workload 144 ± 8 watts). As with all previous experimental chapters, heart rate and RPE were measured every 15 min throughout the exercise, with expired air measured continuously. The last 5 min of each 10 min segment was used to calculate substrate utilisation. After completion of the exercise bout, participants recovered for 30 min (showered if desired) before they ingested a standardised meal. Consistent with the methods in chapter 4, the meal was 800 g (2 cans) of vegetable soup (1584 kJ (376 kcal)), containing 6.8 g fat, 66.4 g carbohydrate, 8.8 g protein. Subjective feelings of appetite and substrate utilisation were measured every 15 min post-ingestion for a total period of 2 h. A schematic diagram of the experimental protocol is presented in Figure 21. 24 h energy intake post trial was recorded via a weighed food diary by the participants and later analysed using Nutritics.

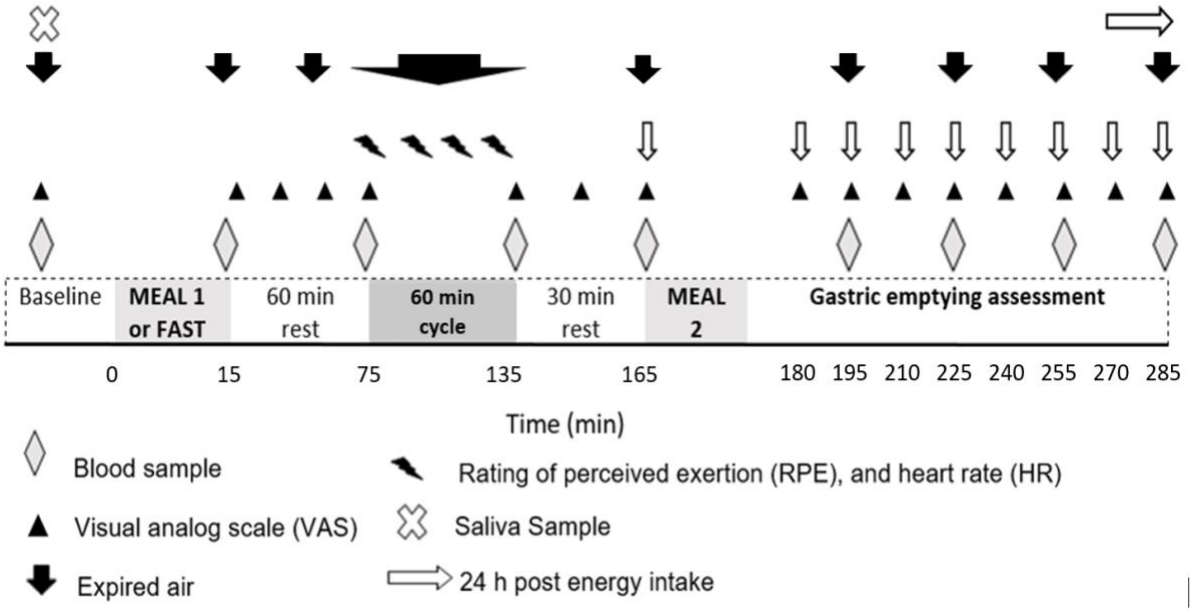

Figure 20: Schematic diagram of the experimental trial protocol, represented in minutes of the trial.

5.2.2. Statistical Analysis

A three-way (trial x time of day x time across trial) repeated-measures analysis of variance (ANOVA) to assess trial (fasted vs. fed) x time of trial (morning vs. evening) x time across trial differences for differences for blood serum concentration of hormones and metabolites, gastric emptying DOB, substrate oxidation, and VAS ratings. Incremental area under the curve (iAUC) was calculated using the Time Series Response Analyser (TSRA) created by Narang et al., (2020). A two-way repeated-measures ANOVA was used to assess iAUC for trial x time of day differences for blood serum concentration of hormones and metabolites, gastric emptying DOB, substrate oxidation, and VAS ratings. A two-way repeated-measures ANOVA was used to assess trial (fasted vs. fed) x time of trial (morning vs. evening) differences for gastric emptying $T_{½}$ and T_{lag} data, salivary melatonin concentration and 24-hour energy intake. Sphericity for repeated measures was assessed, and where appropriate, Greenhouse–Geisser corrections were applied for epsilon <0.75, and the Huynh–

Feldt correction adopted for less severe asphericity. Significant F-tests were followed by dependent Student's t-Tests or one-way repeated measures ANOVA and Bonferroni adjusted pairwise comparisons as appropriate. One-way repeated-measures ANOVA was also conducted for ensuring standardisation of body mass between all trials and mid-point of sleep. All analyses were carried out using IBM SPSS statistics (v25.0 for Windows; SPSS, Chicago, IL). The level of significance was set at $P<0.05$. Descriptive data are expressed as mean ± standard deviation (SD).

5.3. Result

5.3.1. Trial order analysis

Analysis demonstrated that there were no significant differences in responses according to the order of trials that the participants completed (all P>0.05) (Table 11).

Table 11: Trial order analysis, mean ± SD for gastric emptying half time ($T_{1/2}$), and incremental area under the curve (iAUC) for subjective feeling of hunger, and glucose concentrations.

Variables	Trial 1	Trial 2	Trial 3	*Trial 4*	P value
Gastric emptying $T_{1/2}$ (min)	125 ± 41	115 ± 20	144 ± 37	112 ± 38	0.084
Subjective feeling of hunger (mm^{-1} x 285 min)	1039 ± 1649	1824 ± 3044	1070 ± 1255	1030 ± 1343	0.667
Glucose concentrations ($mmol/L^{-1}$ x 285 min)	132 ± 77	192 ± 139	133 ± 132	167 ± 99	0.284

5.3.2. Gastric emptying

A three-way repeated-measures ANOVA reported no trial effect for DOB (P = 0.948), although, a significant time of day (P = 0.002), time effect (P< 0.001) but no interaction effect was observed (P = 0.841; Figure 22a). Two-way ANOVA for iAUC showed a significant time of day effect for DOB (P=0.003), but no significant trial effect (P = 0.912) or interaction (trial x time of day) (P = 0.878) (Figure 22b). No significant main effect of trial (P = 0.973) or trial x time of day interaction effect (P = 0.511) was observed for T_{lag}, although a significant time of day effect was found (P < 0.001). T_{lag} was slower in the evening trials compared to the morning trials (AM-FASTED vs. PM-FASTED; AM-FED vs. PM-FED; 64 ± 16 vs. 81 ± 17 min, P = 0.005 and 61 ± 17 vs. 84 ± 24 min, P = 0.041, respectively; Figure 23a). Two factor ANOVA demonstrated a time-of-day effect for $T_{½}$ (P = 0.040), but no main effect for trial (P = 0.117) or trial x time of day interaction effect (P = 0.441). $T_{½}$ was slower in the PM-FASTED trial compared to AM-FASTED (145 ± 37 vs 115 ± 21 min, P = 0.036) (Figure 23b).

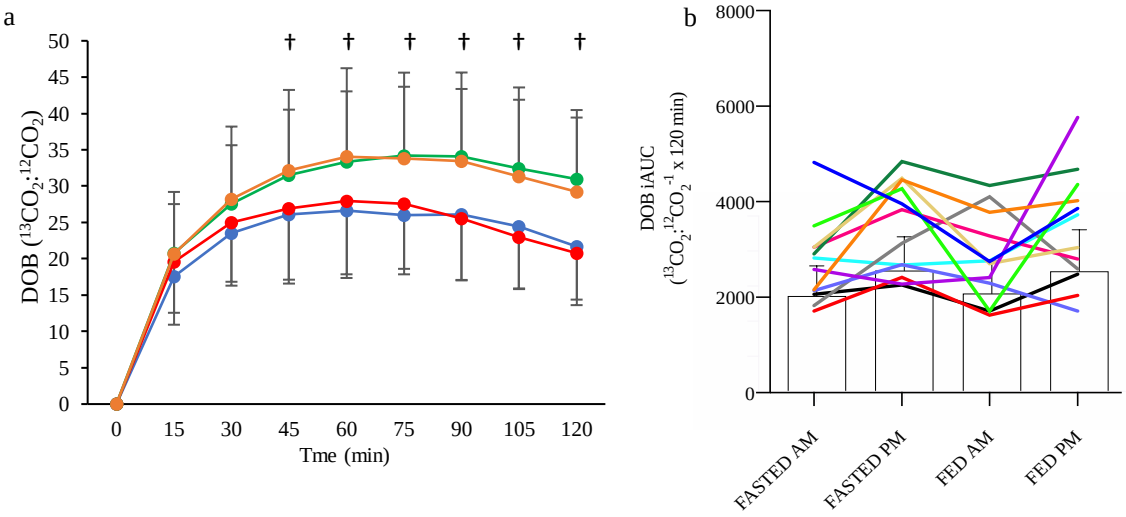

Figure 21: Gastric emptying assessment. a) Gastric emptying delta over baseline (DOB) and b) DOB iAUC, for all four trials of MEAL 2 (800 g vegetable soup). The blue line corresponds to AM-FASTED, red line = AM-FED, green line = PM-FASTED, orange = PM-FED. † indicates diurnal significance between morning and evening trial (i.e. AM vs. PM). Values represent mean ± SD; n=12.

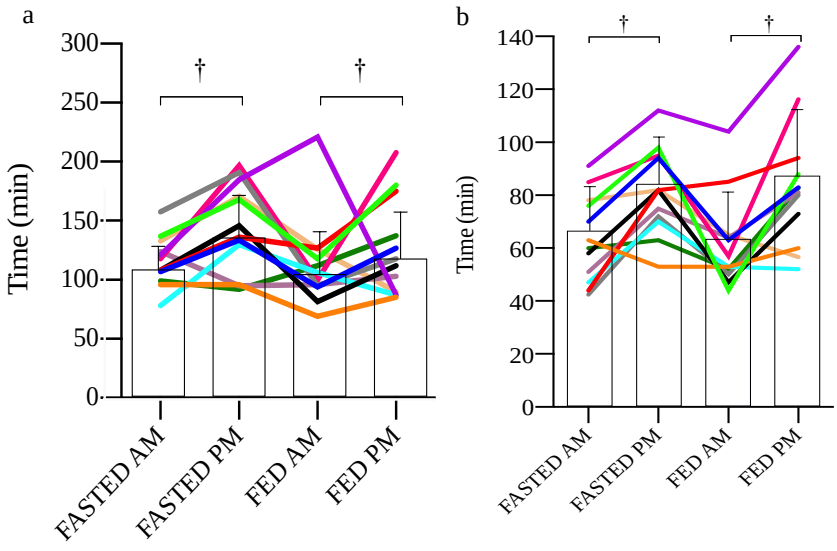

Figure 22: Gastric emptying assessment, AM-FASTED, AM-FED, PM-FASTED, and PM-FED a) Gastric emptying half time ($T_{½}$) and time of maximal emptying rate (T_{lag}) for all four trials of MEAL 2 (800 g vegetable soup). † indicates diurnal significance between morning and evening trial (i.e. AM vs. PM). Values represent mean ± SD; n = 12.

5.3.3. Gut hormones

No significant main effect for trial (P = 0.508), time of day (P = 0.571), or interaction effect (trial x time of day x time) (P = 0.652) was observed for ghrelin concentrations, although a significant main effect for time was observed (P < 0.001) (Figure 24a). A significant main effect for trial (P = 0.009), time (P < 0.001) was observed for GLP-1 concentrations, although no significant main effect for time of day (P = 0.182) or interaction effect (P = 0.073) (Figure 24b). No significant main effect for trial (P = 0.561), time of day (P = 0.367), or interaction effect (P = 0.200) for insulin concentrations, although a significant main effect for time was observed (P < 0.001) (Figure 24c). A significant time of day effect (P = 0.002), and time effect (P = 0.012) was observed for pancreatic polypeptide (PP) concentrations, although no main effect for trial (P = 0.078) or interaction effect (P = 0.347) was observed (Figure 24d). Peptide tyrosin tyrosin (PYY) was measured but not presented/anaylsed due to insufficient analysis.

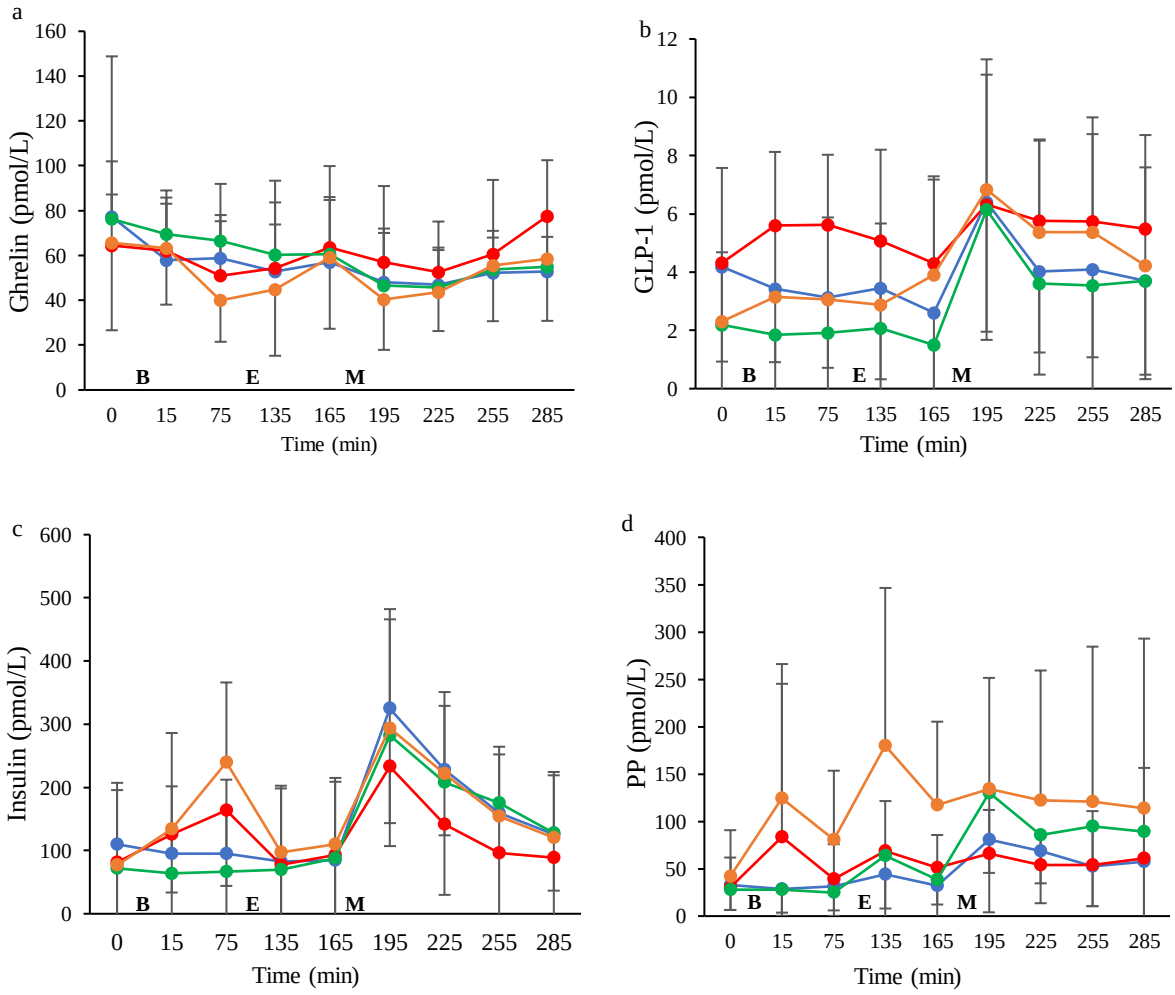

Figure 23: Hormonal responses during trials. Serum concentrations of a) ghrelin, b) glucagon-like peptide-1 (GLP-1), c) insulin, d) Pancreatic polypeptide (PP). Values represent mean ± SD; n=12. The blue line corresponds to AM-FASTED, red line = AM-FED, green line = PM-FASTED, orange = PM-FED. '**B**' indicates breakfast period, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, '**E**' indicates Exercise period, where participants completed a 1 h cycle at 60% of their $\dot{V}O_{2peak}$, and '**M**' indicates standardised lunch meal where 800 g vegetable soup was ingested.

Serum insulin iAUC concentrations showed a significant trial effect (P = 0.010), time of day (P = 0.034), and interaction effect (trial x time of day) (P = 0.040). iAUC for insulin concentrations were greater in the PM-FED trial (2731 ± 16422 pmol/L) compared to AM-FASTED (13013 ± 7346 pmol/L $^{-1}$ x 285 min; P = 0.031) and AM-FED (13532 ± 6390 pmol/L $^{-1}$ x 285 min; P = 0.047). Similarly, PP iAUC showed a significant trial effect (P = 0.026), time of day (P = 0.001), and trial x time of day interaction effect (P = 0.048). iAUC for PP concentrations were greater in the PM-FED trial (21245 ± 19412 pmol/L) compared to AM-FASTED (4686 ± 4967 pmol/L $^{-1}$ x 285 min; P = 0.018) and AM-FED (7610 ± 7795 pmol/L $^{-1}$ x 285 min; P = 0.017). GLP-1 iAUC showed a significant trial effect (P = 0.009), time of day (P = 0.017), but no trial x time of day interaction (P = 0.991). Ghrelin iAUC concentrations showed no significant trial effect (P = 0.388), time of day (P = 0.076), or trial x time of day interaction effect (P = 0.635) (Figure 25).

