Does the Intensity, Mode, and Timing of Exercise Affect Postprandial Gastrointestinal Function, Metabolic Responses and Energy Intake in Healthy Men?

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Abstract

The global prevalence of obesity has dramatically increased and has become a major economic burden for western countries; therefore, health professionals are looking at strategies to control this increase in body weight. Despite the well-established physiological effects of exercise, such as increased muscle oxygenic capacity and fuel utilisation being well documented, there is limited research available investigating how the human body responds to a change in exercise characteristics. Manipulating exercise intensity, mode, or timing has become a popular strategy for controlling metabolic health and might support improved weight management programs. The ability of the human body to respond to nutritional intervention after exercise may determine how fat stores are regulated and in what manner the body responds postprandially. This may hold the key to how the body recovers and adapts after exercise, however, this area of research remains ambiguous.

Through a series of studies on human volunteers this thesis is aimed at enhancing our understanding of how different exercise characteristics (intensity, mode, or timing) might affect gastrointestinal function, metabolic responses, appetite, and energy intake (EI), and as a result support the development of novel non-pharmacological interventions for weight management.

The main findings of this thesis were as follows. Gastric emptying rate (GER) is similar 30 min after continuous and intermittent exercise at a low intensity (40% $\dot{V}O_{2peak}$) and at a moderate intensity (60% $\dot{V}O_{2peak}$). Repeated bouts of continuous exercise cause food within the stomach to empty faster when compared to a one-off exercise bout matched at a high intensity of 70% $\dot{V}O_{2peak}$. Intermittent exercise > 40% $\dot{V}O_{2peak}$ and continuous exercise > 60% $\dot{V}O_{2peak}$ reduces subjective feelings of hunger immediately post-exercise. Although, continuous exercise < 50% $\dot{V}O_{2peak}$ has no effect. A subsequent meal following both intermittent and continuous exercise at various intensities abolishes any compensatory effects in subjective feelings of hunger. Acylated ghrelin increases immediately after continuous exercise < 50% $\dot{V}O_{2peak}$ whereas continuous exercise >70% $\dot{V}O_{2peak}$ and intermittent exercise at peak power output (PPO) decreases acylated ghrelin. Furthermore, postprandial acylated ghrelin increases after multiple exercise bouts compared to a one-off continuous bout

at the same intensity, although this also leads to an increase in EI within the first 24-h. EI was also found to be increased after moderate intensity intermittent exercise. Intermittent exercise >40% $\dot{V}O_{2peak}$ increases blood glucose during and immediately after exercise. Conflicting evidence revealed continuous exercise triggered a spike in blood glucose after a calorific meal more so than intermittent exercise causing blood glucose to remain elevated during recovery periods. Substrate utilisation shifts to predominantly fat oxidation after continuous and intermittent exercise at various intensities between 40- 70% $\dot{V}O_{2peak}$ while a small calorific meal diminishes this increase in the postprandial period.

The role of manipulating exercise characteristics through intensity, mode, or timing may hold positive implications for weight management practices in healthy and overweight populations. Future work is warranted to investigate the influence of ingesting whole foods/meals after exercise to better recognise the changes during the postprandial period; on GER, appetite, and appetite regulatory hormones over an extended duration to explore the effects on energy balance and metabolic health in the long-term.

Declaration

This is to certify that the material contained in this thesis has been produced by the author, has not been accepted in substance for any other degree, and is not currently submitted in candidature for any other academic award. Two Chapters within this thesis have been published (see page iii for more information) and all four research Chapters within this thesis have been presented as an abstract at different international conferences (see page iv for more information).

Publications

Mattin LR, Yau AMW, McIver VJ, James LJ, Evans GH. (2018). The Effect of Exercise Intensity on Gastric Emptying Rate, Appetite and Gut Derived Hormone Responses after Consuming a Standardised Semi-Solid Meal in Healthy Males. Nutrients. 10(6): 787. (https://www.mdpi.com/2072-6643/10/6/787).

Mattin LR, McIver VJ, Yau AMW, James LJ, Evans GH. (2020). A Comparison of Intermittent and Continuous Exercise Bouts at Different Intensities on Appetite and Postprandial Metabolic Responses in Healthy Men. Nutrients. 12(8): 2370.

Conferences

Mattin LR, Yau AMW, James LJ, Evans GH. (2016). Is appetite and gastric emptying effected by different exercise intensities after consuming a standardised calorie semi-solid meal. *19th-21st December 2016, ISENC International sports + Exercise Nutrition Conference, Newcastle.*

Mattin LM, McIver VJ. Yau, A. James, LJ. Evans, GH. (2018). A comparison of the effect of high and low intensity intermittent and continuous exercise on gastric emptying rate, appetite, and substrate utilisation in healthy males. Europhysiology. The QEII Centre, London, UK: *The physiology Society*, 2018:376-7"

Mattin LR, McIver VJ. Yau, A. James, LJ. Evans, GH. (2019). The effects of multiple exercise bouts on appetite and metabolic response to food ingestion. The Physiological Society annual conference. Aberdeen Exhibition and conference center, Aberdeen, UK: *The physiology Society*."

Mattin LR, Ishihara K, McIver VJ, Yau AMW, James LJ, Evans GH (2020). How does a 'twice-a-day approach' to a daily exercise retain, effect gastrointestinal hormonal, appetite regulation, and metabolic response after consuming a standardised breakfast and lunch respectively after each exercise bout in healthy males. Europhysiology. ESTREL, Congress Center, Berlin, Germany." Canceled due to COVID-19.

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At points, this journey has felt like “space oddity”, and at times, I have felt myself calling for ground control and even maybe Major Tom, so without hesitation. PhD thesis it’s time to say goodnight and, goodbye.

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List of Abbreviations and symbols

°	Degree Celsius
µl	Microlitre
µM	Micromole
$^{12}CO_2$	Carbon 12 carbon dioxide
$^{13}CO_2$	Carbon 13 carbon dioxide
Acetyl-coA	Acetyle coenzyme a
ACSM	American College of Sport Medicine
AgRP	Agouti-related protein
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
AUC	Area under the curve
BBB	Blood brain barrier
BM	Body mass
BMI	Body mass index
^{13}C	Carbon 13
CABF	Celiac artery blood flow
CGM	Continuous glucose monitoring
CHO	Carbohydrate
CKK	Cholecystokinin
cm	Centimetre
CNS	Central nervous system
CO_2	Carbon Dioxide
CON	Continuous

DOB	Delta over baseline
DOMS	Delayed onset muscle soreness
DPP-IV	Dipeptidyl peptidase IV
EE	Energy expenditure
EI	Energy intake
EIMD	Exercise-induced muscle damage
ELISA	Enzyme-linked immunosorbent assay
et al.	*et alii*
ETEE	Estimated trial energy expenditure
FABP	Fatty acid-binding protein
Fat_{max}	Maximal fat oxidation
FFA	Free fatty acids
G	Gram
GER	Gastric emptying rate
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
h	Hour
H^+	Hydrogen ion
HIIE	High intensity intermittent exercise
HIIT	High intensity intermittent training
HR	Heart rate

ICV	Intracerebroventricular
I-FABP$_2$	Intestinal fatty acid binding protein 2
INT	Intermittent
IOM	Institute of medicine
IR	Infrared
K^+	Potassium ion
kcal	kilocalorie
kg	Kilogram
kJ	Kilojoules
L	Litre
Ltd	Limited
m	Metre
MC4R	Melanocortin receptor 4
mg	Miligram
MICE	Moderate intense continuous exercise
min	Minute
mL	Millilitre
mm	Millimetre
mmol	Millimoles
MRI	Magnetic resonance imaging
n	Participant number
Na^+	Sodium ion
NEFA	Non-esterified fatty acid
N^2p	Partial eta squared
NPY	Neuropeptide Y

O_2	Oxygen
pmol/L	Picomoles Per Litre
P	Probability
pH	Potential Hydrogen
POMC	Proopiomelanocortin
PPO	Peak power output
PP	Pancreatic polypeptide
PYY	Peptide tyrosin tyrosin
RBE	Repeated bout effect
REE	Resting energy expenditure
RER	Respiratory Exchange Ratio
rER	Rough endoplasmic reticulum
RMR	Resting metabolic rate
rpm	Revolutions/min
RPE	Rating of perceived exertion
SD	Standard Deviation
sec	Second
SEM	Standard error of the mean
sER	Smooth endoplasmic reticulum
SPSS	Statistical Package for Social Sciences
SIT	Sprint interval training
T_{Core}	Core body temperature
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
VCO_2	Volume of Carbon Dioxide
VO_2	Volume of oxygen
VO_2max	Maximum oxygen uptake
VO_2peak	Peak oxygen uptake
VTA	Ventral tegmental area
W	Watts
WB	Wellbeing
WHO	World Health Organisation
y	Year