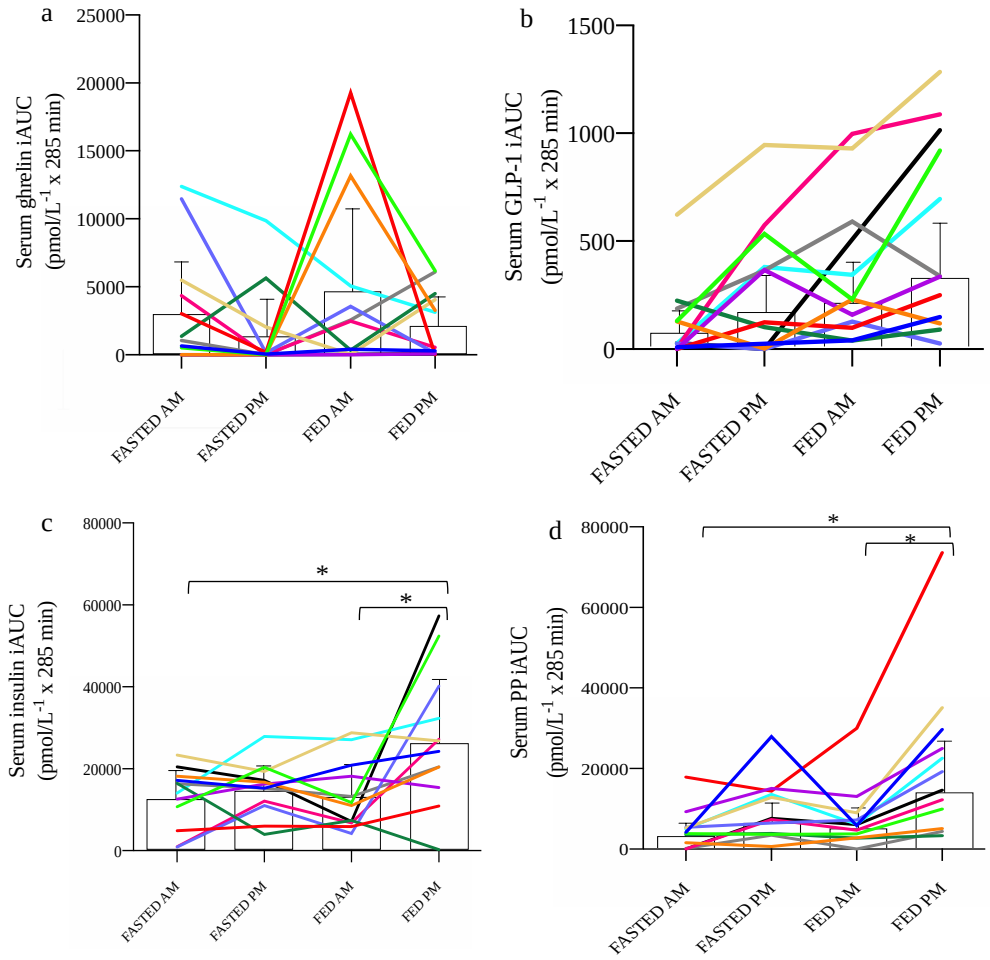

Figure 24: Hormonal responses during both trials. iAUC serum concentrations of (a) Ghrelin (n=12), (b) Glucagon like peptide-1 (GLP-1), (c) Insulin, and (d) Pancreatic polypeptide (PP). Values represent mean ± SD; n = 12. * indicates significant ($P < 0.05$) difference between trials.

5.3.4. Metabolic markers

A significant main effect for trial ($P = 0.002$), time of day ($P = 0.039$), time ($P < 0.001$) and interaction effect ($P = 0.002$) was observed for glucose concentrations. Glucose concentrations were higher in AM-FED compared to PM-FASTED post-breakfast (4.8 ± 0.4 vs. 4.3 ± 0.3 mmol/L; $P = 0.022$), however pre-exercise AM-FED was significantly lower compared to PM-FED (3.7 ± 0.7 vs. 4.9 ± 1.4 mmol/L; $P = 0.043$). Post-exercise, PM-FED glucose concentrations were significantly lower compared to AM-FASTED (4.2 ± 0.4 vs. 4.7 ± 0.2 mmol/L; $P = 0.006$). Pre-soup ingestion, glucose concentrations were significantly lower in the PM-FASTED trial compared to those observed in the AM-FED trial (4.2 ± 0.1 vs. 4.7 ± 0.4 mmol/L; $P = 0.048$). Glucose concentrations during the AM-FED trial remained significantly lower at 30 min and 60 min post-soup compared to all trials and PM-FASTED remained higher at 90 min than PM-FED (all $P<0.05$) (Figure 26a).

A significant main effect for trial ($P = 0.011$), time of day ($P < 0.001$), time ($P < 0.001$), was observed for NEFA concentrations, although no interaction effect was found ($P = 0.223$). (Figure 26b). No significant main effect for trial ($P = 0.083$), time of day ($P = 0.410$), or interaction effect ($P = 0.738$), although a main effect for time was observed for triglycerides concentrations ($P < 0.001$) (Figure 26c). Similarly, no significant main effect for trial ($P = 0.274$), time of day ($P = 0.177$), or interaction effect ($P = 0.606$) was observed for cholesterol concentrations, although a main effect for time was found ($P < 0.001$) (Figure 26d).

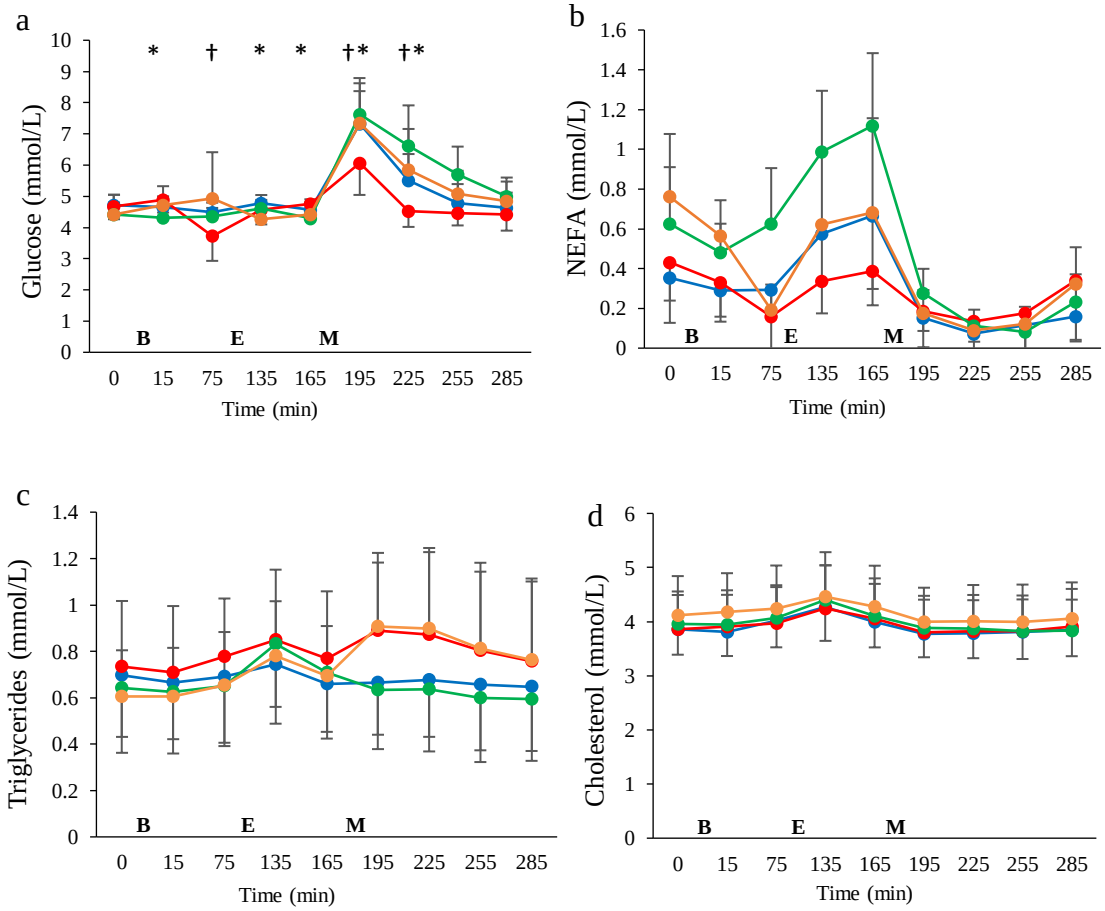

Figure 25: Metabolic responses during trials. Serum concentrations of a) glucose, b) non-esterified fatty acids (NEFA), c) triglycerides, and d) cholesterol. Values represent mean ± SD; n=12. The blue line corresponds to AM-FASTED, red line = AM-FED, green line = PM-FASTED, orange = PM-FED. * indicates significance ($P < 0.05$) versus corresponding time point in other trial (i.e. FASTED vs. FED), † indicates diurnal significance between morning and evening trial (i.e. FED AM vs. FED PM). '**B**' indicates breakfast period, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, '**E**' indicates Exercise period, where participants completed a 1 h cycle at 60% of their $\dot{V}O_{2peak}$, and '**M**' indicates standardised lunch meal where 800 g vegetable soup was ingested.

Glucose iAUC showed a significant trial effect (P = 0.012), time of day effect (P = 0.001), and trial x time of day interaction effect (P = 0.042). Glucose iAUC was significantly higher in the evening, for PM-FASTED and PM-FED compared to AM-FASTED (P = 0.001; P = 0.012, respectively) and AM-FED (P < 0.001; P < 0.001, respectively) (224 ± 102 mmol/L^{-1} x 285 min and 215 ± 128 mmol/L^{-1} x 285 min vs. 118 ± 73 mmol/L^{-1} x 285 min and 68 ± 66 mmol/L^{-1} x 285 min; P < 0.001). Glucose iAUC for AM-FED was significantly lower than all three other trials (P<0.005). Triglycerides iAUC shown a significant trial effect (P = 0.004), but no time of day (P = 0.130) or interaction effect (P = 0.394). No significant trial effect (P = 0.873), time of day effect (P = 0.961), and interaction effect (P = 0.957) was found for cholesterol iAUC. NEFA iAUC concentrations shown a significant trial effect (P = 0.001), but no time of day effect (P = 0.100), or interaction effect was observed (P = 0.086) (Figure 27).

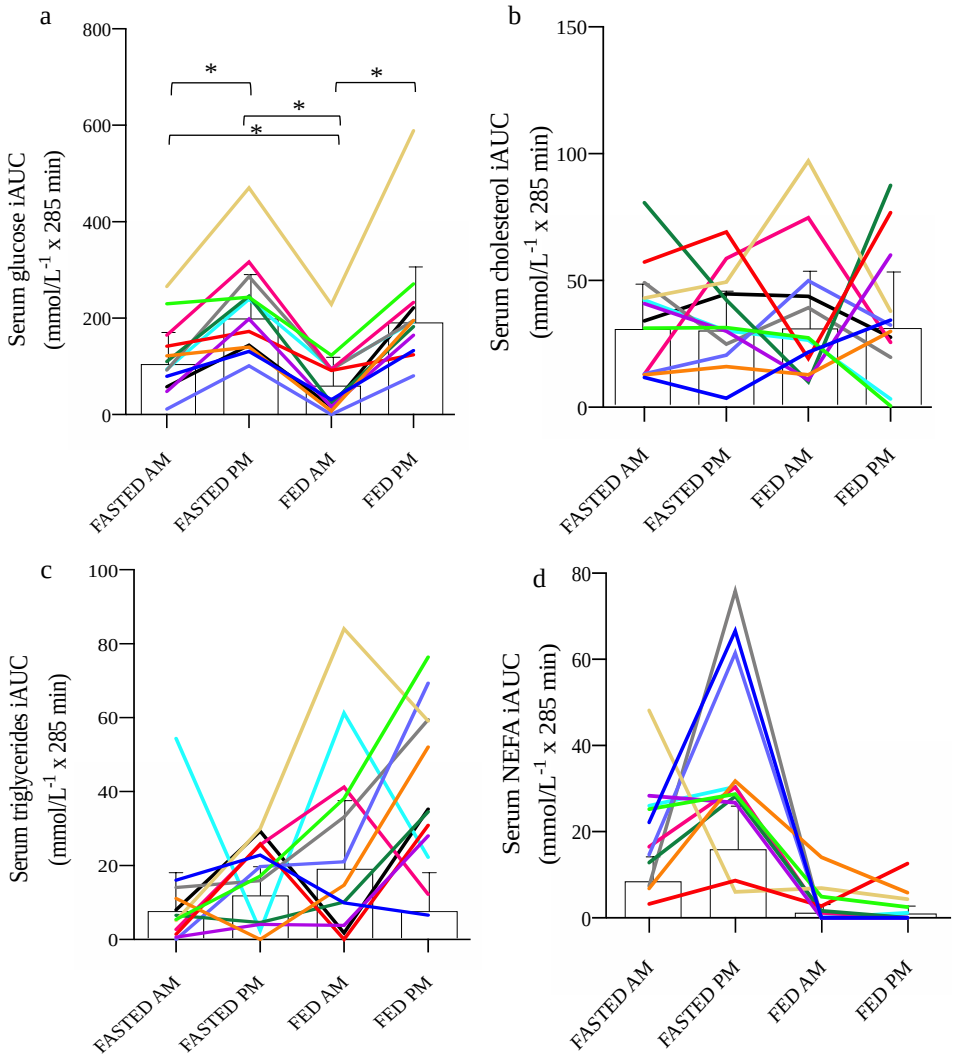

Figure 26: Metabolic responses during trials. iAUC serum concentrations of (a) Glucose, (b) Cholesterol, (c) Triglycerides and (d) Non-esterified fatty acids (NEFA). Values represent mean ± SD; n=12. * indicates significant ($P < 0.05$) difference between trials.

5.3.5. Appetite

A significant main effect for trial ($P = 0.021$), time of day ($P < 0.001$), time ($P < 0.001$), although no interaction effect (trial x time of day x time) ($P = 0.228$) was observed for feelings of hunger (Figure 28a). A significant main effect for trial ($P = 0.016$) and time ($P < 0.001$) for fullness, although no main effect for time of day ($P = 0.344$) or interaction effect (trial x time of day x time) ($P = 0.172$) (Figure 28b). No main effect for trial ($P = 0.120$), time of day ($P = 0.433$), or interaction effect (trial x time of day x time) for PFC ($P = 0.709$), only main effect observed was for time ($P < 0.001$) (Figure 28c). No main effect for bloated for trial ($P = 0.082$), time of day, ($P = 0.488$), or interaction effect ($P = 0.684$), only main effect for time ($P < 0.001$) was observed (Figure 28d). Likewise, no main effect for trial ($P = 0.072$), time of day ($P = 0.164$), or interaction effect ($P = 0.454$) was observed for food satisfaction again, only main effect observed for time ($P < 0.001$) (Figure 28e).

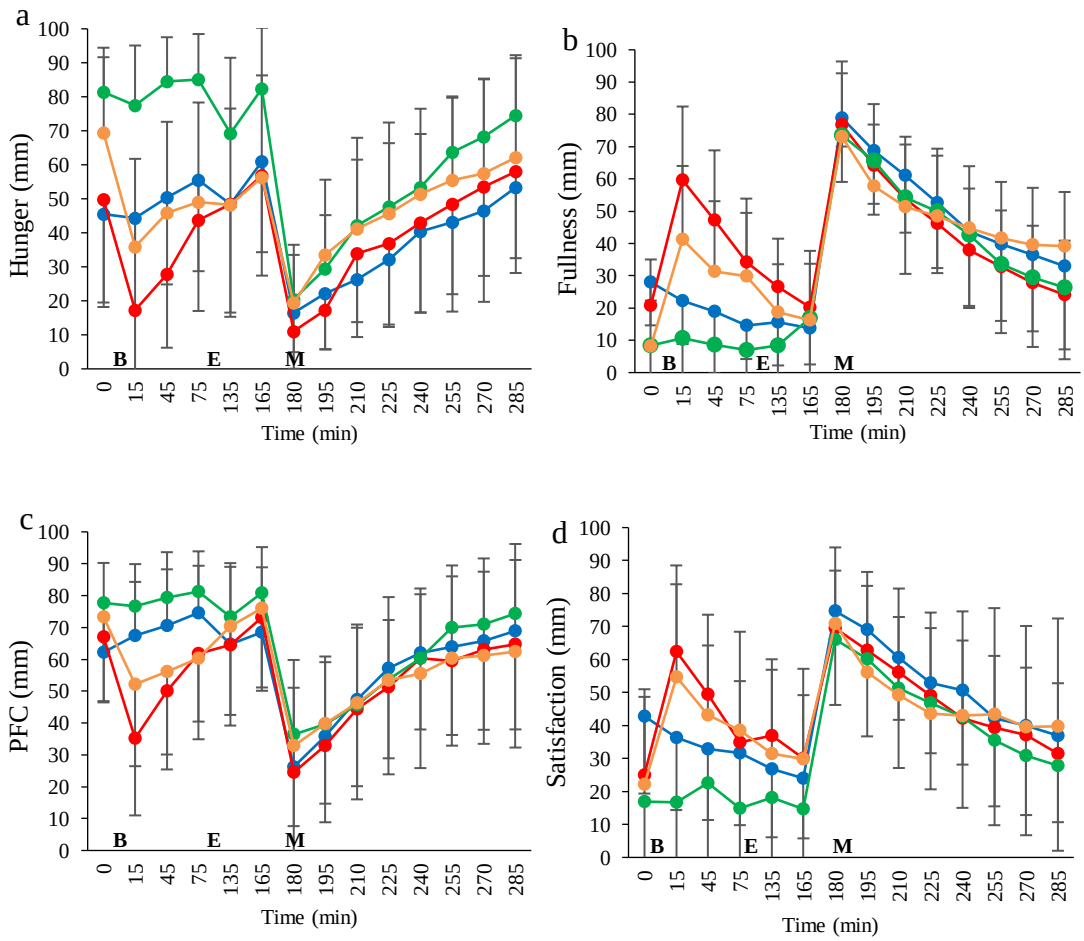

Figure 27: Appetite scores during trials, assessed by 100 mm visual analogue scale (VAS); (a) Hunger, (b) Fullness, (c) Prospective food consumption (PFC), and (d) Food satisfaction. Values represent mean ± SD n=12. The blue line corresponds to AM-FASTED, red line = AM-FED, green line = PM-FASTED, orange = PM-FED. '**B**' indicates breakfast period, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, '**E**' indicates Exercise period, where participants completed a 1 h cycle at 60% of their $\dot{V}O_{2peak}$, and '**M**' indicates standardised lunch meal where 800 g vegetable soup was ingested.