CHAPTER 1.0

GENERAL INTRODUCTION

The global prevalence of obesity has dramatically increased becoming a leading health concern (Finucane et al., 2011), contributing to approximately 2.8 million deaths each year world-wide (Rouhani et al., 2016). The sharp increase in the prevalence of obesity has risen with around ~35% of men being defined as obese; BMI $\geq$ 30kg/m^2 (Bray et al., 2018). Changes in lifestyle have contributed to reduced physical activity at work and home causing a reduction in energy expenditure (Brock et al., 2009) combined with a daily increase in food of ~500 KCal per day since the 1970s (Woodward et al., 2021, Chan and Woo, 2010). The rapidly increasing prevalence of obesity has prompted health professionals to enhance weight management strategies to control the increase in body weight by excess fat accumulation (Bray et al., 2018, Church et al., 2011, Brock et al., 2009, Heath et al., 2006). Obesity has serious implications for public health and is a major health concern which is linked to several chronic diseases such as cardiovascular and metabolic disorders, in particular atherosclerosis and type II diabetes (Poirier et al., 2006), which results in considerable health implications if untreated (Di Angelantonio et al., 2016) and in extreme cases early death (Rodgers et al., 2018). Therefore, finding efficient and effective exercise strategies to increase physical activity to improve weight management may be critical for improving health.

Recent trends have suggested the obesity epidemic is related to the substantial decline in physical activity (Church et al., 2011, Brock et al., 2009). In its most basic form, weight gain is simply the end-product of increased energy accumulation. Therefore understanding how exercise can be incorporated into the general populations daily routine may well become a prominent strategy for increasing physical activity and reducing body weight (Heath et al., 2006). Exercise can profoundly affect weight loss by increasing energy expenditure and subsequently creating a negative energy balance (Catenacci and Wyatt, 2007). Given that there are somewhat mixed findings in regard to how acute bouts of exercise impact subsequent

energy intake (EI). Schubert et al, (2013) conducted a meta-analysis on absolute and relative EI when using different acute methods of exercise (walking, running, cycling, swimming or resistance exercise) demonstrating minor differences in metabolic, endocrine and neural signalling pathways with the most diverse differences discovered after swimming (King et al., 2011b). In addition, individuals who engage in less physical activity or who have a lower fitness level are more likely to experience an anorexia effect immediately post-exercise after all forms of exercise (Schubert et al., 2013). The term "exercise-induced anorexia" has been used to describe the reduction in perceived hunger felt in the time period after exercise (Blundell et al., 2003).

Investigations involving exercise that require greater metabolic and mechanical demand, such as running, have shown a more potent suppression in perceived hunger and a higher energy expenditure from the exercise prescribed (Broom et al., 2007, King et al., 2010a). Therefore, to prevent inconsistencies between investigations within this thesis cycling was selected to be the main method of exercise. It is important to add that cycling is the preferred form of exercise for commuting to work (Wardman et al., 2007) with males cycling more than females (Unwin, 1995). The potential health gain from increasing the level of cycling is widely recognised and is an excellent form of aerobic exercise (Morris et al., 1990). Information has also been gathered suggesting further benefits of cycling which are cheap, environment friendly and a time saving mode of transport (Wardman et al., 2007, Noland, 1995, Unwin, 1995). Cycling has increased in recent year as an enjoyable form of indoor activity at home or within a gym (Chavarrias et al., 2019). It has been suggested that any person wishing to increase their physical activity level from a sedentary state, should in general start by using low-impact exercise such as cycling, walking or swimming (Vincent and Vincent, 2013). Although, even with walking programmes in people with BMIs ranging from 25-40 Kg/m^2, 32% of participants reported musculoskeletal complaints (Janney and Jakicic, 2010). Independently using the term 'exercise' in relation to weight loss remains controversial. Therefore, it is important to define the terminology (mode, intensity, and timing) used to describe exercise characteristics within this thesis. Mode identified exercise methods described as continuous (CON) or intermittent (INT). Intensity conveyed the relative exertion participants worked at in relation to maximum oxygen

uptake ($\dot{V}O_{2Max}$) using the American College of Sports Medicine (ACSM), classifications as a guideline; Low < 45% $\dot{V}O_{2Max}$, Moderate (MOD) 46-63% $\dot{V}O_{2Max}$, High 64-90% $\dot{V}O_{2Max}$, and Very High >91% $\dot{V}O_{2Max}$ (Garber et al., 2011). Finally, timing expressed when exercise bouts were conducted within a trial day, or the sequence exercise was conducted in. Manipulating exercise characteristics (mode, intensity, and timing) might be one theory why acute appetite response and subsequent food intake fluctuate in the initial hours following an acute exercise bout.

Nutritional strategies that are designed for athletic groups/ individuals endeavour to ensure adequate availability of muscles fuel stores by producing sufficient levels of adenosine triphosphate (ATP) according to the demands of the event (Burke, 2021). Although, post-exercise strategies strongly intend to optimise muscle glycogen content by supplying carbohydrate (Burke et al, 2018) and optimising muscle adaptation by increasing amino-acids within the diet (Witard et al., 2021, Phillips, 2014b), possible more so using leucine (Katsanos et al., 2006, Paddon-Jones et al., 2006). Over the last decade, opinions between the scientific literature and the media surrounding nutritional interventions to stimulate recovery after exercise have consistently suggested immediate calorie replacement within all populations to manipulate the anabolic window (Aragon and Schoenfeld, 2013). It is important to consider populations who participate in exercise for health and wellbeing reasons may have vastly different nutritional requirements than athletes. Unfortunately, any nutrient dense food or drink that is ingested after most forms of exercise might abolish or influence the overall energy deficit achieved by an exercise bout. In reality, there is often minimal provision of nutrition advice provided when it comes to post-exercise strategies for the general public. What athletes consume post-exercise may depend on their long-term approach and the phase of training that is being focused on in that particular training period, whereas the main emphasis for non-athlete adults is weight loss (Yumuk et al., 2015). Understanding how the human body responds in the hours after exercise may be mitigated by the timing of food ingestion and/or the macronutrient composition provided within the diet is an important consideration. This therefore opens an interesting debate, whether an untrained healthy population responds physiologically in the same manner as a highly physically trained population.

Periodized nutrition strategies used with athletic individuals is uncommon in healthy to overweight populations (Jeukendrup, 2017a). Individuals who perform exercise to improve health-related changes don't usually contemplate; pre, within or post session nutritional requirements. Given the current understanding that nutrient availability can modulate an increase in available energy in excess of requirements, it is somewhat surprising that very few investigations have examined the role of mixed macronutrient meal ingestion after exercise with a healthy population, with the outcome of modifying body composition (fat loss or muscle gain). Hence, well-defined guidance is required to optimise the relationship between energy expenditure and EI by expanding our awareness about the role of post-exercise nutrition and how the food that is consumed in the hours after exercise might influence how our body stores and utilises energy.

For several decades, research has mostly focused on adaptations in skeletal muscle, although there are numerous adaptations in other organs that might be influenced by nutritional intake in the hours after exercise. The phase after consuming nutrient dense material is known as the "postprandial period". Such changes in this period are often overlooked or receive significantly less consideration, such as changes in the gastrointestinal tract (GI). Examples include but are not limited to changes in the stomach and the small intestine. A well-functioning GI system, delivers nutrients to the body to form energy and any impairment in this system can delay this response (Cheng et al., 2010). Therefore, any nutrient or fluid consumed is required to first pass through the pyloric sphincter from the stomach into the duodenum (small intestines). This process is known as gastric emptying rate (GER) has been used as a method to assess how fast an ingested food or solution empties from the stomach. Several scenarios have been used to enhance how the stomach adapts to nutrient delivery and it has been suggested that the 'gastric emptying' process can be trained, with the intestinal tract being highly adaptive when delivery of nutrients is required (Yau et al., 2014, Jeukendrup and McLaughlin, 2011). The ability to recover after exercise might be limited by the capability of the stomach to deliver nutrients to the small intestine. Unsurprisingly, more information is required to identify if GER is a limiting step in the delivery of nutrients after exercise. It is important to enhance awareness of gastric emptying, by eliminating this process as a potential mechanism responsible for

negative metabolic changes in the hours or days after exercise. For this reason, GER is the central focus within this thesis. By examining how the body responds to a mixed macronutrient meal (whole foods), after exercise might improve strategies to control metabolic changes within the postprandial period.