Hunger iAUC ratings showed no significant effect of trial (P = 0.171), or interaction effect (trial x time of day) (P = 0.170), but a significant time of day effect was observed (P = 0.016). No significant effect trial effect for prospective food consumption iAUC ratings (P = 0.267), time of day (p = 0.129), or interaction effect was found (P = 0.150). iAUC for fullness ratings had a significant trial effect (P = 0.0001), time of day (P = 0.035), but no interaction effect (P = 0.998). Food satisfaction iAUC was significantly different between trials (P = 0.005) but no significant time of day (P = 0.183), or interaction effect observed (P = 0.266) (Figure 29).

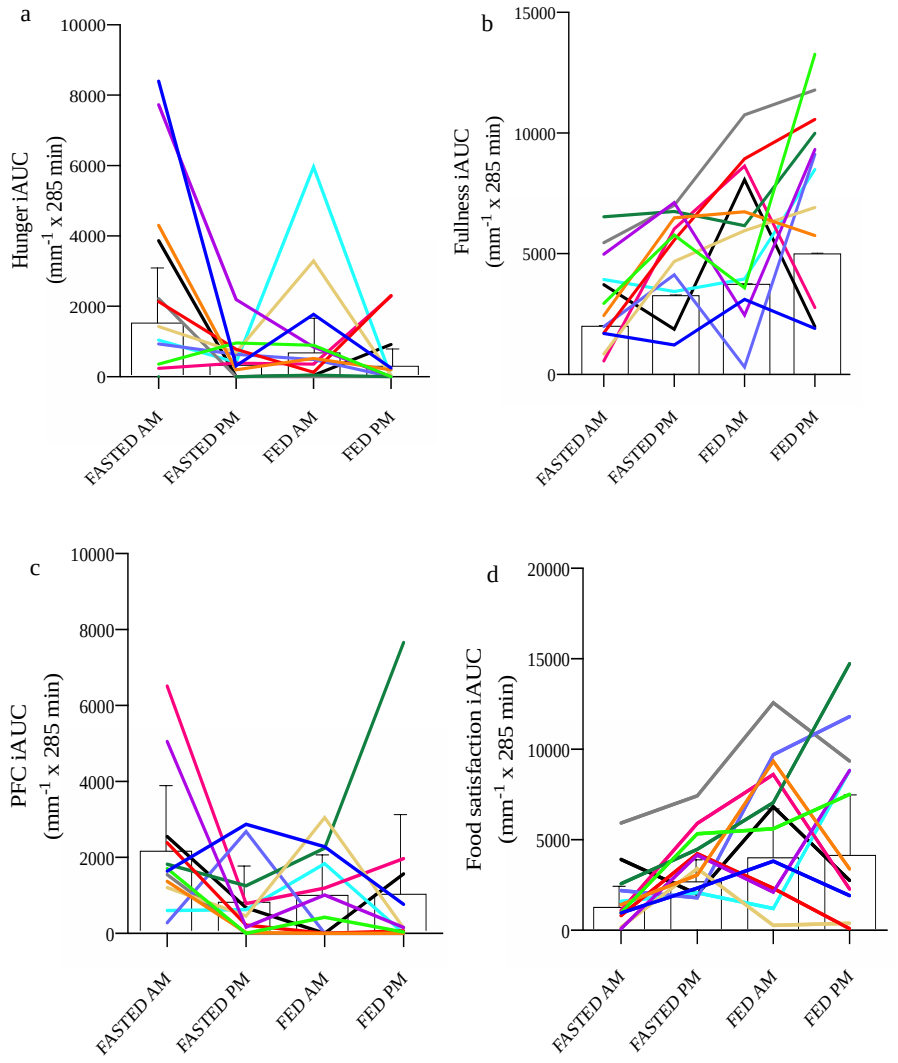

Figure 28: iAUC appetite scores during trials, assessed by 100 mm visual analogue scale (VAS); (a) Hunger, (b) Fullness, (c) Prospective food consumption (PFC), (d) Food satisfaction. Values represent mean ± SD n=12.

5.3.6. Substrate oxidation

A significant main effect of trial ($P<0.001$), and time ($P<0.001$), but no time of day effect ($P = 0.773$) or interaction effect (trial x time of day x time) was observed for fat oxidation ($P= 0.077$) (Figure 30a). Fat oxidation iAUC showed a significant trial effect ($P = 0.001$) and trial x time of day interaction effect ($P = 0.039$), but no time of day effect observed ($P = 0.670$) (Figure 30b). Fat oxidation iAUC was greater in both AM-FASTED and PM-FASTED trials compared to AM-FED ($P = 0.001$; $P = 0.001$, respectively) and PM-FED ($P = 0.001$; $P = 0.007$, respectively) (29 ± 10 g/min^{-1} x 285 min and 23 ± 8 g/min^{-1} x 285 min vs. 7 ± 3 g/min^{-1} x 285 min and 11 ± 8 g/min^{-1} x 285 min, respectively; $P < 0.001$).

A significant main effect for trial ($P<0.001$) and time ($P<0.001$), but no time of day ($P = 0.449$) or interaction effect (trial x time of day x time) ($P = 0.581$) for CHO oxidation (Figure 30c). iAUC for CHO oxidation showed a significant trial effect ($P = 0.001$), but no time of day ($P = 0.955$), or interaction effect was observed ($P = 0.537$) (Figure 30d).

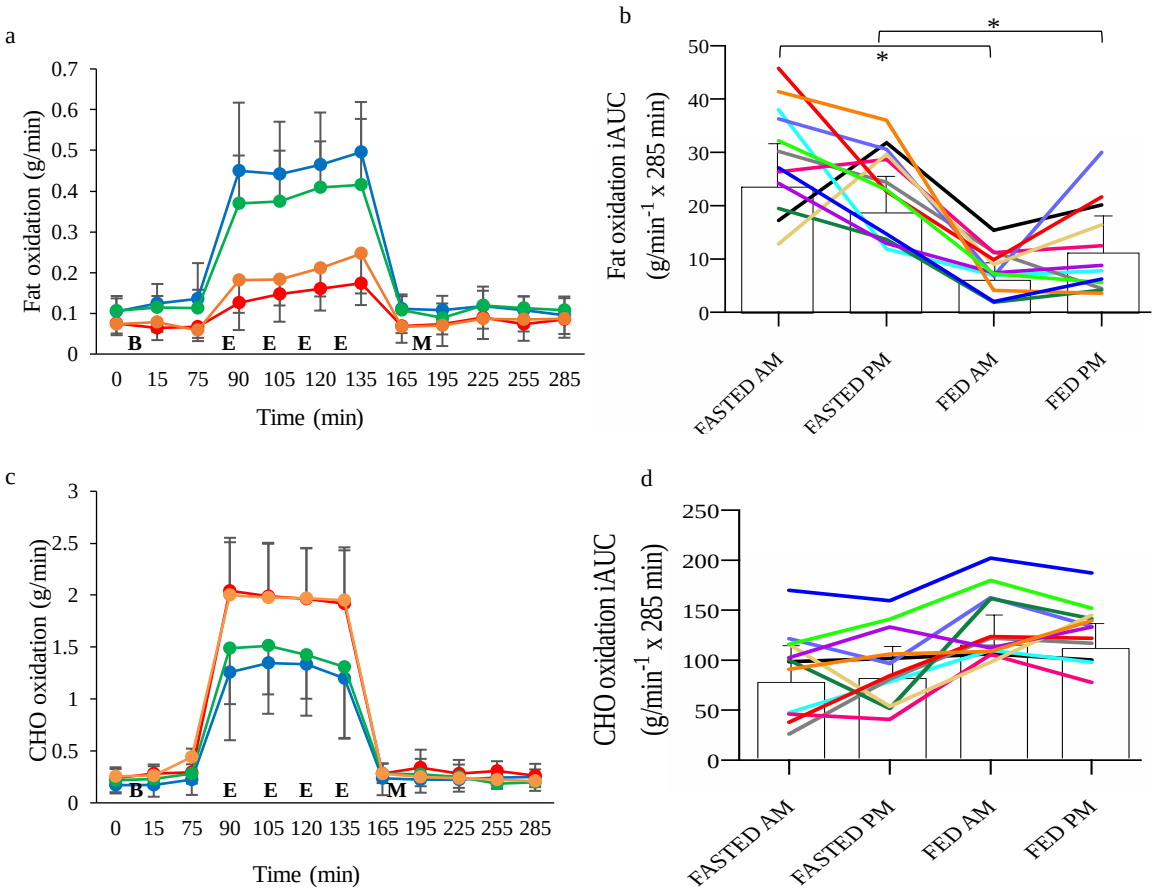

Figure 29: Substrate utilisation; Fat oxidation (a), iAUC for fat oxidation (b) and carbohydrate oxidation (c), iAUC for carbohydrate oxidation (d) during the trials, AM-FASTED, AM-FED, PM-FASTED, and PM-FED. The blue line corresponds to AM-FASTED, red line = AM-FED, green line = PM-FASTED, orange = PM-FED. Values represent mean ± SD; n = 12. * indicates significant ($P < 0.05$) difference between trials. '**B**' indicates breakfast period, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, '**E**' indicates Exercise period, where participants completed a 1 h cycle at 60% of their $\dot{V}O_{2peak}$, and '**M**' indicates standardised lunch meal where 800 g vegetable soup was ingested.

5.3.7. Physiological measurements during the exercise bout

No significant differences between HR, RPE and VO_2 measurements were seen across both trials ($P>0.05$) (Table 12).

Table 12: Illustrates the physiological responses during the cycling exercise within all four trials.

	FASTED-AM					**FED-AM**				
Time (min)	0	15	30	45	60	0	15	30	45	60
RPE	6±0	12±4	13±2	13±2	13±3	6±0	12±2	13±2	13±2	13±2
HR (bpm)	75±3	143±5	145±4	146±2	147±2	74±2	142±2	143±2	145±2	146±3
VO_2 (L/min)	4±5	27±8	27±3	27±4	27±3	5±5	27±6	27±3	27±4	27±7
VO_2 (%)	11±1	60±8	60±4	60±3	60±5	13±4	60±1	60±5	60±3	60±6
	FASTED-PM					**FED-PM**				
Time (min)	0	15	30	45	60	0	15	30	45	60
RPE	6±0	12±2	13±4	13±4	13±5	6±0	13±4	13±4	13±3	13±3
HR (bpm)	76±3	142±5	145±3	146±3	146±2	75±2	144±2	145±4	145±2	146±2
VO_2 (L/mn)	6±6	28±9	27±3	57±6	27±6	5±3	27±5	27±4	27±6	27±6
VO_2 (%)	14±5	60±8	60±8	60±5	60±4	13±9	59±7	60±5	60±9	61±3

Rating of perceived exertion (RPE), heart rate (HR) and oxygen consumption (VO_2)

5.3.8. 24 h energy intake

A two-way factor ANOVA observed no significant main effect for trial (P = 0.388), time of day (P = 0.065), or interaction effect (trial x time of day) (P = 0.409) for 24 h energy intake (Table 13).

Table 13: 24 h post-trial energy intake and macronutrient breakdown for all participants (n=12).

	AM-FASTED	AM-FED	PM-FASTED	PM-FED
Energy intake (kcal)	2861 ± 618	3062 ± 523	2585 ± 703	2607 ± 733
Protein (g)	134 ± 24	172 ± 40	131 ± 30	150 ± 49
Carbohydrate (g)	330 ± 109	301 ± 68	246 ± 107	275 ± 83
Fat (g)	112 ± 45	130 ± 46	119 ± 42	117 ± 41

Values expressed as mean ± SD

5.3.9. Salivary melatonin

A two factor ANOVA demonstrated a main effect of time of day (P = 0.004), no main effect of trial (P = 0.756) and no interaction effect (P = 0.888) for saliva melatonin levels. Salivary melatonin concentration was significantly different between morning and evening trials (AM-FASTED, AM-FED vs. PM-FASTED, PM-FED; P<0.001). No significant differences between trials for midpoint of sleep (P = 0.152) was detected (Table 14).

Table 14: Melatonin measurements (mean ± SD).

	AM-FASTED	AM-FED	PM-FASTED	PM-FED
Melatonin (pg/mL)	12.4 ± 9.7 *	12.7 ± 8.2*	2.8 ± 1.0*	2.9 ± 1.7*
Midpoint of sleep	02:35 ± 0:04	02:25 ± 0:05	02:37 ± 0.01	02:42 ± 0.02

* indicates significant difference to corresponding trial (AM-FASTED vs. PM-FASTED).

5.4. Discussion

The primary findings observed a delay in GER in the evening. Glucose iAUC concentrations were greater in the two evening trials in comparison to that of morning trials. Pancreatic polypeptide (PP) iAUC and insulin concentrations iAUC were greater in the PM-FED trial compared to the morning trials. Fat oxidation iAUC was greater in the fasted trials compared to the fed trials, regardless of time of day. No differences were observed in appetite or 24 h energy intake post trials in response to fasted versus fed exercise. Except for PP and insulin, the findings refute the hypothesis that all appetite regulatory hormones differ and follow diurnal variation corresponding with gastric emptying rate in lean individuals.

No studies have previously assessed the diurnal variation on gastrointestinal function, metabolic markers and appetite regulation in response to fasted versus non-fasted exercise in lean individuals. Diurnal variations are apparent in gastrointestinal absorption rate and GER, with gastric emptying being slower in the evening (Goo et al., 1987; Grammaticos, Doumas and Koliaskos, 2015; Orr et al., 2004). This is consistent with the current findings, with a slower gastric emptying rate observed in the evening. Furthermore, Cardoso-Júnior et al., (2007) observed that lean individuals have a slower gastric emptying rate in comparison to overweight individuals. Several investigations have reported obese individuals to have a rapid gastric emptying rate (Zahorska-Markiewicz et al., 1986; Wright et al., 1983; Tosetti et al., 1996; Vazquez et al., 2006), whilst a delayed gastric emptying rate in overweight individuals in other studies (Di Ciaula, Wang, Portincasa, 2012; Buchholz et al., 2013). It would be interesting for future studies to investigate whether gastric emptying is also affected by diurnal variation in overweight populations.

A novel aspect of the current study was that appetite regulatory hormones were also measured alongside GER to explore if differences in GER correspond to appetite regulatory

hormones, following a diurnal variation. A previous study found that fasting at various times of the day (0800-1700 h or 1200-2100 h) did not change the suppression of ghrelin or the rise in PYY, GLP-1 postprandially (Hutchison et al., 2019). The results of this study are in line with previous studies, with no differences observed between fasted versus fed exercise or time or day, excluding PP iAUC concentrations being greater during the PM-FED trial compared to the other three trials. The present investigation found no differences in ghrelin, GLP-1 or insulin concentrations between trials. Similar to the current findings for subjective appetite and energy intake, Betts et al., (2014), investigated the effects of the casual links between breakfast behaviours and energy balance in free-living lean men and reported no metabolic adaptation to breakfast consumption with limited subsequent suppression of appetite than with fasting. Comparable findings have also been found more recently with neither breakfast nor fasted prior to exercise also affect post-exercise energy intake (Edinburgh et al., 2019).

Glucose iAUC were higher during the evening in comparison to the morning trials. This is consistent with previous investigations that have found glucose concentrations in the evening trials to be higher than those in the morning in healthy young adults (Takahashi et al., 2018). Meal timing has been indicated to be an important factor in regulating glucose concentrations, insulin sensitivity, and with postprandial glucose levels reporting to be higher in the evening compared to the morning (Bandín et al., 2015; Bo et al., 2015b; Morris, Yang, et al., 2015b; Takahashi et al., 2018). Other studies have suggested that consuming food later in the evening can be associated with obesity and metabolic syndrome (Arora & Taheri, 2015; Reutrakul et al., 2013; Yu et al., 2015). However, the physiological mechanism for glucose metabolism in the postprandial period between morning and evening remains unclear. Potential mechanisms for this diurnal variation of metabolic differences could be due to insulin sensitivity decreasing later in the day (Cauter et al., 1989; Lindgren et al., 2009; Scheer et al., 2009). However, it is

important to highlight that the pre-trial meal was not standardised across participants before the trial and any diurnal variations in measurements could be influenced by this. Although, the diets 24 h before all trials were standardised within subjects and all subjects conducted the identical fasting timeframe before the trials to minimise any effects on the outcomes measured. Furthermore, hormonal levels of GLP-1 (acts as stimulate for insulin secretion) is elevated during the postprandial period (Drucker & Nauck, 2006), and has also been shown to elevate during the afternoon (Gil-Lozano et al., 2015; Mingrone et al., 2009; Salera et al., 1983). However, no differences in GLP-1 were found between trials as well as no other differences in other metabolic or hormonal markers were found. Differences in digestion, absorption and metabolism in the stomach and intestines that are modulated by diurnal variation could also be a possible mechanism contributing to these differences (Vaughn et al., 2014), which could correspond with the slower GER observed in the current findings in the evening.