It is suspected that a slower emptying rate may delay the appearance of nutrients in the circulation that might contribute to satiety (a feeling or condition of being full after eating food). The regulation of GER is therefore perceived as an important factor in appetite control (Delzenne et al., 2010). The crosstalk between the gut and the brain, might be critical in controlling appetite and energy balance as specific brain regions such as the brainstem and the hypothalamus receive signals through the vagus nerve in the form of gut hormones secreted from the enteroendocrine cells of the stomach or intestines (Cork, 2018). Importantly, the drive to consume food does not happen by chance and must be triggered to cause the behavioural process of eating. These physiological triggering events might arise from low blood glucose, low GI activity and an empty stomach; from lack of recent ingestion (Blundell et al., 2020) or a change in emptying rate. Nevertheless, these signals lead to a potential change in biomarkers, which are responsible for central and peripheral signals that might trigger an appetite response after exercise or a period of food restrictions (Murphy and Bloom, 2006). Around ~20 peptide hormones are located in the GI tract (Ahlman and Nilsson, 2001) and a large number of these are thought to influence EI (Murphy and Bloom, 2006). Essentially, five main GI hormones will be focused on within the thesis; acylated ghrelin, glucagon-like peptide-1 (GLP-1), peptide tyrosine tyrosine (PYY), insulin and pancreatic polypeptide (PP), see section 2.5. Gastrointestinal Hormones for more information.

The regulation of food intake is complex, involving hormonal signalling from the gastrointestinal system associated organs such as the liver and pancreas and adipose tissue e.g. fat cells (Zouhal et al., 2019). Aldiss et al, (2018) suggests that adipose tissue is not merely an energy store, but could also be seen as a key regulator of endocrine activity especially after intense exercise. However, there has been limited research focusing on how a healthy untrained population responds to nutritional interventions in the hours after exercise. Considerable attention has been given to the theory that muscle glycogen depletion must be restored in the first two hours following

intense exercise (Friedman et al., 1991) as it might therefore elicit greater lipid oxidation rates in the following 24 h period (De Feo et al., 2003). Research suggests that consuming carbohydrate in a state of low glycogen results in an increased glycogen resynthesis rate prioritising storage rather than direct energy use (Jeukendrup, 2017b). The different rates of substrate utilisation during and after exercise have clear implications for potential fat loss (De Feo, 2013). This theory warrants further investigation as increased oxidation of lipids after an exercise bout might in principle increase total active calorie expenditure boosting exercise induced weight-loss.

Food intake and energy expenditure are homeostatically regulated (Blundell et al., 2020, Mani et al., 2019, Zouhal et al., 2019) and changes to this process are thought to respond to an acute lack of immediately available nutrients that will meet the long-term needs of the body to restore equilibrium (energy balance). Limited research has targeted changes in GER, although there is growing interest within the literature committed to recognising the role of gut hormones in regulating appetite and satiety. This area is complex, and research into gastric emptying and gut hormone responses remains relatively unexplored (Crabtree and Blannin, 2015, King et al., 2010b, Wasse et al., 2013b) after exercise in healthy untrained populations.

In summary, research focusing on gastrointestinal responses and appetite regulation needs to further expand and provide definitive conclusions on gut and intestinal hormonal markers related to energy balance. The adaptive response to exercise is determined by a combination of factors: the duration, intensity and type of exercise (Jeukendrup, 2017a). The manner in which GI hormones interact after different exercise stimulus might in theory be one mechanism to explain the physiological responses seen within the postprandial period. Additionally, eliminating methodological differences between investigations, such as fasting participants prior to exercise, using the same method of exercise (cycling) and using the same manner in which food is provided to participants in a holistic and realistic approach to energy homoeostasis will allow a more comprehensive comparison between the investigations within this thesis. Grasping a better understanding of gut hormones would potentially facilitate the development of exercise and dietary interventions to modulate the prevalence of obesity, by improving effective strategies to control

weight. The mechanisms responsible for changes in appetite as a consequence of exercise are not well defined, suggesting further attention is needed to determine what process gastric emptying plays within appetite control.

1.2. Thesis Aims and Objectives

The aim of this thesis is to determine whether there is a connection between gastric emptying rate (GER) and a consequence of, 1) adjustment in exercise intensity, 2) using an intermittent mode of exercise or 3) timing of exercise by conducting multiple exercise bouts. These aims will be examined in 4 studies reported in Chapters 4, 5, 6, and 7 using healthy men:

- Study 1, reported in Chapter 4 investigated whether changing the intensity of an exercise bout was associated with alterations in appetite regulation, metabolic responses and GER following a post-exercise semi-solid meal.

- Study 2, reported in Chapter 5 questioned whether variations in exercise mode conducted at the same intensity influenced GER, appetite regulation, glucose concentrations, and 24-h EI.

- Study 3, reported in Chapter 6 was undertaken to establish whether a one-off exercise bout would evoke different GI function, appetite regulatory hormone response and 24-h EI, compared to a split exercise bout conducted at the same intensity.

- Study 4, reported in Chapter 7 examined whether changing the timing of exercise by performing multiple modes of exercise (continuous and intermittent) during the same day were associated with variations in GI function, appetite, and metabolic responses. This study also examined post-exercise wellbeing to elucidate any recovery effects for EI.

CHAPTER 2.

REVIEW OF LITERATURE

Current literature has been reviewed, in relation to the GI system and the different GI-phases that combine leading to changes in GER and intestinal absorption. Changes in hormone regulation, appetite, energy balance and the impact of exercise, when intensity, mode or timing is manipulated have been discussed and evaluated. Further information regarding the potential mechanisms and physiological differences when exercising in a fasted state have been considered in relation to metabolic adaptations. This Chapter concludes by reviewing the literature relating to the recovery process in the postprandial period.

2.1. Is there an Optimal Exercise Protocol?

There is a belief that physical activity drives up perceived hunger and increases food intake (Blundell et al., 2003). This is thought to be achieved by altering the hedonic response to food and adjusting macronutrient preference or food choices (Simon et al., 2017). Therefore, the effectiveness of exercise to induce weight loss is a controversial topic, and considerable interest in the effect of exercise on appetite-regulating hormones after short-term acute exercise bouts have witnessed a significant surge in popularity (Hazell et al., 2016, Deighton and Stensel, 2014). Previous investigations have mainly focused on continuous exercise to influence post-exercise metabolism and appetite, by predominantly prescribing exercise bouts of 30-120 min at an intensity between 35-85% $\dot{V}O_{2Max}$ (Schubert et al., 2013) (Table 1). Although, within these investigations minimal analysis has been conducted using multiple gut hormones after exercise; the most popular being ghrelin, GLP-1 and PYY. Additionally Martins et al, (2007a) and Ueda et al, (2009b) are the only two investigations to date that have considered using multiple hormones (five) after an acute cycling bout, with very few investigations also observing GER as a marker of appetite control (please see Table 1).

Additional forms of physical activity such as intermittent exercise might also manipulate appetite regulation in the postprandial period. Therefore, high-intensity exercise is at the forefront of the health and fitness industry, predominantly in the form of high-intensity intermittent exercise (HIIE) or high-intensity intermittent training (HIIT) (Hazell et al., 2016). Hence, high to moderate intermittent exercise might disrupt appetite regulation and decreases fat mass by increasing exercise-induced energy expenditure (Skelly et al., 2014, Townsend et al., 2014, Hazell et al., 2012) or decreased post-exercise EI (Sim et al., 2014). HIIE and HIIT, which consists of repeated high-intensity exercise at >80% peak power output (PPO) for 30-60 seconds, separated by 4-6 min recovery periods, with sessions lasting 10-30 min (Astorino and Schubert, 2018). In addition, other high intensity sessions have been adopted, such as "all out" training intensities of ≥100% PPO for ≤30 seconds known as sprint interval training (SIT).