The minimal differences in appetite regulatory hormones in the current study corresponds with the lack of differences between subjective feelings of hunger and 24 h energy intake post trials, which could suggest, that fasted exercise regardless of the time of day, produces a greater negative daily energy balance and could be a beneficial strategy to induce a short-term energy deficit. However, it is important to note that the lack of changes observed in appetite hormones becoming apparent may be due to the intensity of exercise not being high enough to elicit physiological significant differences in hormonal responses, or the short measurement over time post-exercise (2 h) (Ueda et al., 2009).

In conclusion, gastric emptying rate is slower in the evening in lean individuals. Fasted exercise did not elicit any compensatory effects on appetite regulatory responses, subjective feelings of hunger, or 24 h energy intake, thus sustaining the exercise-induced energy deficit on the day of exercise. These findings suggest that fasted exercise could be a useful method for

inducing a short-term negative energy balance in lean individuals and could serve as a practical tool for weight management. Future work is required exploring whether these findings would resemble or differ in overweight populations.

6.0. THE EFFECT OF FASTED VERSUS NON-FASTED CYCLING AT DIFFERENT TIMES OF THE DAY ON METABOLIC RESPONSES, GASTROINTESTINAL FUNCTION, AND APPETITE IN OVERWEIGHT MEN

Some preliminary data was accepted as a poster communication at ACSM 2020, San Francisco, May 2020.

6.1. Introduction

It was shown in chapter 4 and 5 that GER is slower in response to a meal following fasted exercise in the evening, in normal weight and lean individuals; however, whether this is also apparent in overweight individuals is unclear. There is considerable variability in body mass within western societies (Li et al., 2015). A complex variety of factors influence energy intake (EI) and energy expenditure (EE). These factors may contribute to the wide variability of whole-body mass observed between individuals. With this in mind, subjective appetite, appetite-regulatory hormones, and energy intake following exercise could be altered by body fat. This has previously been observed with appetite-regulatory hormones, as body fat increases fasting levels of acylated ghrelin, PYY and GLP-1 being lower and postprandial changes are less sensitive (Considine et al., 1996; Coutinho et al., 2018; Karra & Batterham, 2010), whilst leptin and insulin concentrations are elevated (Bagdade et al., 1967; Coutinho et al., 2018). Due to these notable differences, previous exercise studies measuring appetite, appetite-regulatory hormone and EI have been conducted on individuals with a variety of body compositions.

The findings on the effect of exercise on appetite in overweight individuals has been inconsistent, with some studies showing an exercise-induced suppression in appetite in overweight individuals (Douglas et al., 2017; Holmstrup et al., 2013; Martins et al., 2015; Sim et al., 2014; Ueda, Yoshikawa, Katsura, Usui, Nakao, et al., 2009; Unick et al., 2010), whilst others have shown no differences in subjective appetite (Larsen et al., 2017; Martins et al., 2015). The variations in appetite and EI responses are challenging to determine, but differences in meal composition and/or timing, as well as the mode and intensity of exercise are all probable factors that contribute to these inconsistencies. Additionally, appetite-regulatory hormones post-exercise have yielded conflicting findings. The reduction in acylated ghrelin

with the rise of PYY, GLP-1 and PP from aerobic exercise has been observed with overweight/obese individuals (Larsen et al., 2017; Holmstrup et al., 2013).

Whether there is an optimal time for meal and exercise timing on appetite regulation and GER in overweight individuals is unknown, and if they respond differently to fasted versus non-fasted exercise compared to lean populations from chapter 5 is unclear. Therefore, the aims of this study were to 1) explore the effects of fasted exercise versus non-fasted exercise on GER, appetite regulatory hormones, metabolic responses, and appetite regulation in overweight men and 2) to explore the diurnal variation of these outcomes in response to fasted exercise in overweight individuals. It was hypothesised that overweight individuals would exhibit compensatory changes in appetite following fasted versus non-fasted exercise-induced energy deficits.

6.2. Methods

6.2.1. Participants

Twelve overweight men completed four experimental trials in a randomised order (Table 12). Following WHO criteria, an overweight individual was defined by having a BMI and body fat percentage between 25.0–29.9 $kg.m^{-2}$ and 20-25%, respectively. A sample size estimation was performed using gastric emptying data from Goos et al., (1978) and data from chapter 5. A medium effect size was 0.6 and 0.7, respectively, with a required sample size of N = 8 and N = 12, respectively. Fat oxidation data from Iwayama et al., (2015) revealed an effect size of 0.8, which is large, requiring a sample size of N = 10. Total energy intake data from Bachman et al., (2016) revealed an effect size of 0.8, which is considered large, requiring a sample size of N = 12. Therefore, the sample size of 12 was decided to be an adequate for the main objective of this study. Participant characteristics shown in Table 15.

Table 15: Participant characteristics.

	Overweight (*n = 12*)
Age (years)	26 ± 4.
Height (cm)	176 ± 7
Weight (kg)	88 ± 11
BMI ($kg \cdot m^{-2}$)	28 ± 4
Body fat (kg)	24 ± 4
Fat free mass (FFM) (kg)	64 ± 8
Body fat (%)	23 ± 1
FFM (%)	77 ± 1
$\dot{V}O_{2peak}$ (ml/kg/min)	42 ± 6
PA levels per week (min)	168 ± 19
Systolic BP (mmHg)	128 ± 8
Diastolic BP (mmHg)	87 ± 6

Values expressed as mean ± SD

6.2.2. Experimental trials

The trials and study design conducted within this study were identical to those carried out within chapter 5 of this thesis, with the only difference the participant criteria/selection being overweight men. Participants completed four experimental trials in a randomised crossover fashion. Two-morning trials (08:00 h-13:00 h) fasted (AM-FASTED) and non-fasted (AM-FED) and two evening trials (15:00 h-20:00 h) fasted (PM-FASTED) and non-fasted (PM-FED).

On the morning trials, participants were required to fast from 00:00 the evening before, and on the evening trials, participants were required to have breakfast and then fast from 07:00 except for plain water consumption. Baseline assessments of appetite, substrate utilisation and a blood sample were collected using procedures outlined in general methods.

Following baseline measurements, participants ingested the test 'breakfast' in FED within a 15 min period or remained fasted in FASTED. The test 'breakfast' and test meal after exercise was identical to what was prescribed in chapters 4 and 5 (30 g of breakfast cereal with 125 mL of semi-skimmed milk, and a croissant and 800 g of vegetable soup, respectively). Post-breakfast ratings of appetite, substrate utilisation and blood sample were measured at the end of the 15 min breakfast period. Participants then rested for 1 h before commencement of the exercise protocol. During this 1 h rest period, further measures of appetite were taken every 15 min and substrate utilisation every 30 min. The exercise protocol involved 60 min of cycling on a Lode Corival ergometer at a workload intensity of 60% $\dot{V}O_{2peak}$ pre-determined in the preliminary trial (average workload of 139 ± 11 watts). As with all previous experimental chapters, heart rate and RPE were measured every 15 min throughout the exercise, with expired air measured continuously. The last 5 min of each 10 min segment was used to calculate substrate utilisation. After completion of the exercise bout, participants recovered for 30 min (showered if desired) before they ingested a standardised meal. Subjective feelings of appetite and substrate utilisation were measured every 15 min post-ingestion for a total period of 2 h. A schematic diagram of the experimental protocol is presented in Figure 31. 24 h energy intake post trial was recorded via a weighed food diary by the participants and later analysed using Nutritics Dietary Analysis.

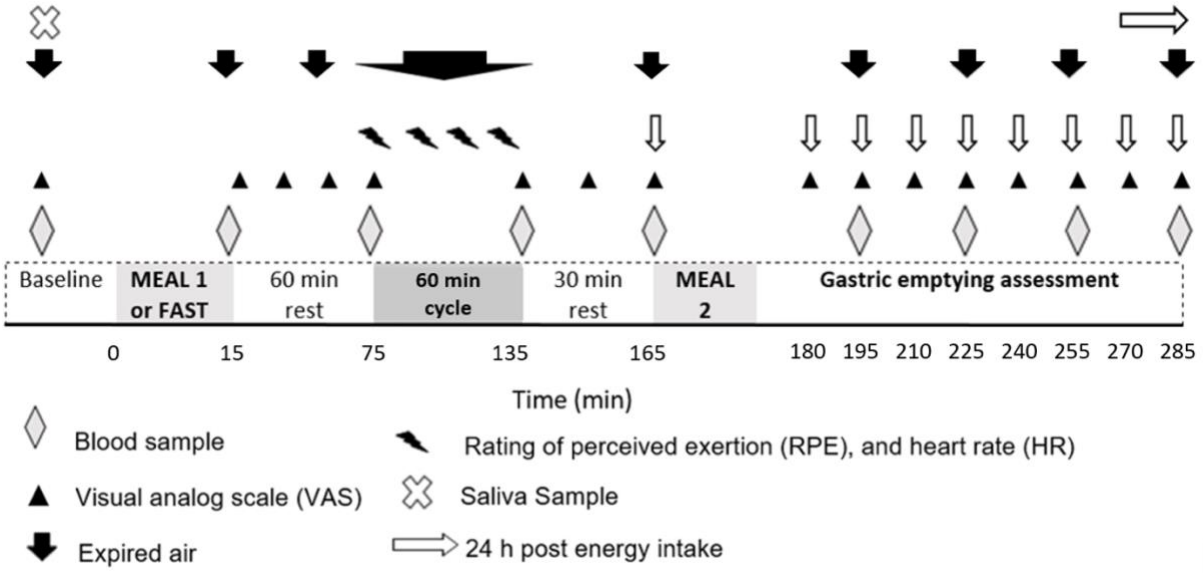

Figure 30: Schematic diagram of the experimental trial protocol, represented in minutes of the trial.

6.2.3. Statistical Analysis

A three-way (trial x time of day x time across trial) repeated-measures analysis of variance (ANOVA) to assess trial (fasted vs. fed) x time of trial (morning vs. evening) x time across trial differences for blood serum concentration of hormones and metabolites, gastric emptying DOB, substrate oxidation, and VAS ratings. Incremental area under the curve (iAUC) was calculated using the Time Series Response Analyser (TSRA) created by Narang et al., (2020). two-way repeated-measures ANOVA was used to assess iAUC for trial x time of day differences for blood serum concentration of hormones and metabolites, gastric emptying DOB, substrate oxidation, and VAS ratings. A two-way repeated-measures ANOVA was used to assess trial (fasted vs. fed) x time of trial (morning vs. evening) differences for gastric emptying $T_{½}$ and T_{lag} data, salivary melatonin concentration and 24-hour energy intake. Sphericity for repeated measures was assessed, and where appropriate, Greenhouse–Geisser corrections were applied for epsilon <0.75, and the Huynh–Feldt correction adopted for less severe asphericity. Significant F-tests were followed by dependent Student's t-Tests or one-way repeated measures ANOVA was also conducted and Bonferroni adjusted pairwise comparisons as appropriate. One-way repeated-measures ANOVA was also conducted for ensuring standardisation of body mass between all trials and mid-point of sleep. All analyses were carried out using IBM SPSS statistics (v25.0 for Windows; SPSS, Chicago, IL). The level of significance was set at $P<0.05$. Descriptive data are expressed as mean ± standard deviation (SD).

6.3. Results

6.3.1. Trial order analysis

Analysis demonstrated that there were no significant differences in responses according to the order of trials that the participants completed (all P>0.05) (Trial 16).

Table 16: Trial order analysis, mean ± SD for gastric emptying half time (T1/2), and incremental area under the curve (iAUC) for subjective feeling of hunger, and glucose concentrations.

Variables	Trial 1	Trial 2	Trial 3	*Trial 4*	P value
Gastric emptying $T_{1/2}$ (min)	114 ± 47	114 ± 32	117 ± 46	116 ± 40	0.995
Subjective feeling of hunger (mm^{-1} x 285 min)	1439 ± 1901	1635 ± 1788	1254 ± 2109	1429 ± 1329	0.915
Glucose concentrations ($mmol/L^{-1}$ x 285 min)	206 ± 102	215 ± 147	159 ± 105	163 ± 74	0.494

6.3.2. Gastric emptying

No significant main effect of trial (P = 0.450), time of day (P = 0.554), time (P = 0.224) or an interaction effect (trial x time of day x time) (P = 0.344) was observed for DOB (Figure 32a). No significant trial effect (P = 0.193), time of day effect (P = 0.058), or interaction effect (P = 0.902) found for DOB iAUC (Figure 32b). No significant main effect of trial (P = 0.848), time of day (P = 0.964), or trial x time of day interaction effect (P = 0.898) for T_{lag} was observed (Figure 33a). Two factor ANOVA also revealed no significant main effect for trial (P = 0.367), time of day (P = 0.531), or trial x time of day interaction effect (P = 0.118) for $T_{½}$ (Figure 33b).

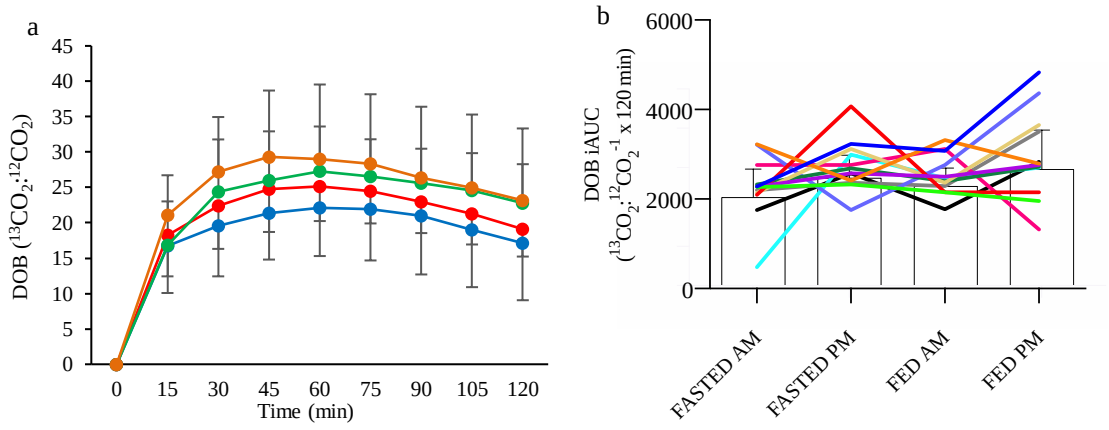

Figure 31: Gastric emptying assessment, for all four trials AM-FASTED, AM-FED, PM-FASTED, and PM-FED a) Gastric emptying delta over baseline (DOB) and b) iAUC for DOB of MEAL 2 (800 g vegetable soup) following 60 min of cycling either fasted (FASTED) or after breakfast consumption (FED). Values are mean ± SD; n=12. The blue line corresponds to AM-FASTED, red line = AM-FED, green line = PM-FASTED, orange = PM-FED.

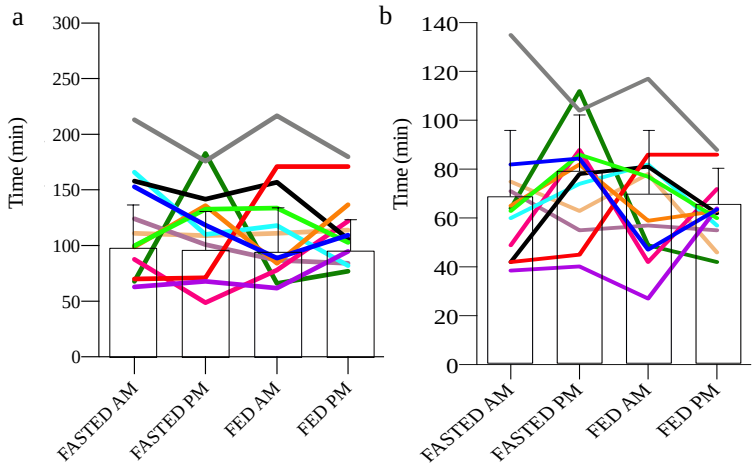

Figure 32: Gastric emptying assessment, AM-FASTED, AM-FED, PM-FASTED, and PM-FED a) Gastric emptying half time (T½) and b) time of maximal emptying rate (Tlag) for all four trials of MEAL 2 (800 g vegetable soup). Values represent mean ± SD; n = 12.

6.3.3. Gut hormones

No significant main effect of trial (P = 0.628), time of day effect (P = 0.281), or interaction effect (P = 0.561) was seen for ghrelin concentrations, but a significant time effect was observed (P = 0.001) (Figure 34a). A significant main effect of trial (P = 0.007) was revealed for GLP-1 concentrations, but no significant time of day (P = 0.083), time (P = 0.053),

or interaction effect (P = 0.517) was observed (Figure 34b). A significant main effect of trial (P = 0.030) and time (P = 0.004), but not time of day (P = 0.908), or interaction effect (P = 0.624) was observed for insulin concentrations (Figure 34c). A significant main effect trial (P = 0.001), time of day (P = 0.002), time (P < 0.001), but no interaction effect (P = 0.124) was observed for PP concentrations (Figure 34d). No significant main effect of trial (P = 0.709), time of day effect (P = 0.830), time (P = 0.245) or interaction effect (P = 0.352) was found for PYY concentrations (Figure 34e).