Nevertheless, concerns have been raised with the safety of using HIIT and SIT methods in clinical populations (Weston et al., 2014). Gillen et al, (2014) agrees that HIIT protocols may not be safe or tolerable for many individuals and require specialised equipment to complete exercise of this nature. For this reason, a more appropriate variant known as modified low-volume HIIT has been employed to eliminate this issue, which consists of intervals at ~85-90% HR_{max} separated by 1-2 min of rest (Gillen and Gibala, 2014, Hood et al., 2011). Vitally, Little et al, (2010) used low-volume HIIT with healthy, untrained individuals discovering improved muscle metabolic capacity, and suggesting that more research is needed to investigate whether low-volume HIIT or HIIE can improve markers of metabolic health in healthy individuals.

The scientific literature has suggested that intermittent exercise in the form of HIIT may result in greater weight loss, by reducing the perception of appetite in the post-exercise period, when compared to continuous endurance exercise (Boutcher, 2011, Heydari et al., 2012, Trapp et al., 2008). Although, when comparing exercise matched for energy expenditure, these bouts typically result in HIIE being evidently shorter in duration than continuous endurance exercise bouts (Weston et al., 2014). Therefore, in an effort to guide future research, it is important to consider how exercise alters appetite-regulating hormones released from the GI tract, with knowledge of the

potential mechanisms involved, which might suggest exercise intensity and mode are an important aspect in the regulation of appetite, which maintains body fat mass.

The majority of gut hormones which catalyse appetite fluctuation, food intake and energy balance also stimulate the process of gastric emptying by hormonal signals and activity (Liu et al., 2019). Continuous endurance exercise performed on a bike has been shown to influence GER of a solid meal consumed post-exercise (Clegg et al., 2007) and drinking a 600ml solution pre-exercise is unaffected when GER was measured after exercise (Feldman and Nixon, 1982). These two investigations measured gastric emptying as a marker of appetite regulation in response to a cycling exercise bout, while minimal evidence has been collected on the changes in GER after HIIE. Similarly, continuous exercise has been shown to influence gastric emptying, although these changes in GER were assumed by changes in gut hormones which regulate GER, not by directly measuring GER (Zouhal et al., 2019), suggesting that, more research is needed in response to different exercise bouts provoking a change in GER after exercise.

Finally, the acute exercise bouts that have been prescribed have resulted in inadequate clarity regarding an optimal exercise protocol. Furthermore, there is an insufficient definition to describe the bout of exercise employed, as vastly different terms have been used within the literature when prescribing cycling as a form of exercise with terminology such as, ‘strenuous’ ‘high’ ‘intense’ ‘aerobic’ ‘moderate’ ‘vigorous’ being used to describe an exercise bout performed at 70% $\dot{V}O_{2Max}$ and astonishingly in some cases no terminology has been used at all to describe the exercise bout being conducted (please see summary in Table 1). This has caused confusion in pinpointing the exercise intensity or mode, that specifically affects appetite regulation and hormonal control after exercise. Future studies need to clarify exercise protocol terminology and the impact of various forms of exercise that have recently engaged the public interest such as intermittent exercise in the form of interval or HIIT.

Table 1. Acute Exercise Literature which used Cycling to effect Appetite, Food Intake, Energy Balance or GER

Study	Participants	Duration	Intensity	Definition	Gut Hormones	GER
Feldman et al. (1982)	M & W (2,3)	45 min	50 % $\dot{V}O_{2Max}$ 70 % $\dot{V}O_{2Max}$	Strenuous Strenuous	Gastrin	↔ 600 mL Liquid Consumed Pre-EXE (EXE &CON)
King et al. (1994)	M (12)	60 min	30 % $\dot{V}O_{2Max}$ 70 % $\dot{V}O_{2Max}$	Low High	*NR*	*NR*
King et al. (1996)	W (13)	50 min	70 % $\dot{V}O_{2Max}$	Intense	*NR*	*NR*
Hubert et al. (1998)	W (11)	40 min	70 % $\dot{V}O_{2Max}$	NR	*NR*	*NR*
Melby et al. (2002)	W (13)	75 min	65 % $\dot{V}O_{2Max}$	Mod	*NR*	*NR*
Clegg et al. (2007)	M (8)	60 min	60 % HR_{Max}	Mod	*NR*	↑ Post-EXE Solid Meal $T_{1/2}$ (208 ± 98; CON 238 ± 137)
Erdmann et al. (2007)	M & W (2,5)	30 min	50 W 100 W	Low High	Ghrelin, Insulin	*NR*
	M& W (4,3)	30, 60,120 min	50W	Low	Ghrelin, Insulin	*NR*
Martins et al. (2007a)	M & W (6,6)	60 min	65 % HR_{Max}	NR	Ghrelin, Insulin, GLP-1, PYY, PP	*NR*
Ueda et al. (2009b)	M (14)	60 min	50 % $\dot{V}O_{2Max}$	Aerobic	Glucagon, Ghrelin, Insulin, GLP-1, PYY	*NR*

Ueda et al. (2009a)	M (10)	30 min 75 min	50 % $\dot{V}O_{2Max}$ 75 % $\dot{V}O_{2Max}$	Mod High	Insulin, GLP-1, PYY	*NR*
Laan et al. (2010)	M& W (9, 10)	35 min	70 % HRR	Aerobic	*NR*	*NR*
Becker et al. (2012)	M (8)	60 min	70 % $\dot{V}O_{2Max}$	Aerobic	Ghrelin	*NR*
Hagobian et al. (2012)	M & W (11, 10)	60 min	70 % $\dot{V}O_{2Max}$	Acute EXE	Insulin, Ghrelin, Insulin, PYY	*NR*
Jokisch et al. (2012)	M (10)	45 min	65-75 % HR_{Max}	NR	*NR*	*NR*
Deighton et al. (2013b)	M (12)	60 min; 10 x 4 min	60 % $\dot{V}O_{2Max}$ 85-90% $\dot{V}O_{2Max}$	SSE HIIE	PYY	*NR*
Hazell et al. (2017)	M (10)	30 min 30 min 6 x 30s	65 % $\dot{V}O_{2Max}$ 85 % $\dot{V}O_{2Max}$ *100%	Mod High SIT	GLP-1, PYY	*NR*
Holliday et al. (2017c)	M (12)	15, 30, 45 min	80 % $\dot{V}O_{2Max}$	High	Ghrelin, GLP-1,PYY	*NR*
Benedetti et al. (2021)	M (30)	60 min	70 % $\dot{V}O_{2Max}$	Mod/ Vig	Ghrelin, Insulin,PYY	*NR*

Abbreviations and symbols: M; Men; W, women; Mod; moderate intensity; EXE = Exercise, SSE; steady state exercise; * All-out, 100%; HII, high-intense intermittent exercise, SIT; sprint interval training; Vig, vigorous; HRR; heart rate reserve; $\dot{V}O_{2Max}$, maximum oxygen consumption; MR_{Max}, heart rate max; CON, control; GLP-1, glucagon-like peptide-1; PYY, peptide YY; PP, pancreatic polypeptide; *NR*; Not Reported; ↑, increased; ↔, unchanged.

2.2. Function of the Gastrointestinal System

The gastrointestinal (GI) system comprises of a long passageway known as the alimentary canal which starts at the mouth and ends with the anus passing through the oesophagus, stomach, small intestine, large intestine, and rectum. Associated organs to the digestive system are the liver, gallbladder and pancreas (Nigam and Knight, 2017). Along the course of this journey, food is broken down and nutrients are extracted while waste material is disposed of as faeces (Van de Graaff, 1986). The primary functions of the GI tract are characterised as four distinct processes; digestion, absorption, excretion and protection (Cheng et al., 2010).

There are three main stages to gastric secretion; cephalic, gastric and intestinal phase (Katschinski, 2000). Starting with the cephalic phase, which begins before food enters the stomach by thought, sight, smell, taste and chewing (Feldman and Richardson, 1986). The gastric phase begins in the stomach where gastric glands secrete different products from several different cells (Table 2) and the simple columnar epithelium forms folds know as gastric pits. The secretion of gastric acid and intrinsic factor from the parietal cells make the initial content of the stomach very acidic > 2 pH (Dockray, 1999). Intrinsic factor is a glycoprotein which helps the absorption of folate (vitamin B_{12}) in the GI tract (Pavelka and Roth, 2010). The function of the acidic environment within the stomach is to kill potential pathogens in a non-immunological defence (Hunt et al., 2015) and begin the structural breakdown of proteins. Furthermore, pepsinogen enzymes react to the low pH and start activating pepsin. The peptide bounds of ingested protein are not directly affected by the acidic gastric acid conditions within the stomach but are digestive by pepsin which is optimised by the low pH (Van de Graaff, 1986).