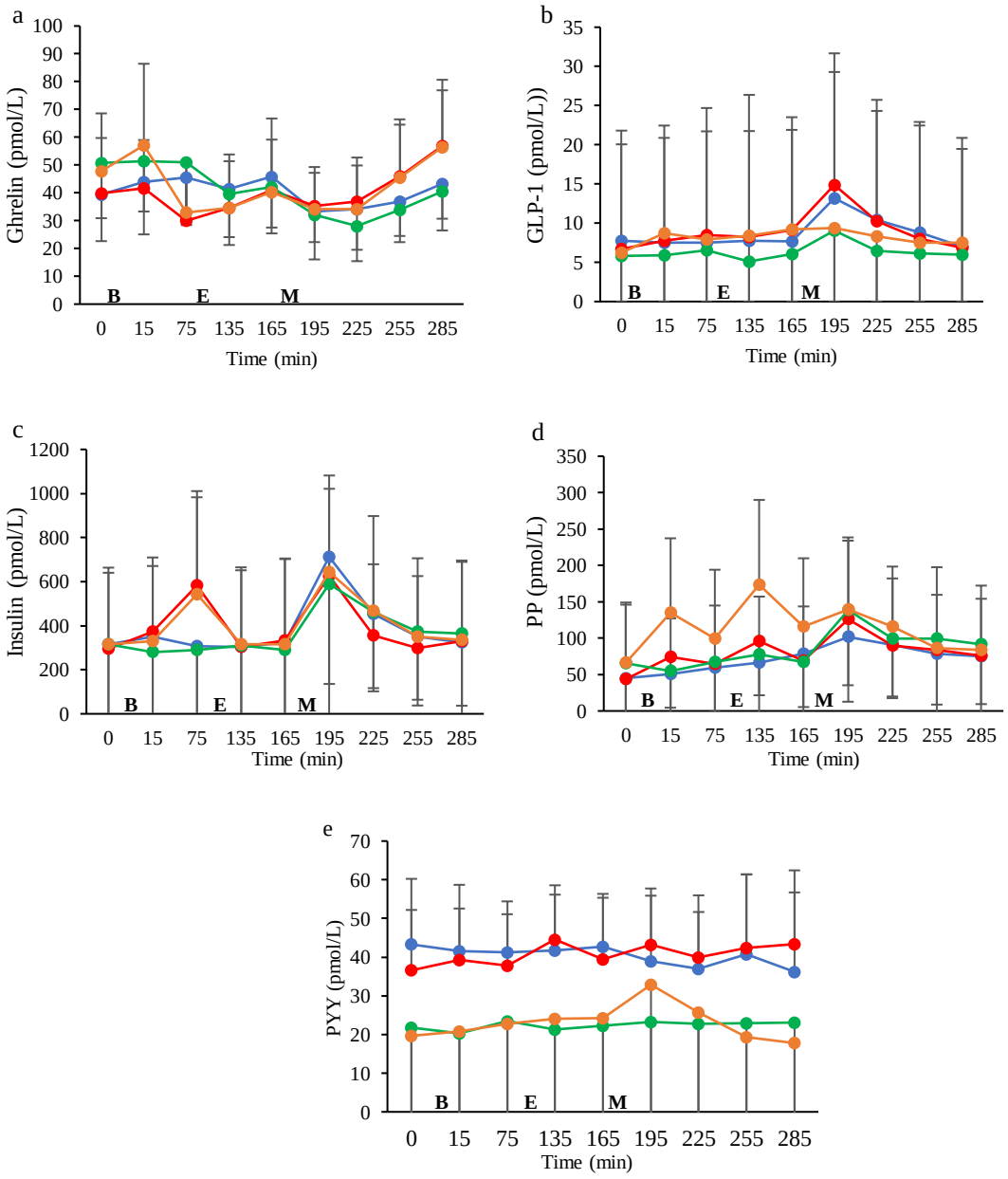

Figure 33: Hormonal responses during trials. Serum concentrations of a) ghrelin (n=12), b) glucagon-like peptide-1 (GLP-1) (n=12), c) insulin (n=12), d) Pancreatic polypeptide (PP) (n=11) and e) Peptide tyrosin tyrosin (PYY) (n=6). Values represent mean ± SD. The blue line corresponds to AM-FASTED, red line = AM-FED, green line = PM-FASTED, orange = PM-FED. '**B**' indicates breakfast period, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, '**E**' indicates Exercise period, where participants completed a 1 h cycle at 60% of their $\dot{V}O_{2peak}$, and '**M**' indicates standardised lunch meal where 800 g vegetable soup was ingested.

Serum ghrelin iAUC concentrations showed no significant trial effect (P = 0.871), time of day effect (P = 0.296), or trial x time of day interaction (P = 0.121). GLP-1 iAUC concentrations showed a significant trial effect (P = 0.019), but no time of day (P = 0.528) or interaction effect (P = 0.754). Similarly, a significant trial effect (P = 0.008), but no time of day (P = 0.708), or interaction effect (P = 0.884) was observed for insulin iAUC. No significant trial effect (P = 0.183), time of day effect (P = 0.122), or interaction effect (P = 0.444) was observed for PYY iAUC. However, a significant trial effect (P = 0.006), time of day (P = 0.034), and interaction effect (P = 0.021) for PP iAUC concentrations was observed. PP iAUC concentrations was greater in the PM-FED trial compared to AM-FASTED (P = 0.033) and PM-FASTED (P = 0.015) (19029 ± 4026 vs. 8089 ± 1909 and vs. 8378 ± 2570 pmol/L $^{-1}$ x 285 min).

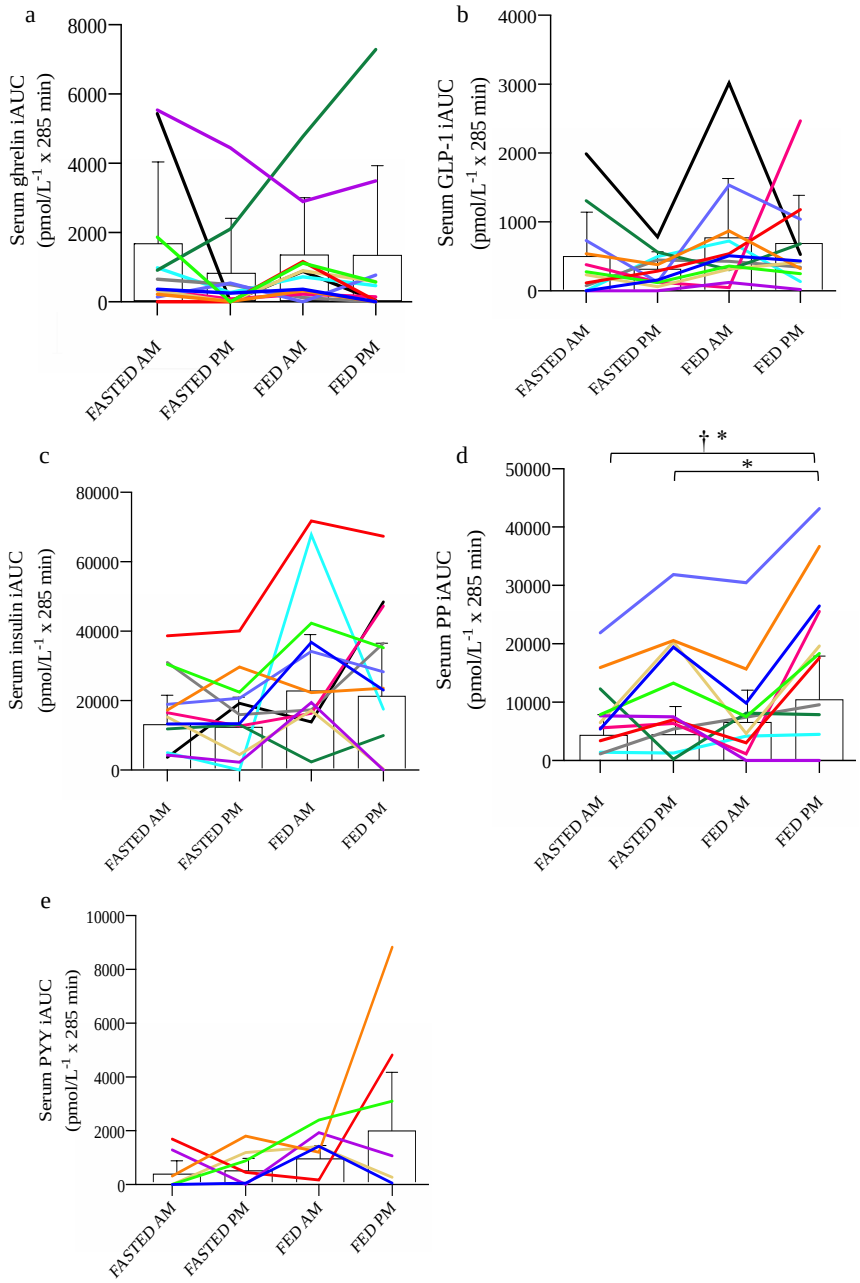

Figure 34: Hormonal responses during both trials. iAUC serum concentrations of a) Ghrelin (n=12), b) glucagon-like peptide-1 (GLP-1) (n=12), c) insulin (n=12), d) Pancreatic polypeptide (PP) (n=11) and e) Peptide tyrosin tyrosin (PYY) (n=6). Values represent mean ± SD. * indicates significance ($P < 0.05$) versus corresponding time point in other trial (i.e. FASTED vs. FED), † indicates diurnal significance between morning and evening trial (i.e. AM vs. PM).

6.3.4. Metabolic markers

No significant main effect for trial (P = 0.653), time of day (P = 0.276), or interaction effect (P = 0.082) was observed for glucose concentrations, but a main effect of time was observed (P < 0.001) (Figure 36a). A significant main effect for trial (P < 0.001), time of day (P = 0.003), and time (P < 0.001) was observed for NEFA concentrations, although no interaction effect (P = 0.165) observed (Figure 36b). A significant main effect was found for trial (P = 0.041), and time (P = 0.023) for triglycerides concentrations, but no time of day (P = 0.192) or interaction effect (P = 0.385) observed (Figure 36c). A significant main effect was found for time of day (P = 0.041) and time (P < 0.001) for cholesterol concentrations, but no trial (P = 0.069) or interaction effect (P = 0.376) observed (Figure 36d).

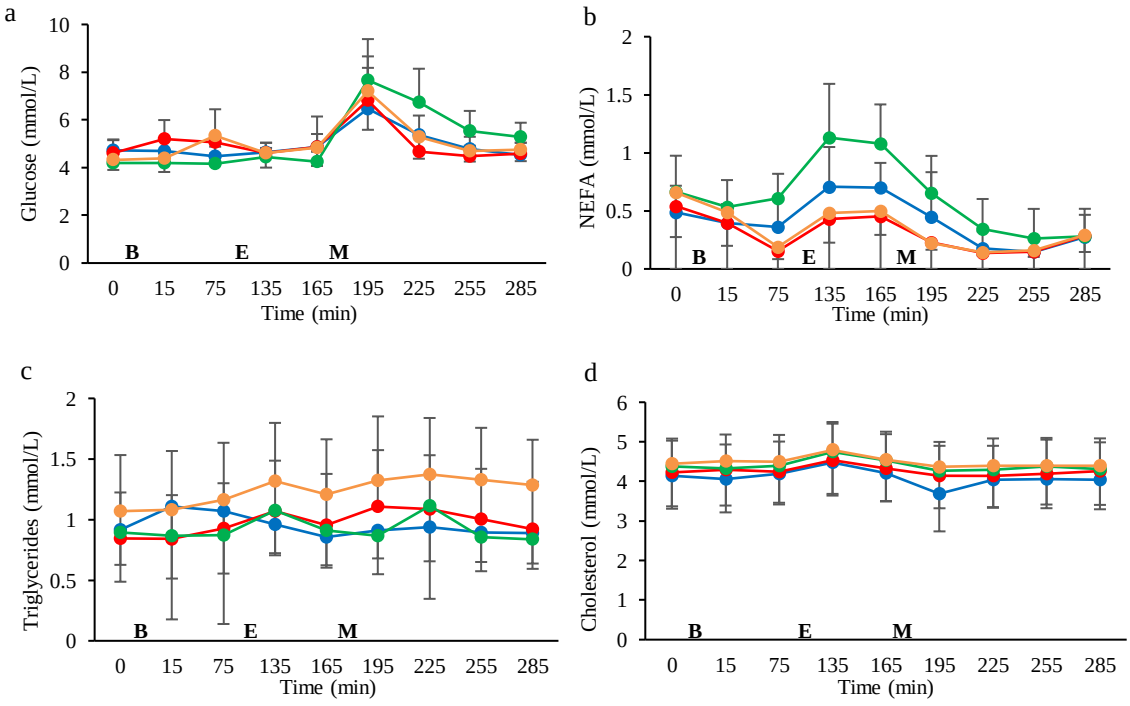

Figure 35: Metabolic responses during trials. Serum concentrations of a) glucose, b) non-esterified fatty acids (NEFA), c) triglycerides, and d) cholesterol. Values represent mean ± SD; n=12. The blue line corresponds to AM-FASTED, red line = AM-FED, green line = PM-FASTED, orange = PM-FED. '**B**' indicates breakfast period, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, '**E**' indicates Exercise period, where participants completed a 1 h cycle at 60% of their $\dot{V}O_{2peak}$, and '**M**' indicates standardised lunch meal where 800 g vegetable soup was ingested.

Serum glucose iAUC concentrations showed no significant trial effect (P = 0.470), or trial x time of day interaction (P = 0.111), but a time-of-day effect was observed (P = 0.001). No significant trial effect (P = 0.103), time of day (P = 0.969), or interaction effect (P = 0.673) was observed for triglycerides iAUC. No significant trial effect (P = 0.408), time of day effect (P = 0.685), or interaction effect (trial x time of day) (P = 0.332) was observed for cholesterol iAUC. NEFA iAUC concentrations showed a significant trial effect (P = 0.001), but no time of day (P = 0.137) or interaction effect (P = 0.128) (Figure 37).

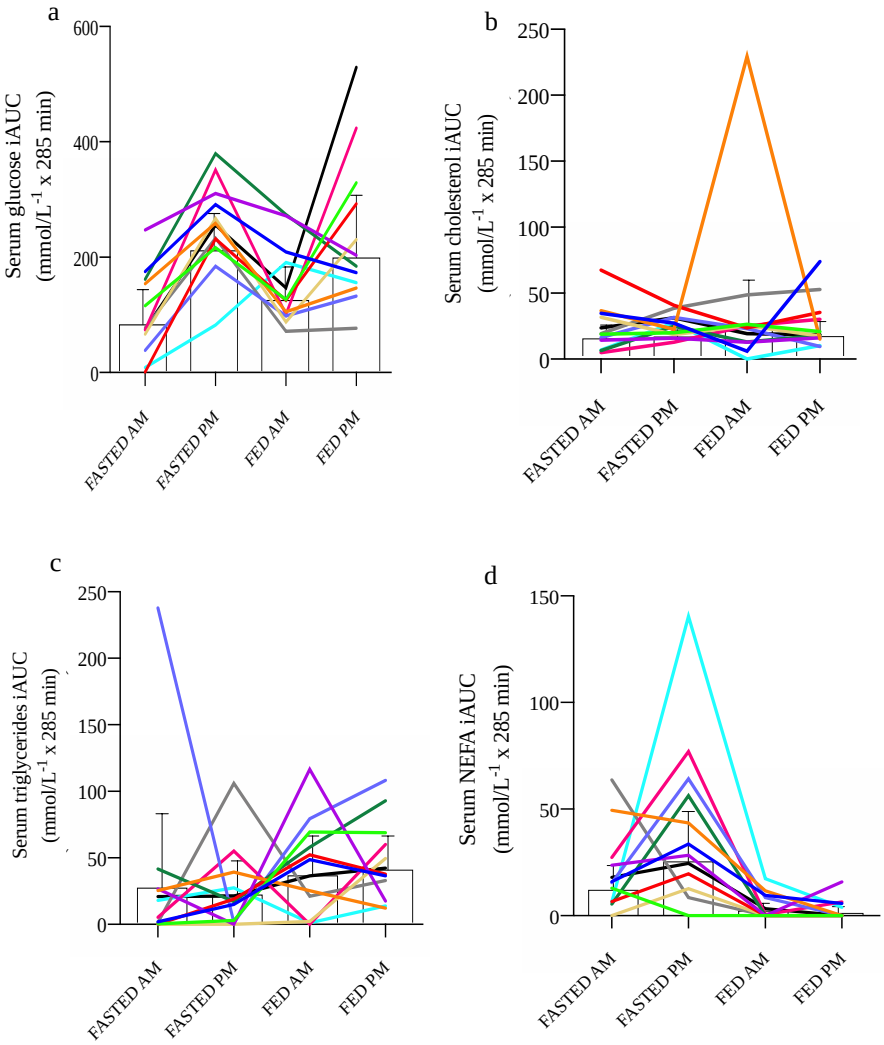

Figure 36: Metabolic responses during trials. iAUC serum concentrations of (a) Glucose, (b) Cholesterol, (c) Triglycerides and (d) Non-esterified fatty acids (NEFA). Values represent mean ± SD; n=12.

6.3.5. Appetite

A significant main effect for trial (P = 0.002), time of day (P = 0.028), time (P<0.001), although no interaction effect observed (trial x time of day x time) (P=0.221) (Figure 38a). A significant main effect for trial (P = 0.002) and time (P < 0.001) for fullness, although no main effect for time of day (P = 0.270) or interaction effect (trial x time of day x time) (P = 0.320) (Figure 38b). A significant main effect for trial (P = 0.026), time of day (P = 0.005), time (P < 0.001) and interaction effect (trial x time of day x time) (P = 0.152) for PFC (Figure 38c). A significant main effect for trial (P = 0.001), time (P < 0.001), but no time of day (P = 0.434), or interaction effect (trial x time of day x time) (P = 0.636) for food satisfaction (Figure 38).

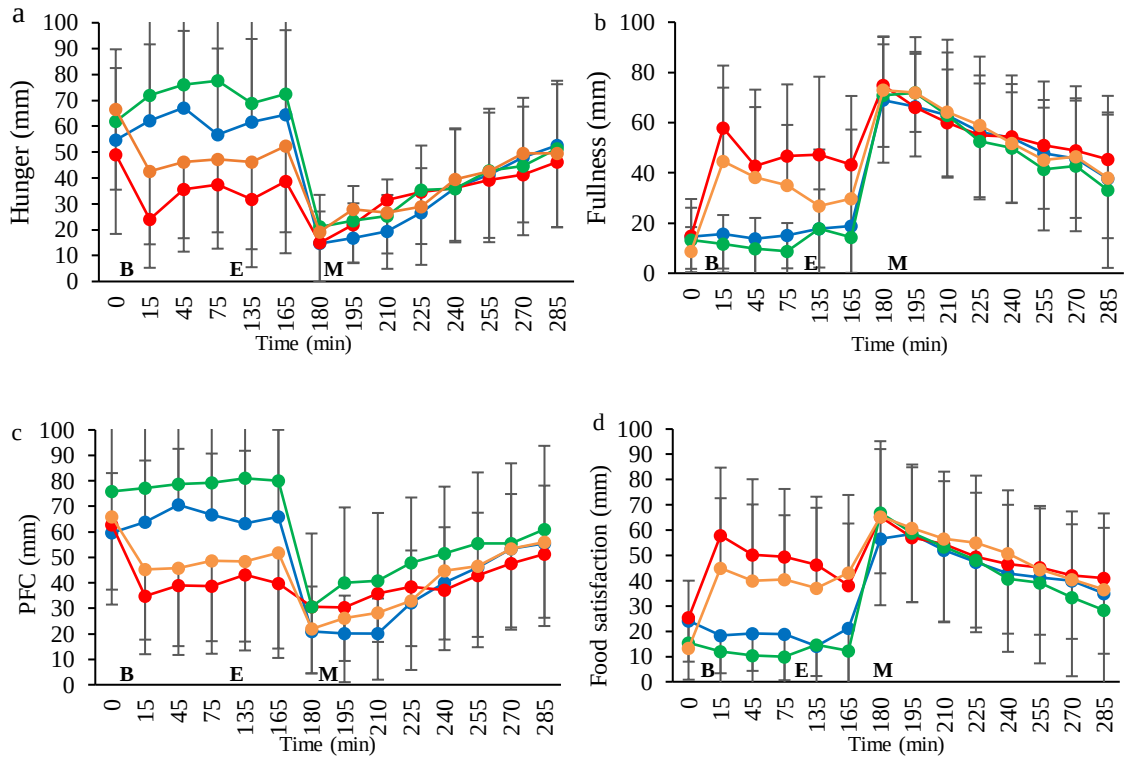

Figure 37: Appetite scores during trials, assessed by 100 mm visual analogue scale (VAS); (a) Hunger, (b) Fullness, (d) Prospective food consumption (PFC), (d) Food satisfaction. Values represent mean ± SD n=12. The blue line corresponds to AM-FASTED, red line = AM-FED, green line = PM-FASTED, orange = PM-FED. '**B**' indicates breakfast period, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, '**E**' indicates Exercise period, where participants completed a 1 h cycle at 60% of their $\dot{V}O_{2peak}$, and '**M**' indicates standardised lunch meal where 800 g vegetable soup was ingested.

A significant trial effect for hunger ratings for iAUC was observed (P = 0.002), but no time of day effect (P = 0.246), or interaction effect (P = 0.348). Similarly, a significant trial effect observed for fullness, PFC and food satisfaction (P = 0.036, P = 0.048, P = 0.050, respectively), but no time of day (P = 0.566, P = 0.648, P = 0.777, respectively), or interaction effect observed (P = 0.444, P = 0.779, P = 0.342, respectively) (Figure 39).

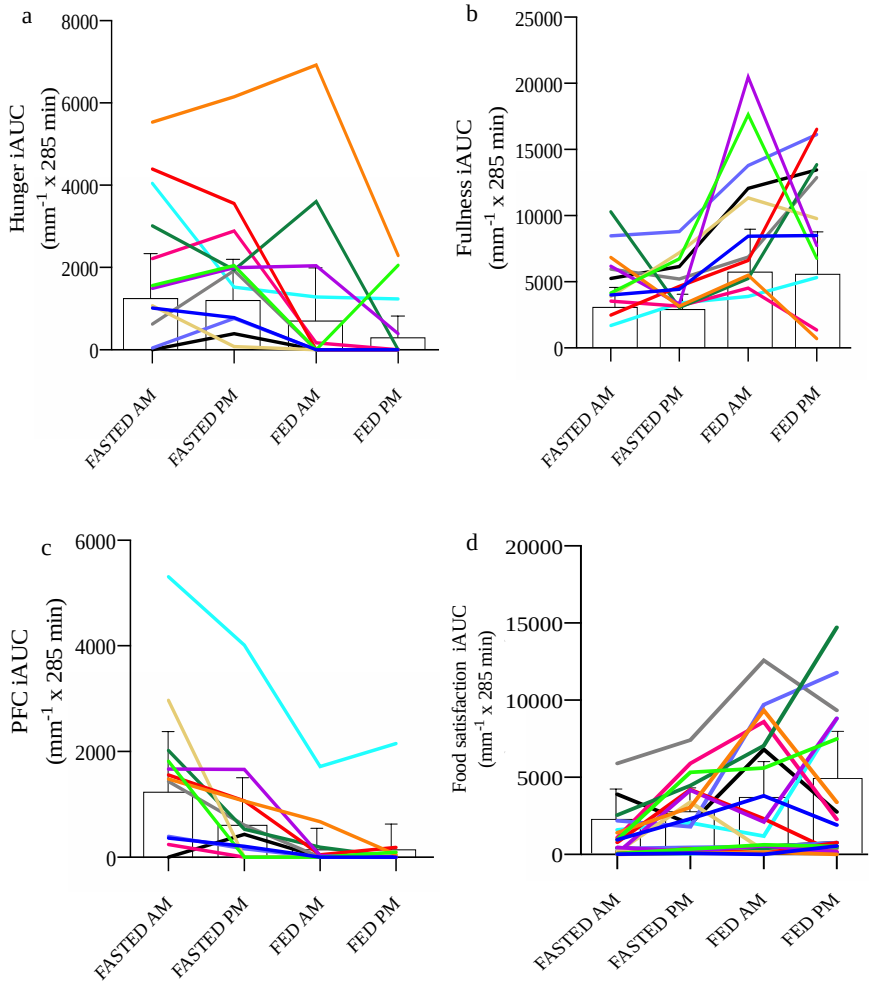

Figure 38: iAUC appetite scores during trials, assessed by 100 mm visual analogue scale (VAS); (a) Hunger, (b) Fullness, (c) Prospective food consumption (PFC), (d) Food satisfaction. Values represent mean ± SD n=12.

6.3.6. Substrate utilisation

A significant main effect of trial (P < 0.001) and time (P< 0.001), although no time of day (P = 0.353), or interaction effect (P = 0.732) was observed for fat oxidation (Figure 40a). A significant trial effect (P = 0.001), but no time of day effect (P = 0.976), or interaction effect (P = 0.437) was observed for fat oxidation iAUC (Figure 40b). A significant main effect for trial (P<0.001) and time (P<0.001), but no significant time of day effect (P = 0.782), or interaction effect (P = 0.083) was observed for CHO oxidation (Figure 40c). A significant trial effect (P = 0.004), but time of day effect (P = 0.124), or interaction effect (P = 0.162) was observed for CHO iAUC (Figure 40d).

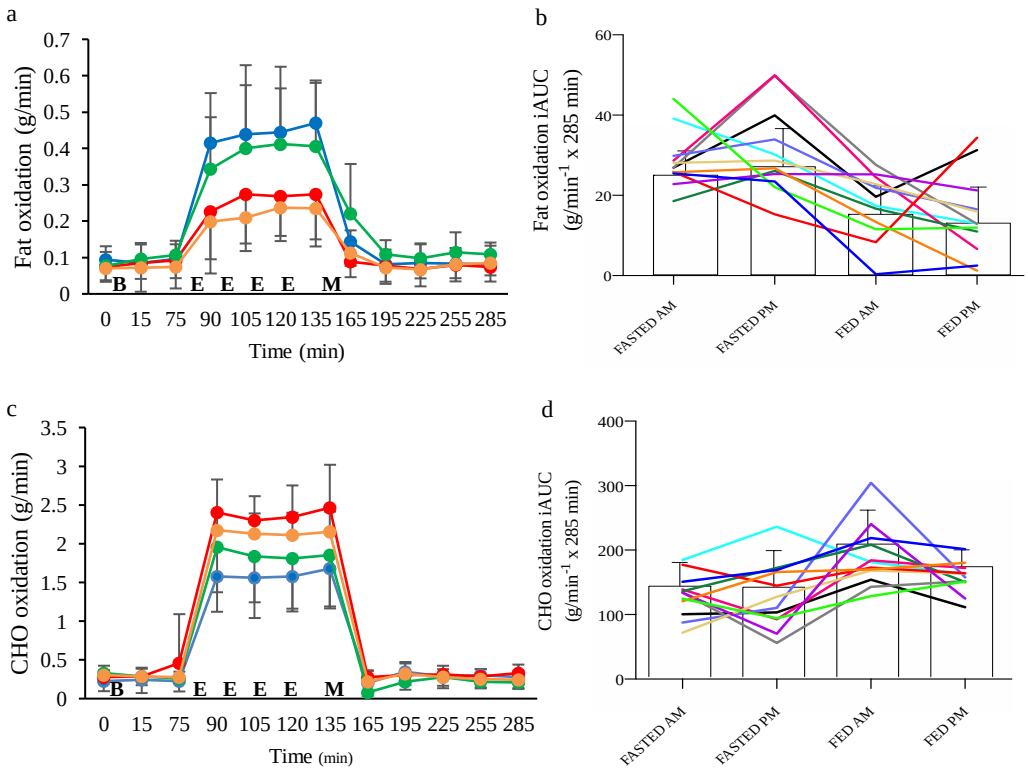

Figure 39: Substrate utilisation; Fat oxidation (a), iAUC for fat oxidation (b) and carbohydrate oxidation (c), iAUC for carbohydrate oxidation (d) during the trials, AM-FASTED, AM-FED, PM-FASTED, and PM-FED. The blue line corresponds to AM-FASTED, red line = AM-FED, green line = PM-FASTED, orange = PM-FED. Values represent mean ± SD; n = 12. '**B**' indicates breakfast period, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, '**E**' indicates Exercise period, where participants completed a 1 h cycle at 60% of their $\dot{V}O_{2peak}$, and '**M**' indicates standardised lunch meal where 800 g vegetable soup was ingested.

6.3.7. Physiological measurements during the exercise bout

No significant differences between HR, RPE and VO_2 measurements were seen across and between trials (all P>0.05) (Table 17).

Table 17: Illustrates the physiological responses during the cycling exercise within all four trials.

	FASTED-AM					**FED-AM**				
Time (min)	0	15	30	45	60	0	15	30	45	60
RPE	6±0	12±4	13±2	13±2	13±3	6±0	12±2	13±2	13±2	13±2
HR (bpm)	75±3	143±5	145±4	146±2	147±2	74±2	142±2	143±2	145±2	146±3
VO_2 (L/min)	4±5	24±8	25±3	25±4	24±3	5±5	25±3	25±3	25±4	25±7
VO_2 (%)	11±1	59±8	60±4	60±3	60±5	13±4	60±1	60±5	60±3	60±6
	FASTED-PM					**FED-PM**				
Time (min)	0	15	30	45	60	0	15	30	45	60
RPE	6±0	12±2	13±4	13±4	13±5	6±0	13±4	13±4	13±3	13±3
HR (bpm)	76±3	142±5	145±3	146±3	146±2	75±2	144±2	145±4	145±2	146±2
VO_2 (L/mn)	3±6	24±8	25±3	25±6	25±6	4±3	25±5	25±4	25±6	25±6
VO_2 (%)	8±5	60±6	60±8	60±5	60±4	10±9	59±7	60±5	60±9	61±3

Rating of perceived exertion (RPE), heart rate (HR) and Oxygen Consumption (VO_2)

6.3.8. 24 h Energy intake

A two-way factor ANOVA observed a significant main effect for time of day (P = 0.043), but no significant main effect was observed for trial (P = 0.145), or interaction effect (trial x time of day) (P = 0.096) for 24 h energy intake (Table 18). A one-way ANOVA was further conducted to compare trials on time of day, but no significant differences were found between AM and PM trials (all P>0.05) (Table 18).

Table 18: 24 h post-trial energy intake and macronutrient breakdown for all participants (n=12).

	AM-FASTED	AM-FED	PM-FASTED	PM-FED
Energy intake (kcal)	2963 ± 443	3230 ± 430	2701 ± 648	2728 ± 700
Protein (g)	150 ± 21	176 ± 28	145 ± 24	169 ± 25
Carbohydrate (g)	314 ± 84	332 ± 53	262 ± 91	275 ± 75
Fat (g)	122 ± 35	132 ± 45	119 ± 42	123 ± 38

Values expressed as mean ± SD

6.3.9. Salivary melatonin

A two factor ANOVA demonstrated a main effect of time of day (P = < 0.001), no main effect of trial (P = 0.617) and no interaction effect (P = 0.901) for saliva melatonin levels. Salivary melatonin concentration was significantly different between morning and evening trials (AM-FASTED, AM-FED vs. PM-FASTED, PM-FED; P<0.001, Table 19). No significant differences between trials for midpoint of sleep (P = 0.267).

Table 19: Melatonin measurements for all participants (mean ± SD).

	AM-FASTED	AM-FED	PM-FASTED	PM-FED
Melatonin (pg/mL)	15.2 ± 7.2 *	15.8 ± 9.5*	4.9 ± 2.4*	5.3 ± 2.2*
Midpoint of sleep	02:40 ± 0:07	02:30 ± 0.06	02:32 ± 0.04	02:42 ± 0.03

* indicates significant difference to corresponding trial (AM-FASTED vs. PM-FASTED).

6.4. Discussion

The primary findings were that GER in overweight individuals was not affected and did not follow a diurnal variation. Fasted exercise or timing of exercise did not elicit any differences in subjective feeling of hunger, appetite regulatory hormones, metabolic markers or 24 h energy intake post trials. However, pancreatic polypeptide (PP) iAUC was greater in the PM-FED trial compared to AM-FASTED and PM-FASTED trials. The present findings refute the hypothesis that overweight individuals will demonstrate compensatory changes in appetite following fasted versus non-fasted exercise-induced energy deficits.

To the investigator's knowledge, no studies have assessed the diurnal variation in gastrointestinal function, metabolic markers and appetite regulation, in response to fasted versus non-fasted exercise in overweight individuals. Within the previous experimental chapter, lean individuals displayed a slower gastric emptying rate in the evening, however this was not apparent in the current study for overweight individuals. A possible explanation for the no differences in gastric emptying rate may be due to the greater body fat (Baudry et al., 2012; Cardoso et al., 2007). The lack of time-of-day effect in GER rate following a meal post-exercise fasted versus fed in overweight individuals within the current study, along with no compensatory effects in appetite markers or 24 h energy intake following all trials represents novel insights for potential appetite regulation and may hold practical implications for weight management.

Previous investigations have compared appetite-regulatory hormone responses to acute exercise in both lean and overweight/obese individuals (Douglas et al., 2017; Marzullo et al., 2008; Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009). In line with the current findings, both Douglas et al., (2017) and Ueda et al., (2009) found similar hormonal responses between lean and overweight individuals. In contrast, the findings of Marzullo et al., (2008) found a

reduced level of ghrelin response in overweight individuals compared to lean individuals. Also, non-exercise investigations have reported that overweight individuals display a lessened meal-induced increase in PYY and GLP-1 levels (Le Roux et al., 2006; Verdich et al., 2001) and diminished suppression of acylated ghrelin in the postprandial period (Le Roux et al., 2006). However, no differences in ghrelin, GLP-1 or PYY concentrations were observed between trials within the current study, although PP iAUC concentrations were higher in the PM-FED trial compared to fasted trials. Additionally, a post-hoc power analysis was performed using the GLP-1 data and showed that 14 subjects are required to observe any meaningful differences. In addition to this, gastric emptying data was also analysed and resulted in 13 subjects, whilst glucose concentrations required 16 subjects to detect any meaningful differences. The current study recruited 12 subjects and therefore it is important to consider that the lack of differences observed may be due to a type II error. Especially for PYY, where only half of the sample size (n = 6) was included due to insufficient data analysis, which may account for the no differences found between trials. The lack of differences observed compared to previous studies could be due to several factors. Such as, a type II statistical error, the intensity of exercise not being high enough to stimulate such physiological responses, a variation in body fat percentages and BMI, and/or the different test meals implemented (Chandarana et al., 2009; Douglas et al., 2017).

No differences were observed for metabolic markers within the current findings. Insulin resistance increases with higher body fat contribution and has been linked with disrupted satiety signalling in the postprandial phase (Flint et al., 2007), and lower ghrelin levels (Rask et al., 2001). This may correspond to the lack of differences observed within hormones and metabolites in the overweight individuals of the current study. The lack of differences between appetite, metabolic markers, and appetite regulatory hormones for fasted versus fed exercise in

overweight individuals may suggest that fasted exercise produces a negative daily energy balance. However, it's important to highlight that the absolute food portion provided in the trials could proportionally be less food compared to relative to body mass (i.e., potentially less food to what they normally ingest) for the current study participants who have greater adiposity. This could be a potential reason why there were no differences in compensatory responses of appetite between the fasted versus fed trial and concluding that fasted exercise is a useful tool to aid weight loss requires further investigation. Though, a previous investigation found little change in energy intake from those that consumed breakfast than fasting, and no changes in weight change and most health outcomes, other than in obese individuals (Chowdhury et al., 2016). Therefore, fasted exercise in the morning or evening could be a valuable tool to induce a short-term energy deficit. However, performing exercise at a higher intensity than performed in the current study would be beneficial to confirm if no compensatory effects in appetite still occur.