Finally, the duodenum responds to the delivery of chyme by activating further enzymes, hormones and neural reflexes, which is known as the intestinal phase (Adler et al., 1991). The three phases overlap and interact contributing to overall GI motor response to food ingestion (Katschinski, 2000).

Table 2: Stomach Mucosal Cell's Gastric Secretion

Cell	Secretion	Region of Stomach	References
Goblet	Mucus	Fundus, Cardiac, pyloric	Pelaseyed (2014)
Parietal	Gastric acid (HCI) Gastric intrinsic factor (GIF)	Fundus, Body	Allen (2005)
Chief	Pepsinogen, Gastric Lipase	Fundus, body	Hunt (2015)
Argentaffin	Serotonin, Histamine	Fundus, cardiac, pyloric	Nagai (1976)
G Cell	Gastrin	Pyloric antrum	Hunt (2015)

2.2.1. Gastrointestinal Wall

The human gastrointestinal wall consists of four main layers; the mucosa, sub-mucosa, muscularis propria, and serosa. The mucosa is the innermost layer, which surrounds the lumen of the GI tract. The rough, longitudinal folds which increase the surface area play an important role in the overall digestion process, with the support from epithelial cells, which brace gastric glands that secrete different gastric juices and a lamina propria binding layer of connective tissue (Bornhorst and Singh, 2012). External to these components there are two thin layers of smooth muscle called the muscularis mucosae. The mucosa is fundamental across the length of the GI tract as within the stomach it allows distension which increases capacity, absorption within the small intestine, and water extraction from the large intestine. With goblet cells secreting mucus throughout the GI tract (Van de Graaff, 1986).

The sub-mucosa is a very vascular layer containing a large network of capillary blood vessels where nutrients released from digestive food forming chyme within the lumen are absorbed through the mucosa and enter the vessels of the submucosa. It also contains the submucosal plexus (plexus of Meissner). The GI tract is innervated by sympathetic and parasympathetic branches of the autonomic nervous system. The vagus nerves supply parasympathetic stimulation through the celiac ganglion to the stomach, pancreas, gallbladder and small intestine, whereas the large intestine receives impulses from the inferior mesenteric ganglia from the sacral region (Fung and Kong, 2018). Stimulation of the parasympathetic components increase peristaltic activity and muscle tone.

The muscularis layer has two main muscle layers. The inner circular layer moves food peristaltically through the intestine and mechanically grinds and crushes food, at the same time as mixing digestive chemicals through the chyme. The longitudinal layer forces the chyme further along the digestive system (Cheng et al., 2010). These movements are controlled by the myenteric plexus (plexus of Auerbach), which is found between the two muscle layers (Van de Graaff, 1986). Therefore, the outer serosa layer is the final lining of the GI tract which is a protective layer containing fibrous connective tissue which prevent external penetration.

2.2.2. Mouth

The process of digestion begins with the ingestion of a food product that are broken down both mechanically and chemically, using enzymes to separate food into simple components. The mouth is mainly responsible for food fragmentation (Bornhorst and Paul Singh, 2014). The initial chemical breakdown of food begins in the oral cavity during mastication, where ingested solid and semi-solid foods are broken down into small particles using teeth, and the injection of salivary enzymes amylase (breakdown of carbohydrates) and lingual lipase (breakdown of fats) start the digestion process of food; although saliva is also important to help the bolus travel from the mouth to the stomach by swallowing, via the oesophagus (Foegeding et al., 2011).

2.2.3. Stomach

The stomach is the second major compartment where food is mechanically separated and therefore the stomach is critical for the release of nutrients and the formation of chyme. The stomach is a muscular J-shaped organ, which frequently has different variations. It is located beneath the diaphragm in the left hypochondriac epigastric region of the abdominal cavity. This organ is divided into four major compartments: cardia, fundus, corpus (body) and pylorus (Figure 1). The cardia is the first area of the stomach which is separated from the oesophagus by the lower oesophageal sphincter. The dome-shaped area of the upper curvature of the stomach is the fundus, which is often filled with air (Liu et al., 2021). The body which is the largest portion and usually stores food and the pylorus is the lowest section of the stomach that is divided into two parts the antrum that mixes the food with digestive

juice and the pylorus canal that controls the transport of food into the duodenum (Somaratne et al., 2020).

The shape of the stomach depends on the contents and surrounding organs of the abdominal cavity (Kojima and Kangawa, 2005). However, individual variations can be affected by age, body type and respiratory phase (Liu et al., 2021). The stomach when empty is a slender cylindrical organ which will increase in size and shape after consuming fluid and food by the anterior wall increasing the area attached to the abdominal wall. During inspiration the stomach freely moves downwards, whilst it will be elevated in expiration regardless of the content or size (Burdan et al., 2012). The stomach can be subdivided into the proximal and distal regions. The proximal region represents the cardia, fundus and half of the corpus (body), regulates the gastric emptying of liquids and receives the food from the oesophagus (Urbain et al., 1989). The distal region contains the remaining half of the corpus (body), and the pylorus which mixes, folds and grinds predominantly solid food in order for it to be pumped through the pyloric sphincter into the small intestine (Kelly, 1980).

The movement of chyme through the digestive system is known as peristalsis or the antral contraction wave which begins in the stomach (Singh and Singh, 2010). As food enters the stomach an involuntarily contraction and relaxation of smooth muscle in the gastric wall is underpinned by an omnipresent electrical activity of the membrane potential, that passes through the GI musculature in a coordinated fashion (Cheng et al., 2010). The slow waves from muscle moment within the stomach, serve to move the smooth muscle cells membrane potential from a state of low probability, allowing sufficient Ca^{2+} influx leading to smooth muscle contraction (Sanders, 2008). This causes a ‘slow wave’ to occur at the fundus and advances towards the pylorus in regular-peristaltic contractions which start shallow at the beginning and gradually strengthens as it progresses towards the pylorus sphincter (Liu et al., 2021). When the sphincter is closed the food is pushed back into the body of the stomach allowing mixing process to begin. This will continue until the chyme is emptied from the stomach, known as gastric emptying. During a fed state 2-3 antral contraction waves happen approximately every minute (Bornhorst and Singh, 2012).

Understanding the mechanisms that govern nutrient release from food within the stomach may predict the nutrient bioavailability (Somaratne et al., 2020). How solid

foods are broken down and digested within the stomach remains a fundamental research area, as uncertainties surrounding the specific mechanisms within the stomach, such as gastric juice diffusion, nutrient release and particle size reduction process (Drechsler and Ferrua, 2016), might all be contributing factors affecting GER pre-post exercise.