In conclusion, gastric emptying rate is not affected by time of day in overweight individuals. Fasted exercise did not provoke compensatory effects on appetite regulatory responses or subjective feelings of hunger during the trial, or 24 h post-trial in overweight individuals, therefore sustaining the exercise-induced energy deficit on the day of exercise. These findings support fasted exercise as a strategy for inducing a short-term negative energy balance in overweight individuals. Additional work is required to explore the longer-term effects of fasted versus on-fasted exercise on appetite regulation and weight control in overweight individuals.

7.0. GENERAL DISCUSSION

The prevalence of overweight and obesity is becoming epidemic and continues to be a major health concern worldwide. Sufficient exercise and nutrition play an equally important role in weight management and metabolic health (Astrup, 2013; Witard & Ball, 2018). Research on many dietary strategies has been conducted to identify and tackle the obesity epidemic by modulating appetite and food intake. One of these strategies is intermittent fasting, an intervention which has become increasingly popular in recent years, promoting metabolic health and weight management, whilst exercise alone induces many health benefits. Consequently, interest in the effects of fasted exercise has grown in both the scientific field and the general public. However, exercising in the fasted state compared with that of a postprandial state for reducing total energy intake during the day and thus, creating a negative energy balance to promote weight loss remains unclear, and the role of gastric emptying in appetite and satiety is often overlooked. A better knowledge of fasted exercise on gastric emptying rate, gut hormone, and metabolic secretion in response to a subsequent meal post-exercise can help develop dietary interventions. This overall aim of the thesis was to add further understanding of fasted exercise by assessing GI function and metabolic and appetite responses to fasted and non-fasted exercise. The results of the experimental chapters presented in this thesis will be discussed collectively below.

The findings within this thesis add novel insights into the effect of fasted versus non-fasted exercise on GER in response to a subsequent meal post-exercise. No differences were observed between fasted and fed exercise on GER in the morning across all four experimental chapters (chapter 3-6). However, the effect of fasted and non-fasted exercise on GER does seem to be affected by the time of day, typically a slower GER in the evening, at least within

the normal weight (chapter 4) and lean group (chapter 5) of the thesis. This is consistent with previous studies that observed a slower gastric emptying rate in the evening (Grammaticos, Dourmas and Kliakos, 2015; Goo et al., 1987). A slower gastric emptying may be favourable for reducing food intake and prolonged satiety (Zhu, Hsu and Hollis, 2013; Jones et al., 1997), which could have positive weight-loss implications.

The mechanisms behind the reduced rate of GER in the evening has been questioned by many. In the digestive tract, a wide range of vital functions and mechanisms display diurnal variations (Codoñer-Franch & Gombert, 2018). Emerging evidence has suggested that the circadian clocks regulate the digestive process, which could be the potential cause of the delayed gastric emptying in the evening (Codoñer-Franch & Gombert, 2018; Hoogerwerf, 2010). All studies within this thesis used a soup meal that contained a large liquid component. This may affect the emptying rate compared to a solid meal with a more significant delay of emptying with solid food than liquids (Hellstrom et al., 2006). Due to this, the volume and energy content of the lunch meal was increased after chapter 3. However, this did not influence GER in the overweight group (chapter 6) which suggests that the nutritional profile and absolute portion size (instead of relative to body mass) was still relatively low for the subjects recruited. It is also important to highlight that the last meal effect was not standardised, as a result, the differences in food intake between participants pre-trial could attribute to the diurnal variations observed. However, food diaries were recorded to ensure standardisation of food consumption 24 h before trials within-subjects and the fasting period before each trial was consistent across all subjects. As well as the time of trials in chapter 4, 5 & 6 (diurnal variation studies) were in different phases of the circadian cycle (established by melatonin concentrations).

Furthermore, the choices of food supplied for the breakfast and lunch meal may not be appropriate and reflect an accurate representation of what the subjects would consume later in the day and early evening. In addition, if the test meal was solid food, this may have led to differences in GER results. The amount of body fat has been suggested to affect the rate of which food is emptied from the stomach, indicating that overweight/obese individuals may have different osmolar and size receptors for detecting foodstuff compared to those who are lean (Wright et al., 1983). Previous studies reported obese individuals to have a rapid GER (Zahorska-Markiewicz et al., 1986; Wright et al., 1983; Tosetti et al., 1996; Vazquez et al. 2006). Whilst some studies showing a slower GER in overweight individuals (Di Ciaula, Wang, Portincasa, 2012; Buchholz et al., 2013). However, previous studies have employed different gastric emptying measurements and foods, which could account for inconsistencies in results observed, making it difficult to draw accurate conclusions. The greater adiposity of subjects within chapter 6 could have possibly overpowered any diurnal variation. Additionally, although studies were different between chapters 3 and 6, the body composition was similar, which could have some connection for the lack of differences found in GER and appetite responses.

The overweight group were recreationally active, shown by PA minutes being 168 ± 19 whilst lean 174 ± 29 min and fitness levels similar 42 vs 45% $\dot{V}O_{2peak}$. This was because participant criteria for all studies were recreationally active, so comparisons between lean and overweight can be made. Thus, any differences between lean results and overweight would be due to body composition and not because of differences in PA levels. Although within-group effects cannot be accurately used to conclude the presence versus absence of effects between groups, as data was not analysed between groups. However, it is essential to highlight that these lack of differences in chapter 6 may not be due to the increased adiposity but only down to type

II error (Rothman, 2010). Overall, the studies within this thesis are the first to investigate the effect of GER following fasted versus non-fasted exercise within various population groups. Adding novel insights into the second meal metabolism, indicating that the subsequent meal post-exercise demolishes any compensatory effects in appetite response in two different conditions (fasted versus fed) and population groups (lean and overweight).

No differences in appetite hormone responses across the experimental chapters, except PP iAUC concentrations were higher in the PM-FED than morning and evening fasted exercise in both the lean and overweight groups. Elevated PP levels have been shown to induce satiety and increase energy expenditure (Wynne, Stanley & Bloom, 2004) which could make fed exercise in the evening an appealing option to aid weight loss. Also, elevated PP levels are shown to reduce gut motility (Batterham et al., 2003), which corresponds to the findings in the lean group with higher PP levels and slower emptying rate in the evening. Insulin iAUC was also higher in the FED-PM trial than fasted exercise trials but only in the lean group (chapter 5). Most previous studies assessing the effect of appetite responses during and after aerobic exercise recruit lean men with no direct comparison of fasted versus fed exercise. The existing studies suggest that appetite markers are briefly reduced following aerobic exercise at intensities higher than 60% $\dot{V}O_{2peak}$ (Vatansever-Ozen et al., 2011; Broom et al., 2009; Broom et al., 2017). No suppression in ghrelin concentrations post-exercise was consistent with previous observations by King et al., (2010). They also failed to observe an immediate difference in ghrelin following an acute bout of walking exercise. However, some studies have shown a suppression in ghrelin concentrations following high-intensity aerobic exercise in overweight individuals (Sim et al., 2014) and increased PYY following high-intensity exercise bout in lean individuals (Deighton et al., 2013). The lack of diurnal variation in ghrelin, PYY and GLP-1 is consistent with a previous non-exercise study that found that fasting at 0800-

1700 h or 1200-2100 h did not change the postprandial suppression of ghrelin, PYY or GLP-1 (Hutchison et al., 2019). As briefly mentioned above when discussing GER results, the lack of differences in appetite and hormonal responses could as well be due to the food amount being a relatively small calorie content for the subjects recruited.

The lack of literature on moderate/low-intensity exercise such as brisk walking was the initial reason to incorporate this mode and intensity within the thesis, which aligns with the study designs of experimental chapters' ecological validity. However, cycling and running can create a higher physiological demand than brisk walking; thus, the reduced physiological challenge imposed by brisk walking within chapter 3 and 4 compared with the exercise of higher intensity exercise may account for these conflicting findings. Due to this, the mode of exercise was changed to cycling to allow for a greater exertion intensity to be elicited. The choice of cycling was also selected due to the population recruited within chapter 6 too; cycling exercise would typically be more feasible and comfortable for the overweight group to manage at 60% $\dot{V}O_{2peak}$ for 1 h when compared to running for example. However, changing the mode of exercise and intensity wasn't significant enough to provoke hormonal responses.

iAUC glucose and insulin concentrations were greater in the evening than the morning in the lean group only (chapter 5). This is consistent with previous studies that have observed higher glucose concentrations in the evening in lean individuals (Takahashi et al., 2018). The lack of differences in other chapters may be due to the increased adiposity. Studies suggest an increase in adipose tissue can be associated with increased insulin resistance and glucose intolerance, impairing glucose metabolism (Gastaldelli et al., 2017). Therefore, the higher adiposity within chapter 3, 4 and 6 could overpower any diurnal variation. Though, NEFA concentrations were higher in the fasted trial than fed in chapter 3, indicating greater fat mobilisation for metabolism, consistent with the knowledge that fat oxidation is increased with

fasting (Achten and Jeukendrup, 2004). However, no differences were found for fat oxidation during fasted exercise. Fat oxidation only elicited a greater response during the fasted trials in the lean group. Generally, metabolic responses appear to be more apparent within the lean group. The lack of differences observed in other chapters compared to the lean group could be due to the varying adiposity affecting outcome measures. As briefly mentioned earlier on, the absence of differences in results may not be owing to the increased adiposity but simply down to type II error. Furthermore, no carry-over effects were present in all four studies, e.g., which excludes the possibility that fat oxidation was only significantly higher during fasted exercise in chapter 3, compared to the other experimental chapters and inconsistencies in other results across studies. All trials were randomised to avoid the risk of the treatment order interacting with treatment, e.g., participants dealing better with fasting on post-prandial appetite responses or during exercise.

The lack of differences from fasted versus non-fasted exercise on appetite hormones and metabolic responses corresponds with the no differences on the subjective feeling of hunger post-exercise or compensatory effects on food intake following 24 h post trials in all chapters. Like the findings in this thesis, previous investigations have found no significant changes in appetite post-exercise for both lean (Douglas et al., 2017; Holliday and Blannin, 2017) and overweight individuals (Larsen et al., 2017; Holmstrup et al., 2013; Sim et al., 2014; Martins et al., 2015; Unick et al., 2010). However, appetite was determined using the VAS scales. The VAS scale is a valid, reliable indicator of the feeling of appetite (Flint et al., 2000), but it can be a poor marker of energy intake (Stubbs et al., 2000). The use of ab libitum meal would have provided more insight on the subsequent appetite intake and response following fasted versus fed exercise, and whether compensatory effects occur if it wasn't a standardised meal. In addition to this, measuring appetite and gut hormone responses over a longer duration

throughout the day rather than only 2 h post-meal would be advantageous for seeing longer post-prandial responses. This would have been interesting, especially within chapter 5 for the overweight group, to indicate whether hunger increases later in the day following fasted exercise.

Overall, the thesis presents novel findings and highlights the importance that exercise prescription should be tailored to target individual needs. For example, based upon the current thesis findings, GER is typically slower in the evening, which could reduce total calorie intake but not in overweight individuals, suggesting those of greater adiposity could be less susceptible to slow GER. Exercise in the evening results in a greater PP concentration, which could increase satiety levels post-exercise in both lean and overweight individuals. Those that are within the lean category may have the additional benefit of increased insulin response too. To stabilise blood glucose concentrations, exercising in the morning fed or fasted may be more beneficial for blood glucose concentrations. Fasted exercise in the morning or evening may result in a greater negative energy balance with no compensatory effects on appetite regulation or energy intake later in the day, which could have positive implications for weight management. Further work is required to understand the long-term impact of fasted versus fed exercise at various times of day on GER, appetite regulation and metabolic health. This will help clarify whether timing has implications on weight management.

8.0. CONCLUSION

The experimental studies discussed within this thesis have extended on the understanding and knowledge of the effect of fasted exercise on gastric emptying rate following a subsequent meal and gut hormone and metabolic responses. Regardless of fasted or fed exercise, the subsequent meal following exercise seems to abolish any compensatory effects in appetite regulatory hormones and subjective feelings of hunger, which is also supported within the 24 h energy intake post-trials, in lean, normal and overweight populations. This suggests that fasted exercise is beneficial for inducing a negative energy balance. The thesis also adds novel insights into the diurnal variation of GER, gut hormone and metabolic markers, and metabolism in response to fasted versus fed exercise in both lean and overweight individuals. The main findings that can be drawn from this work are:

1) GER and appetite regulatory hormones are not affected in the post-prandial stages following exercise in the fasted or fed state after consuming a subsequent meal. Regardless of the time of day, the second meal diminishes any compensatory effects in food intake and appetite markers in normal weight, lean and overweight individuals.

2) GER follows a diurnal variation, typically being slower in the evening than the morning from a subsequent standardised meal following fasted versus fed exercise, but only in lean/normal weight cohorts. GER may be affected by body composition. With no differences observed in the overweight group, suggesting greater adiposity appears to be less sensitive to such differences.

3) Fasted exercise induces an energy deficit without a subsequent compensatory response in appetite 24 h energy intake which could be favourable for weight management in lean and overweight populations.

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10.0. APPENDICES

A.Munich Chronotype Questionnaire

Munich ChronoType Questionnaire (MCTQ)

Instructions:
In this questionnaire, you report on your typical sleep behaviour over the past 4 weeks. We ask about work days and work-free days separately. Please respond to the questions according to your perception of a standard week that includes your usual work days and work-free days.

Personal Data

Date: _____

Name: _____

eMail: _____

Age: _____ years

Sex: female ☐ male ☐

Height: _____ cm

Weight: _____ kg

Country: _____

City: _____

Postal Code: _____

Participant ID:

MCTQ, English, Version 2015-01

MCTQ

I have a regular work schedule (this includes being, for example, a housewife or househusband):

Yes ☐ I work on 1☐ 2☐ 3☐ 4☐ 5☐ 6☐ 7☐ day(s) per week.

No ☐

Is your answer "*Yes, on 7 days*" or "*No*", please consider if your sleep times may nonetheless differ between regular 'workdays' and 'weekend days' and fill out the MCTQ in this respect.

Please use 24-hour time scale (e.g. 23:00 instead of 11:00 pm)!

Workdays

Image 1: I go to bed at _____ o'clock.

Image 2: Note that some people stay awake for some time when in bed!

Image 3: I actually get ready to fall asleep at _____ o'clock.

Image 4: I need _____ minutes to fall asleep.

Image 5: I wake up at _____ o'clock.

Image 6: After _____ minutes I get up.

I use an alarm clock on workdays: Yes ☐ No ☐

If "Yes": I regularly wake up BEFORE the alarm rings: Yes ☐ No ☐

Free Days

Image 1: I go to bed at _____ o'clock.

Image 2: Note that some people stay awake for some time when in bed!

Image 3: I actually get ready to fall asleep at _____ o'clock.

Image 4: I need _____ minutes to fall asleep.

Image 5: I wake up at _____ o'clock.

Image 6: After _____ minutes I get up.

My wake-up time (Image 5) is due to the use of an alarm clock: Yes ☐ No ☐

There are particular reasons why I cannot freely choose my sleep times on free days:

Yes ☐ If "Yes": Child(ren)/pet(s) ☐ Hobbies ☐ Others ☐, for example:_____

No ☐

Participant ID:

MCTQ, English, Version 2015-01

Work Details

In the last 3 months, I worked as a shift worker.

No ☐ Yes ☐ (please continue with "*My work schedules are ...*").

My usual work schedule …

… starts at _____ o´clock.

… ends at _____ o´clock.

My work schedules are …

… very flexible ☐ … a little flexible ☐ … rather inflexible ☐ … very inflexible ☐

I travel to work …

… within an enclosed vehicle (e.g. car, bus, underground). ☐

… not within an enclosed vehicle (e.g. on foot, by bike). ☐

I work at home. ☐

For the commute to work, I need ___ hours and ___ minutes.

For the commute from work, I need ___ hours and ___ minutes.

Time Spent Outdoors

On average, I spend the following amount of time outdoors in daylight (without a roof above my head):

on workdays: _____ hours _____ minutes

on free days: _____ hours _____ minutes

Participant ID:

Stimulants

Please give approximate/average amounts!

		per →	day	/ week	/ month
I smoke	_____ cigarettes ...		☐	☐	☐
I drink	_____ glasses of beer ...		☐	☐	☐
I drink	_____ glasses of wine ...		☐	☐	☐
I drink	_____ glasses of liquor/whiskey/gin etc. ...		☐	☐	☐
I drink	_____ cups of coffee …		☐	☐	☐
I drink	_____ cups of black tea ...		☐	☐	☐
I drink	_____ cans of caffeinated drinks (soft-drinks) …		☐	☐	☐
I take sleep medication _____ times ...			☐	☐	☐

Participant ID:

B. Pittsburgh Sleep Quality

PITTSBURGH SLEEP QUALITY INDEX (PSQI)

INSTRUCTIONS: The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

1. During the past month, when have you usually gone to bed at night?

   USUAL BED TIME _____

2. During the past month, how long (in minutes) has it usually take you to fall asleep each night?

   NUMBER OF MINUTES _____

3. During the past month, when have you usually gotten up in the morning?

   USUAL GETTING UP TIME _____

4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spend in bed.)

   HOURS OF SLEEP PER NIGHT _____

INSTRUCTIONS: For each of the remaining questions, check the one best response. Please answer all questions.