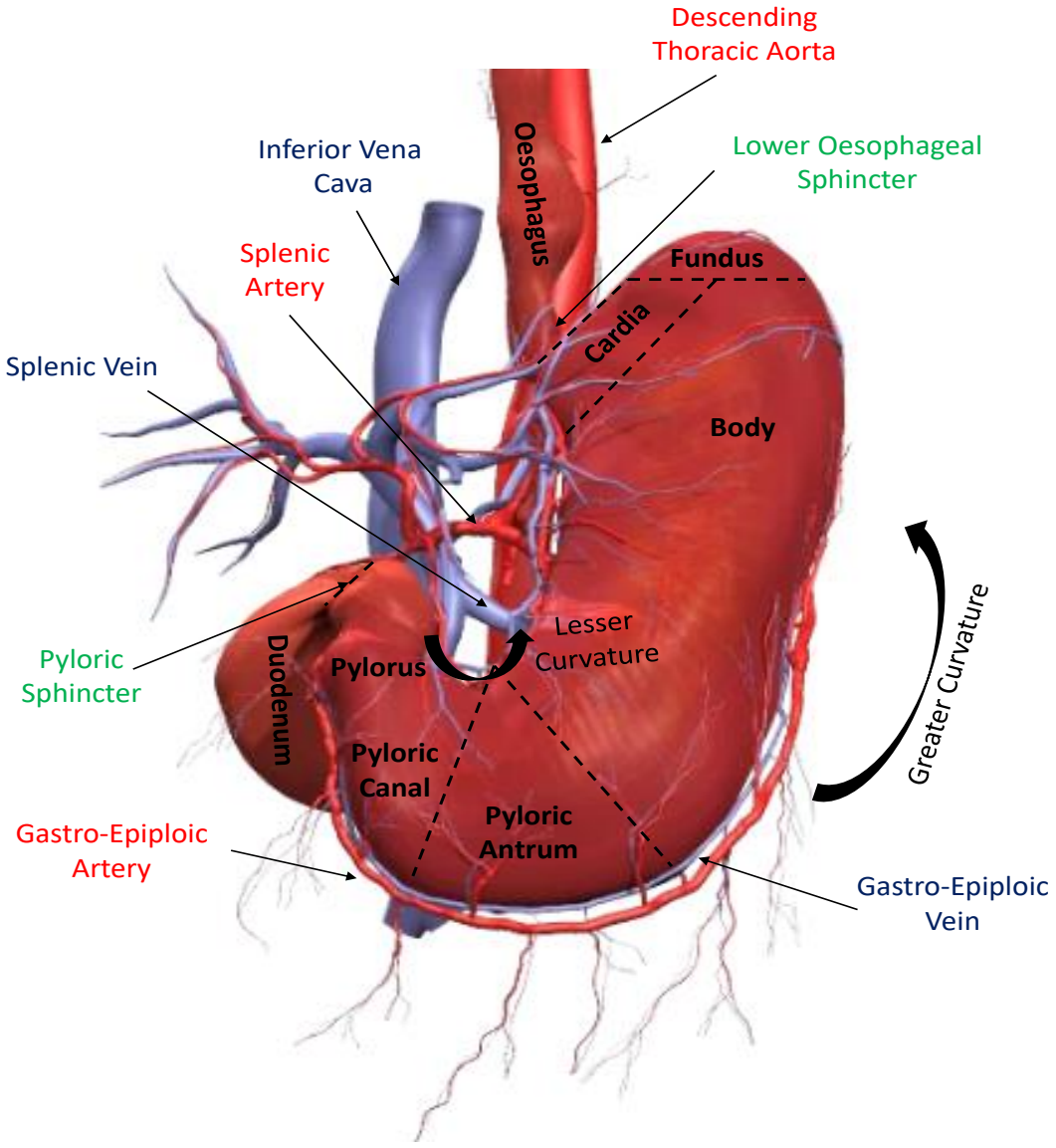

Figure 1: The structural arrangement, and anatomy of the human stomach. Adapted from, Somaratne et al., 2020. Image from, anatomy.tv/ powered by primal pictures. Colours on the diagram represent; Blue; vein, Red; artery, Green; sphincter, and Black anatomy structure.

2.2.4. Small Intestine

The small intestine is on average 4-6 m in length and begins distal to the pylorus it is comprised of three sections duodenum, jejunum, and ileum. Chyme travels through the small intestine at 5-20 mm/s using weak peristaltic contractions (Cheng et al., 2010). This results in a transit time of between 3-5 h for nutrients to be absorbed from the chyme before entering the large intestine. The entry point of the common bile duct and pancreatic duct fuse before entering the duodenum with the sphincter of oddi controlling the flow of bile and pancreatic juice to the small intestine (Knight et al., 2019). Chyme that enters the duodenum is extremely acidic ~2 pH (Dockray, 1999) and this sudden increase in acidity stimulates the release of hormones from the enteroendocrine cells of the duodenum to secrete; secretin and cholecystokinin (CCK) (Fried et al., 1991). Secretin stimulates the liver to release bile, and the pancreas to release pancreatic juice rich in bicarbonate ions, giving it a strong alkaline content (~8 pH) neutralising the acidic conditions (Knight et al., 2019). This occurs for only a short time, as the bicarbonate neutralises the acidic conditions resulting in a negative feedback loop where the effect of secretin inhibits its own secretion (Van de Graaff, 1986). Whereas CCK is released simultaneously to increase the delivery of pancreatic enzymes (trypsin, lipase and amylase) which need a balanced pH to function. CCK also has a number of important functions such as preventing the duodenum from overfilling, by inhibiting gastric emptying (Konturek et al., 1990), stimulating the gallbladder and the sphincter of oddi to release bile, and therefore indirectly increase pancreatic juices (Hansen et al., 2020), and finally activate brunner's glands to continue to secrete a watery fluid containing mucus and bicarbonate ions (Sedano et al., 2015).

However, nutrient absorption occurs mainly within the jejunum and ileum, where the villi are longer and found in greater density (Knight et al., 2019). The mucosa of the small intestine secretes a number of enzymes that are relevant for specific digestion processes such as sucrase, maltase and lactase which split carbohydrates. Peptidases for protein digestion and lipases for breaking fats. These enzymes are formed within the mucosal cells and the majority of the digestive process occurs on the surfaces or the inside of these cell within the digestive system (Van de Graaff, 1986).

2.2.5. Large Intestine

The large intestine is approximately 1.5m long and comprises of the caecum, colon, rectum, anal canal, and the anus. It is connected to the small intestine by the ileocaecal valve. The large intestine structure is much wider than the small intestine and the walls of the mucosa are completely empty of villi but contain goblet cells in abundance (Nigam, 2019). The function of the large intestine is to absorb water from the remaining indigestible chyme (Goulet et al., 2009) and to allow fermentation via gut bacteria, that metabolise polysaccharides into short chain fatty acids and absorb vitamin K and biotin (Yang and Yu, 2018). This is followed by excretion, the large intestine is primarily concerned with desiccation and compaction of waste, with storage in the sigmoid colon and rectum prior to elimination from the GI tract by defecation.

2.3. Absorption of Major Nutrients

Absorption involves transferring digested nutrients, water, and electrolytes from the lumen of the small intestine through the epithelial cells of the villi by active transport and passive diffusion which then enter the capillary network (Goodman, 2010). Understanding how each macronutrient is digested, and how absorption is achieved, is a fundamental characteristic of this thesis as food/meals provided are mixed macronutrient semi-solid meals.

2.3.1. Carbohydrate

Carbohydrates can be ingested in several structures, monosaccharides also called simple sugars (glucose and fructose) which do not have to be broken down any further, disaccharides which are built from multiple monosaccharides (lactose and sucrose) and complex carbohydrates polysaccharides (Starch cellulose) from plants and glycogen from animal cells (Goodman, 2010). Initial digestion of polysaccharides begins within the mouth, using salivary amylase which can digest up to 30-40% of complex carbohydrate before it reaches the small intestine (Goodman, 2010), where pancreatic juice continues to break down complex carbohydrates (starch) using pancreatic amylase which hydrolyses polysaccharides to form disaccharides (maltose) (Southgate, 1995), before absorption of any carbohydrate from polysaccharides, additional enzymatic digestion is required, using a disaccharidases found within the

brush borders of intestinal epithelial cells. Amylopectin and amylose found within potatoes, rice, and bread uses β-glucoamylase and isomaltase to form the monosaccharide (glucose). Sucrose found in table sugar and commercialised desserts use sucrase to form glucose and fructose and lactose found within milk and milk products use lactase to form glucose and galactose (Southgate, 1995). Disaccharidases are highly prevalent within the jejunum to maximise the uptake of the monosaccharides through glucose transporters, contributing to membrane digestion and absorption across epithelial cells (Semenza et al., 1984)

Glucose (and galactose) are transported across the luminal membrane by the Na^+ -coupled secondary active transport symporter (two molecules moving into the cell in the same direction), known as Na^+ -glucose transporter 1 (SGLT1) which allow glucose (and galactose) in conjunction with sodium ions to move into the cytoplasm of the cell (Jeukendrup, 2017b). SGLTs do not directly utilise ATP to transport glucose (and galactose) but rely on an electrochemical gradient generated by Na/K^+ ATP-ase sodium potassium pump located at the basolateral membrane as a source of chemical potential (Ferraris and Diamond, 1997). It is important to add, fructose does not use SGLT1 to enter enterocytes cell, but instead a glucose transporter (GLUTs) specifically GLUT5 via facilitated diffusion (Ferraris and Diamond, 1997). All three monosaccharides (glucose, galactose, and fructose) use GLUT2 which is sodium independent and allows the monosaccharides to leave the intestinal cell on the basolateral side of the cell and enter the systemic circulation. The capacity of GLUT2 to transport monosaccharides across a concentration gradient is believed to be very large (Kellett, 2001, Kellett et al., 2008) as well as the ability of GLUT5 to transport fructose (Kishi et al., 1999), although the ability of SGLT1 to transport glucose (and galactose) into the cell might be a rate limiting step in the absorption of glucose (and galactose) (Jeukendrup and McLaughlin, 2011).

There is little evidence for other carbohydrate transporters to SGLT1 and GLUT5 at the luminal membrane and GLUT2 at the basolateral membrane (Jeukendrup, 2017b). After the monosaccharides are in circulation, other important GLUT's help cells within the body utilis glucose; GLUT1, blood-brain barrier; GLUT2, expressed in beta cells of the pancreas, liver and kidneys; GLUT3, neurons and within the brain cells; GLUT4, is an insulin responsive glucose transporter, that is

found in the heart, skeletal muscle, adipose tissue and liver. It is found deep in the cytoplasm of cells and translocates to the plasma membrane when insulin is located (Navale and Paranjape, 2016).

2.3.2. Protein

Protein is consumed in a number of different ways from animal sources (meat, poultry, fish, eggs, and dairy products) and plant sources (nuts, seeds, legumes, beans, and some cereal/grain) within the diet (Górska-Warsewicz et al., 2018, Laskowski et al., 2018). Each protein consists of a number of amino acids connected by peptide bonds which are hydrolyzed by proteases and peptidases to form tripeptides, dipeptides and single amino acids in the lumen of the GI tract (Wu, 2016).

A variety of proteolytic enzymes are necessary to break down dietary protein into small peptides as certain enzymes break down specific peptide bonds (Goodman, 2010) before getting absorbed into enterocytes. Protein enzymes can be subdivided into endopeptidases (attack certain bonds making large polypeptides) or exopeptidases (cleave off one amino acid from the carboxy or amino terminus) (Heda et al., 2021). Pepsinogen is secreted by the gastric mucosa, also known as a zymogen, as gastric acid alters the conformation so it can become active pepsin (Gritti et al., 2000). Pepsin begins the breakdown of proteins within the stomach before the chyme enters the small intestine, where pancreatic protease enzymes are secreted by the pancreas as further zymogens (Goodman, 2010). Trypsinogen is cleaved to form trypsin by enteropeptidase, an enzyme produced by cells of the duodenum, resulting in the subsequent activation of chymotrypsonogen, proelastase, and procarboxypeptidases by trypsin which catalyses the cleavage of the pancreatic zymogens to form chymotrypsin, elastase, carboxypeptidases and aminopeptidases (Thrower et al., 2006). Active protein enzymes cleave polypeptides into oligopeptides. Any free amino acids begin to be absorbed by specific transport proteins, which facilitate the uptake of amino acids across the intestinal cell via a Na^+ dependent symporter (Bröer, 2008). The oligopeptides are attacked by exopeptidases carboxypeptidases and aminopeptidases which are located on the brush border membrane which form more amino acids and di- and tripeptides which are also absorbed into the enterocytes through the di/tripeptide transporter (PepT1) (Buyse et al., 2001). The H^+ coupled symporter uses the electrochemical gradient from the lumen to the cytoplasm and the

H^+ gradient is maintained by the Na^+/H^+ exchange in the brush border membranes and helped by the removal of Na^+ from the cell from the basolateral membranes Na^+ , K^+ -ATPases pump (Mackenzie et al., 1996), once within the cell the di- and tripeptides are hydrolysed using intracellular peptidases to form free amino acids (Goodman, 2010). These amino acids leave the cell from the basolateral membrane via a Na^+ independent facilitated diffusion transporter and enter the blood.

2.3.3. Fat

The major sources of fat in the UK diet are oils, meat, eggs, fish and dairy products (Gurr et al., 1989). These fats consist of dietary lipids, triacylglycerols, phospholipids, glycolipids and cholesterol and the source of these fatty acids can be subdivided into saturated fatty acid ~51%, monounsaturated 35% and polyunsaturated 14 % of the total fat consumed within the diet (Gurr, 1988).

Digestion of lipids starts within the mouth with lingual lipase and continues in the stomach with gastric lipase, see Table 2 (Hunt et al., 2015). The presence of fat in the duodenum signals the release of CCK and stimulates the gallbladder to contract, releasing bile (Fried et al., 1991), and with the help of secretin, the injection of pancreatic enzymes containing fat digesters, lipases, esterases and procolipase (Ahlman and Nilsson, 2001). As the lumen of the small intestine contains a large volume of water, these partly digested fats which are hydrophobic, move together forming a fat globules that is then further emulsified by bile salts forming emulsion droplets (Sarker et al., 2016). These emulsion droplets then allow procolipase activated by trypsin forming colipase to bind with the dietary fat droplets, supporting the enzyme action of lipase to hydrolyse triglycerides at position 1 and 3 of the glycerol moiety, producing two free fatty acids and one, 2-monoglyceride resulting in the lipolysis proceeds from the outside in further breakdown phospholipids and cholesterol with the help from esterases (Goodman, 2010). During the hydrolysis, emulsion oil-water droplets dissociate into multilamellar liquid crystals, using bile salts once more to form mixed micelles containing; cholesterol, 2-monoacylglycerols, lysophosphoipids, free fatty acids and bile slats (Goodman, 2010). The micelles then move down the small intestine towards the jejunum, where the mixed micelles reach the lipid bilayer of the enterocytes, resulting in the low-pH acidic surface of the brush-border membranes created by the Na^+/ H^+ pump exchanges, enabling lipids and free

fatty acids to diffuse across the lipid bilayer, leaving the bile salts in the lumen which are later absorbed in the terminal ileum. Some long chain fatty acids which cannot pass through the water layer beneath the brush-border membranes are further broken down by bacteria within the colon (large intestine) (Jeppesen and Mortensen, 1998). Some small fatty acids with chain lengths shorter than twelve carbon atoms (glycerol) are absorbed directly into the portal blood, before being metabolised by the liver, not contribute to adipose tissue stores (Gurr et al., 1989).

The separated lipids that transferred across the apical membrane (free fatty acids, monoglycerides and cholesterol) move freely into the cytoplasm before being transported by fatty acid binding proteins (FABPs) to the smooth endoplasmic reticulum (sER) to be reconstituted into triglycerides, by combining free fatty acids and monoacylglycerides using several enzymes (Goodman, 2010). These reformed triglycerides move to the rough endoplasmic reticulum (rER) where microsomal triglyceride transport protein (MTP) catalyses triglyceride, cholesterol, phospholipids and a protein called apolipoprotein B48 (ApoB48) to form a prechylomicron (Mansbach and Gorelick, 2007). This prechylomicron fat partially accepts the rCR simultaneously as the sER releases apoprotein AI (apoAI) combined and completed within the golgi apparatus forming a chylomicron (Siddiqi et al., 2006).

These chylomicrons are secreted into lacteal lymphatic vessels (lacteal lymph) and pass via the thoracic duct to the jugular vein adjacent to the subclavian vein (Randolph and Miller, 2014). The role of the chylomicron is to transport lipids between tissues via the blood with the apolipoprotein stabilising the lipid particle and also providing the tissues with a means to recognising the lipoprotein (Goldstein and Brown, 1977). Large amounts of the chylomicron triacylglycerols content are hydrolysed by lipoprotein lipases in the capillaries of the muscles, adipose tissues and heart cells although some incomplete particles with less triacylglycerol and more cholesterol are taken to the liver to form bile and new membrane structures (Gurr et al., 1989).

2.4. Gastric Emptying

Gastric emptying is a major research area for understanding the process and kinetics of food digestion and absorption (Liu et al., 2021). Gastric emptying is the

process by which the stomach empties its contents into the duodenum, where macronutrients are absorbed through the gut wall, and delivered around the body for metabolic processes (Bornhorst and Paul Singh, 2014). The process of gastric emptying is split into four phases (tonic contractions, peristaltic contractions, retropulsion, and emptying) (Bellmann et al., 2016). As food enters the stomach, tonic contractions move the food towards the distal stomach area, by contracting the proximal stomach. This is then followed by peristaltic movement of the stomach walls, which forces the food towards the pylorus, mixing and grinding the food into chyme; meanwhile the pylorus sphincter contracts and closes, resulting in the arrival of the peristaltic wave forcing the chyme back into the body of the stomach, this action is known as retropulsion which occurs multiple times in order for food to be emulsified with enzymes and gastric juices (Liu et al., 2021). The pylorus partly opens allowing liquid and small particles to empty, while large undigested particles (>2mm) remain in the stomach for further mixing known as a lag phase (Collins et al., 1991). Therefore, gastric emptying is an extremely significant step in the overall production of cellular energy. The process of emptying of the stomach into the small intestine is affected by a multitude of complex factors, with the underlying mechanisms still not completely clear. Even so, it is thought that in most conditions an internal feed-back mechanism is responsible (Delzenne et al., 2010), some details of which is described below.