5. During the past month, how often have you had trouble sleeping because you...

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
(a) ...cannot get to sleep within 30 minutes	☐	☐	☐	☐
(b) ...wake up in the middle of the night or early morning	☐	☐	☐	☐
(c) ...have to get up to use the bathroom	☐	☐	☐	☐
(d ...cannot breathe comfortably	☐	☐	☐	☐
(e) ...cough or snore loudly	☐	☐	☐	☐
(f) ...feel too cold	☐	☐	☐	☐
(g) ...feel too hot	☐	☐	☐	☐
(h) ...had bad dreams	☐	☐	☐	☐
(i) ...have pain	☐	☐	☐	☐
(j) Other reason(s), please describe _____ _____				
How often during the past month have you had trouble sleeping because of this?	☐	☐	☐	☐

PSQI Page 1

	Very good	Fairly good	Fairly bad	very bad
6. During the past month, how would you rate your sleep quality overall?	☐	☐	☐	☐

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
7. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?	☐	☐	☐	☐
8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?	☐	☐	☐	☐

	No problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?	☐	☐	☐	☐

	No bed partner or roommate	Partner/ roommate in other room	Partner in same room, but not same bed	Partner in same bed
10. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?	☐	☐	☐	☐

If you have a roommate or bed partner, ask him/her how often in the past month you have had...

	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
(a) ...loud snoring	☐	☐	☐	☐
(b) ...long pauses between breaths while asleep	☐	☐	☐	☐
(c) ...legs twitching or jerking while you sleep	☐	☐	☐	☐
(d) ...episodes of disorientation or confusion during sleep	☐	☐	☐	☐
(e) Other restlessness while you sleep; please describe	☐	☐	☐	☐

_____

_____

SCORING INSTRUCTIONS FOR THE PITTSBURGH SLEEP QUALITY INDEX:

The Pittsburgh Sleep Quality Index (PSQI) contains 19 self-rated questions and 5 questions rated by the bed partner or roommate (if one is available). Only self-rated questions are included in the scoring. The 19 self-rated items are combined to form seven "component" scores, each of which has a range of 0-3 points. In all cases, a score of "0" indicates no difficulty, while a score of "3" indicates severe difficulty. The seven component scores are then added to yield one "global" score, with a range of 0-21 points, "0" indicating no difficulty and "21 " indicating severe difficulties in all areas.

Scoring proceeds as follows:

Component 1: Subjective sleep quality

Examine question #6, and assign scores as follows:

Response	Component 1 score
"Very good"	0
"Fairly good"	1
"Fairly bad"	2
"Very bad"	3

Component 1 score:_____

Component 2: Sleep latency

1. Examine question #2, and assign scores as follows:

Respo\nse	Score
≤15 minutes	0
16-30 minutes	1
31-60 minutes	2
> 60 minutes	3
Question #2 score:	_____

2. Examine question #5a, and assign scores as follows:

Response	Score
Not during the past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3
Question #5a score:	_____

3. Add #2 score and #5a score

Sum of #2 and #5a: _____

4. Assign component 2 score as follows:

Sum of #2 and #5a	Component 2 score
0	0
1-2	1
3-4	2
5-6	3

Component 2 score:_____

Component 3: Sleep duration

Examine question #4, and assign scores as follows:

Response	Component 3 score
> 7 hours	0
6-7 hours	1
5-6 hours	2
< 5 hours	3

Component 3 score:_____

Component 4: Habitual sleep efficiency

1. Write the number of hours slept (question #4) here:_____
2. Calculate the number of hours spent in bed:

   Getting up time (question #3):_____

   Bedtime (question #1):_____

   _____

   *Number of hours spent in bed:*_____

3. Calculate habitual sleep efficiency as follows:

   (Number of hours slept/Number of hours spent in bed) X 100 = Habitual sleep efficiency (%)

   (_____/_____) X 100 = %

4. Assign component 4 score as follows:

Habitual sleep efficiency %	Component 4 score
> 85%	0
75-84%	1
65-74%	2
< 65%	3

Component 4 score:_____

Component 5: Step disturbances

1. Examine questions #5b-5j, and assign scores for each question as follows:

Response	Score
Not during the past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

5b score: _____
5c score: _____
5d score: _____
5e score: _____
5f score: _____
5g score: _____
5h score: _____
5i score: _____
5j score: _____

2. Add the scores for questions #5b-5j:

Sum of #5b-5j: _____

3. Assign component 5 score as follows:

Sum of #5b-5j	Component 5 score
0	0
1-9	1
10-18-4	2
19-27	3

Component 5 score: _____

Component 6: Use of sleeping medication

Examine question #7 and assign scores as follows:

Response	Component 6 score
Not during the past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

Component 6 score: _____

Component 7: Daytime dysfunction

1. Examine question #8, and assign scores as follows:

Response	Score
Never	0
Once or twice	1
Once or twice each week	2
Three or more times each week	3

Question#8 score: _____

2. Examine question #9, and assign scores as follows:

Response	Score
No problem at all	0
Only a very slight problem	1
Somewhat of a problem	2
A very big problem	3

Question #9 score: _____

3. Add the scores for question #8 and #9:

Sum of #8 and #9: _____

4. Assign component 7 score as follows:

Sum of #8 and #9	Component 7 score
0	0
1-2	1
3-4	2
5-6	3

Component 7 score: _____

Global PSQI Score

Add the seven component scores together:

Global PSQI Score: _____

C. Epworth sleep scale

Epworth Sleepiness Scale

Name: _____ Today's date: _____

Your age (Yrs): _____ Your sex (Male = M, Female = F): _____

How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired?

This refers to your usual way of life in recent times.

Even if you haven't done some of these things recently try to work out how they would have affected you.

Use the following scale to choose the **most appropriate number** for each situation:

0 = would **never** doze
1 = **slight chance** of dozing
2 = **moderate chance** of dozing
3 = **high chance** of dozing

It is important that you answer each question as best you can.

Situation	**Chance of Dozing (0-3)**
Sitting and reading	
Watching TV	
Sitting, inactive in a public place (e.g. a theatre or a meeting)	
As a passenger in a car for an hour without a break	
Lying down to rest in the afternoon when circumstances permit	
Sitting and talking to someone	
Sitting quietly after a lunch without alcohol	
In a car, while stopped for a few minutes in the traffic	

THANK YOU FOR YOUR COOPERATION

D.Ethical approval form study 1 (Chapter 3)

FACULTY OF SCIENCE AND ENGINEERING

Manchester Metropolitan University

MEMORANDUM

TO	Victoria McIver
FROM	Karen Hartley
DATE	7th November 2017
DATE OF EXPIRY:	7th March 2018
SUBJECT	Application for Ethical Approval **(SE1617158)**

On the 7th November 2017 the Head of Ethics for Science & Engineering considered your application for Ethical Approval (**SE1617158**) entitled "Circadian influences on metabolic markers, gastric emptying rate and substrate utilisation during fasted and non-fasted brisk walking". The application has been granted Favourable Opinion and you may now commence the project.

MMU requires that you report any Adverse Event during this study immediately to the Head of Ethics (Professor Tristan McKay) and the Research Degrees Administrator. Adverse Events are adverse reactions to any modality, drug or dietary supplement administered to subjects or any trauma resulting from procedures in the protocol of a study.

An Adverse Event may also be accidental loss of data or loss of sample, particularly human tissue. Loss of human tissue or cells must also be reported to the designated individual for the Human Tissue Authority licence. Please notify Professor Tristan McKay of any issues relating to this.

If you make any changes to the approved protocol these must be approved by the Faculty Head of Ethics. If amendments are required you should complete the MMU Request for Amendment form (found on the Graduate School website) and submit it to the Administrator.

Regards

Karen Hartley
Research Administrator
All Saints North

Page **1** of **1**

E. Ethical approval form study 2 (Chapter 4)

FACULTY OF SCIENCE AND ENGINEERING

Manchester Metropolitan University

MEMORANDUM

TO	Victoria McIver
FROM	Karen Hartley
DATE	7th November 2017
DATE OF EXPIRY:	7th March 2018
SUBJECT	Application for Ethical Approval **(SE1617158)**

On the 7th November 2017 the Head of Ethics for Science & Engineering considered your application for Ethical Approval (**SE1617158**) entitled "Circadian influences on metabolic markers, gastric emptying rate and substrate utilisation during fasted and non-fasted brisk walking". The application has been granted Favourable Opinion and you may now commence the project.

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If you make any changes to the approved protocol these must be approved by the Faculty Head of Ethics. If amendments are required you should complete the MMU Request for Amendment form (found on the Graduate School website) and submit it to the Administrator.

Regards

Karen Hartley
Research Administrator
All Saints North

Page **1** of **1**

F. Ethical approval form study 3 (Chapter 5)

11/10/2018

Project Title: The effect of fasted versus non fasted cycling at different times of day on metabolic responses, gastrointestinal function, and appetite in healthy lean population

EthOS Reference Number: 0855

Ethical Opinion

Dear Victoria Mciver,

The above application was reviewed by the Science and Engineering Research Ethics and Governance Committee and, on the 11/10/2018, was given a favourable ethical opinion. The approval is in place until 30/06/2019 .

Conditions of favourable ethical opinion

Application Documents

Document Type	File Name	Date	Version
Consent Form	Informed Consent Form - Victoria McIver PhD Study 3	29/06/2018	1
Additional Documentation	PSQI - Victoria McIver	29/06/2018	1
Additional Documentation	Epworth Sleepiness Scale - Victoria McIver	29/06/2018	1
Additional Documentation	MCTQ - Victoria McIver	29/06/2018	1
Additional Documentation	Sleep Diary - Victoria McIver	29/06/2018	1
Additional Documentation	Physical activity questionnaire - Victoria McIver	29/06/2018	1
Additional Documentation	Dietary and PA Record Sheet	29/06/2018	1
Additional Documentation	Appetite - VAS scale	29/06/2018	1
Additional Documentation	Medical screening questionnaire - Victoria McIver	29/06/2018	1
Additional Documentation	Advertisement - Study 3 PhD Victoria McIver	13/08/2018	2
Additional Documentation	Participant Feedback Form	13/08/2018	2
Additional Documentation	Direct questions and answers to reviewers comments - Victoria McIver	13/08/2018	2
Recruitment Media	Advertisement - Study 3 PhD Victoria McIver	13/08/2018	2
Information Sheet	Information Sheet - Victoria McIver - Study 3	13/08/2018	2
Information Sheet	Direct questions and answers to reviewers comments - Victoria McIver	14/08/2018	2
Project Proposal	Project proposal_Study3_Victoria McIver	14/08/2018	2

The Science and Engineering Research Ethics and Governance Committee favourable ethical opinion is granted with the following conditions

Adherence to Manchester Metropolitan University's Policies and procedures

This ethical approval is conditional on adherence to Manchester Metropolitan University's Policies, Procedures, guidance and Standard Operating procedures. These can be found on the Manchester Metropolitan University Research Ethics and Governance webpages.

Amendments

If you wish to make a change to this approved application, you will be required to submit an amendment. Please visit the Manchester Metropolitan University Research Ethics and Governance webpages or contact your Faculty research officer for advice around how to do this.

G. Ethical approval form study 4 (Chapter 6)

11/10/2018

Project Title: Implications of fasted cycling exercise on metabolic responses, gastrointestinal function, and appetite, in overweight individuals

EthOS Reference Number: 0878

Ethical Opinion

Dear Victoria Mciver,

The above application was reviewed by the Science and Engineering Research Ethics and Governance Committee and, on the 11/10/2018, was given a favourable ethical opinion. The approval is in place until 01/10/2019 .

Conditions of favourable ethical opinion

Application Documents

Document Type	File Name	Date	Version
Project Proposal	Project proposal_Study4_Victoria McIver	12/07/2018	1
Consent Form	Informed Consent Form - Victoria McIver PhD Study 4	12/07/2018	1
Additional Documentation	Dietary and PA Record Sheet	12/07/2018	1
Additional Documentation	Physical activity questionnaire - Victoria McIver	12/07/2018	1
Additional Documentation	Sleep Diary - Victoria McIver	12/07/2018	1
Additional Documentation	PSQI - Victoria McIver	12/07/2018	1
Additional Documentation	MCTQ - Victoria McIver	12/07/2018	1
Additional Documentation	Epworth Sleepiness Scale - Victoria McIver	12/07/2018	1
Additional Documentation	Medical screening questionnaire - Victoria McIver	12/07/2018	1
Additional Documentation	Appetite - VAS scale	12/07/2018	1
Recruitment Media	Advertisement - Study 4 PhD Victoria McIver	16/08/2018	2
Information Sheet	Information Sheet - Victoria McIver - Study 4	16/08/2018	2
Information Sheet	Direct questions and answers to reviewers comments - Victoria McIver	16/08/2018	2
Additional Documentation	Direct questions and answers to reviewers comments - Victoria McIver	16/08/2018	2
Additional Documentation	Participant Feedback Form	16/08/2018	2

The Science and Engineering Research Ethics and Governance Committee favourable ethical opinion is granted with the following conditions

Adherence to Manchester Metropolitan University's Policies and procedures

This ethical approval is conditional on adherence to Manchester Metropolitan University's Policies, Procedures, guidance and Standard Operating procedures. These can be found on the Manchester Metropolitan University Research Ethics and Governance webpages.

Amendments

If you wish to make a change to this approved application, you will be required to submit an amendment. Please visit the Manchester Metropolitan University Research Ethics and Governance webpages or contact your Faculty research officer for advice around how to do this.

H.Physical activity Questionnaire

Participant Number:

General Physical activity questionnaire

This questionnaire is designed to find out about your physical activity (PA) routines in your everyday life. The questions within the document have been extracted from the general practice physical activity questionnaire (GPPAQ) which is a validated screening tool, used in primary care to assess the physical activity levels of adults (16 to 74 years) (Departmental of Health, 2013). The questions contained within the current questionnaire was first published in April 2013 by the Departmental of Health, however additional questions have been added to suit the purpose of the study.

Please try to answer every question. By answering these questions will provide greater knowledge and understanding of the participants physically activity status. Answer all questions in each section, as this will ensure the most accurate interpretation of your results. You should however leave a question blank if you are unsure of the answer, and discuss this with your researcher.

Participant Number:

Your answers will be treated as strictly confidential and will be used only for the current research. The document will be placed in a secure locked area for the researchers and supervisors use only.

1. Please tell us the type and amount of physical activity involved in your work?

		Please mark one box only
A	I am not in employment (e.g. retired, retired for health reasons, unemployed, full time career etc.)	
B	I spend most of my time at work sitting (such as in an office)	
C	I spend most of my time at work standing or walking. However, my work does not require much intense physical effort (e.g. shop assistant, hairdresser, security guard, childminder, etc.)	
D	My work involves definite physical effort including handling of heavy objects and use of tools (e.g. plumber, electrician, carpenter, cleaner, hospital nurse, gardener, postal delivery workers etc.)	
E	My work involves vigorous physical activity including handling of very heavy objects (e.g. scaffolder, construction worker, refuse collector, etc.)	

2. Do you typically follow the UK government's physical activity guidelines for adults?

	Guidelines for adults aged 19-64 are:	
A	At least 150 minutes of moderate aerobics activity such as cycling or fast walking every week and strength exercise on two or more days a week that work all the major muscles (legs, his, back abdomen, chest, shoulders, and arms). OR 75 minutes of vigorous aerobic activity, such as running or a game of singles tennis every week and strength exercises on two or more days a week that work all the major muscles (legs, hips, back, abdomen, chest, shoulders and arms). OR A mix of moderate and vigorous aerobic activity every week. For example, two 30-minute runs plus 30 minutes of fast walking equates to 150 minutes of moderate aerobic activity, and strength exercises on two or more days a week that work all the major muscles (legs, hips, back, abdomen, chest, shoulders and arms).	
B	No, I don't do any form of physical activity.	
C	I sometimes do, but not every week.	
D	I only partake in half the weekly amount that is recommended.	
E	Yes, I do follow one of the above options for physical activity guidelines.	
F	I do a considerable amount more than the physical activity guidelines. *If so please state what you do for physical activity/exercise and how often you do it?*	

Participant Number:

3. During the *last week*, how many hours did you spend on each of the following activities?
Please answer whether you are in employment or not

Please mark one box only on each row

		None	Some but less than 1 hour	1 hour but less than 3 hours	3 hours or more
A	Physical exercise such as swimming, jogging, aerobics, football, tennis, gym workout etc.				
B	Cycling, including cycling to work and during leisure time				
C	Walking, including walking to work, shopping, for pleasure etc.				
D	Housework/Childcare				
E	Gardening/DIY				

4. How would you describe your usual walking pace? Please mark one box only.

Slow pace (i.e. less than 3 mph) ☐
Steady average pace ☐
Brisk pace ☐
Fast pace (i.e. over 4 mph) ☐

5. When do you prefer to do physical activity/exercise

Morning ☐
Midday ☐
Evening ☐

6. Do you normally fast before conducting physical activity/exercise?

I always fast before any form of physical activity / exercise; 8-h or more ☐ 6-h or more ☐
3-h or more ☐

I only fast if I do any form of physical activity / exercise in the morning ☐

Regardless of time of day, I never fast before any form of physical activity / exercise ☐

I. Standardisation measurements

No significant differences between trials for pre-trial body mass detected (P > 0.05) (Table 16).

Table 20: Standardisation measurements for participants body mass

	Body mass (kg)				
Chapter 3 (FASTED vs. FED)	86 ± 14		86 ± 11		*P = 0.443*
Chapter 4 (FASTED AM vs. FED AM vs FASTED PM vs. FED PM)	82.9 ± 12.5	82.8 ± 12.5	82.7 ± 12.4	82.9 ± 12.5	*P= 0.396*
Chapter 5 (FASTED AM vs. FED AM vs FASTED PM vs. FED PM)	70.9 ± 7.3	71.1 ± 7.3	71.2 ± 7.2	71.1 ± 7.3	*P = 0.230*
Chapter 6 (FASTED AM vs. FED AM vs FASTED PM vs. FED PM)	88. 9 ± 11.3	89.1 ± 11.3	88.9 ± 11.1	88.9 ± 11.3	*P = 0.433*

Values are expressed as mean ± SD