2.4.1. Factors that Affect Gastric Emptying

Liquid foods empty faster in comparison to solid foods, this is because when liquid food is ingested it is quickly distributed throughout the stomach, allowing motor activity of the proximal stomach to force liquid food towards the pyloric sphincter, increasing pressure in the stomach and emptying rate (Goyal et al., 2019). The emptying of noncaloric liquid, such a water and dilute electrolyte solutions, begins immediately in proportion to the volume of liquid in the stomach being expelled in a single exponential manner, while nutrient rich solutions show a linear emptying curve (Collins et al., 1991).

The osmolality has been suggested to influence GER, mainly for solutions with no nutrient content (Hunt and Pathak, 1960), although this is not the case for fluids which contain energy (Rehrer et al., 1993). Using glucose polymers instead of glucose

monomer is a method of changing the osmolality of a solution without effecting the carbohydrate content, however little to no difference in GER has been found regardless of the difference in osmolality (Brouns et al., 1995). Vist and Maughan (1995), support osmolality influencing GER, but the carbohydrate content appears to have a greater influence as the increased osmolality slowed gastric emptying at higher carbohydrate solution concentrations. This is thought to be in response to the osmolality of contents in the upper small intestine also strongly influencing the rate of emptying (Hunt, 1960).

Solid food empties more slowly, as large numbers of indigestible particles sit within the proximal stomach waiting for tonic contractions to mix and separate particles, known as the inter-digestive phase, which results in a slower emptying time, as these larger particles are unable to leave the stomach. High-caloric liquid solutions empty faster than solid food, but slower than low-calorie liquids. Hence, Maurer (2012), suggests there is an interaction between nutrients and small intestinal mucosal receptors, by controlling the amount of chyme which enters the duodenum. Kong and Singh (2008), stated GER ranges between 10-40 mL/min after a meal and subsequently is reduced to 2-4 mL/min as a result in pressure difference between the stomach and the duodenum. Rehrer et al (1989), revealed that gastric emptying falls exponentially with time, and during any time period a constant percentage of the consumed material that is within the stomach would have been emptied.

Energy density has a vital role in the regulation of gastric emptying as Goyal et al, (2019) found that 50% of water emptied from the stomach within 10 min, whereas ~ 40% of a high-calorie liquid remained within the stomach after 2 h of ingestion. Unsurprisingly, gastric emptying is therefore affected by the energy density of the ingested solution and/or the osmolality and energy content of the ingested solution or food which independently affects the rate of gastric emptying and consequently substrate delivery to the small intestines (Noakes et al., 1991). This evidence continues to support the findings of Costill and Saltin (1974), as approximately 65% of a water solution, 50% of a 7% carbohydrate solution and 25% of a 15% carbohydrate solution emptied during each successive 10min period, supporting the fact energy density of the ingested solution plays a major role in how fast the stomach empties. The greater the energy content of a solution (Maughan and

Leiper, 1996, Costill and Saltin, 1974) and/or meal (Hunt and Stubbs, 1975) the slower emptying rate.

Increasing the intragastric volume within a test meal accelerates GER for both liquid and solids meals (Leiper, 2015, Moore et al., 1984, Hunt and Stubbs, 1975, Costill and Saltin, 1974). The steady rate of energy delivery has been associated with a greater meal volume (Hunt et al., 1985). Nevertheless, more recent investigations found that increasing the volume of the meal within the stomach causes gastric emptying to become faster, although energy density delays the emptying rate (Camps et al., 2016, Kwiatek et al., 2009). The rate of gastric emptying increases in proportion to the volume ingested, with the maximal rate of emptying at a volume of 600ml showing a delivery rate up to 40 $ml \cdot min^{-1}$ could be achieved when very large volumes of fluid were ingested. However, this change has also been shown within solid meals regardless of energy content, as the meal volume increases so does the rate of gastric emptying (Moore et al., 1984). Therefore, the volume of the food consumed, and energy density remain strong predictors of GER when consuming food before, during or after exercise.

Macronutrient (carbohydrate, fat, and protein) content of a meal or solution can effect GER, as this usually results in a difference in energy content or density (Hunt and Stubbs, 1975). Hunt (1980), suggests regardless of energy content; fat, carbohydrate, and protein slows down gastric emptying to the same degree when each meal is calorie matched. Increasing the energy content of the ingested meal will increase the osmolality. This osmolality increase results in a slower gastric emptying rate (Vist and Maughan, 1995). Among macronutrients, protein and fatty dense foods are slower at emptying compared to high carbohydrate meal (Tougas et al., 2000, Cecil et al., 1999). The presence of different macronutrients stimulates the secretion of different peptides from the cells within the gut and these will be discussed in more detail within (section 2.5 Gastrointestinal Hormones).

Blood glucose concentrations have been observed to influence gastric emptying (Chang et al., 2010), and this has been shown in healthy and diabetic subjects (Rayner et al., 2001). Subjects that are within a hyperglycaemic state (~15 mmol/L) had a delayed gastric emptying response to solid and liquid substitute meal (Fraser et al., 1990). Schvarcz et al (1997), found even when blood glucose was at a lower blood

glucose range 8 mmol/L, gastric emptying is still slower when compared to baseline glucose values of ~4 mmol/L. Nevertheless, gastric emptying has been shown to be accelerated in periods of hypoglycaemia, this is thought to be a response to increased delivery of nutrients to the small ingestion for absorption (Russo et al., 2005). Interestedly, the gastrointestinal system responds to an increased delivery of macronutrients over a period of time, suggesting a further increase in supply of nutrients to the blood. For example, several different studies have demonstrated carbohydrate absorption can be accelerated by increasing the intake of carbohydrate (glucose) within the diet for more than three days (Cunningham et al., 1991b, Horowitz et al., 1996). Importantly, Yau et al (2014) revealed that ingesting fructose, another monosaccharide for 3 days accelerated GER, for fructose but not glucose. Furthermore, Cunningham et al (1991a) and Castiglione et al (2002), exposed that increasing fats, a different macronutrient, for 14 days within the diet also accelerated gastric emptying of a test meal high in fats although carbohydrate meals were emptied at the same rate before and after a high-fat diet. This strengthens the understanding that the GI system is highly adaptable, as dietary changes can affect how a macronutrient empties from the stomach, showing adaptations in GER within three days of changing a dietary routine, when carbohydrates are increased.

GER has been shown to be affected by the time of day, as diurnal variation has been observed (Kentish et al., 2013). Goo et al (1987) found that healthy males had a delayed gastric emptying half-time after an evening meal (20:00) compared to an meal consumed in the morning (08:00) only within solid food not liquid. More recently, McIver et al (2019), found a semi-solid meal emptied slower in the evening in an fasted state compared to a morning fasted trial. It is thought that differences in the method used when measuring time of day changes to GER have made it difficult to compare between studies, as the choice of meal composition, positioning of the participant, and how often the measurements are taken could have influenced the result (Grammaticos et al., 2015, Szarka and Camilleri, 2009b, Szarka and Camilleri, 2009a). Therefore, within the current thesis GER was measured at a similar time period between 10.30-14.30 in order to prevent time of day difference between emptying rate with a consistent semi solid meal provided.

Other factors that have been shown to influence GER, but are less conclusive, are body posture and temperature of consumed food. Several studies have shown that the temperature of liquid consumed has a slight effect on GER but this only last for ~10 min post ingestion (Sun et al., 1988, Costill and Saltin, 1974). The meal consumed appears to rapidly return to normal core levels, this may be why GER is unaffected (Sun et al., 1988). Others have demonstrated GER was delayed only when a warm liquid meal was consumed (Troncon and Iazigi, 1988). Although, Mishima et al (2009), reported a hot meal accelerated GER, suggesting there is inconsistency within the literature focusing on the effect of meal temperature on GER. This is also the same for postural positioning when measuring GER, as lying in a supine position has been reported to slow emptying compared with sitting or standing (Moore et al., 1988). This was thought to be in releasing to gravity as the intragastric distribution was affected. Whilst other investigations observed no change in GER when postural position was changed (Steingoetter et al., 2006, Doran et al., 1998). Once again, showing inconsistencies may be down to research methods between meal size, volume, or sample size.

2.4.2. How Exercise Affects Gastric Emptying

Gastric emptying could influence exercise-induced changes in appetite and energy intake. This may also contribute to changes in gastric symptoms and the availability of nutrients during exercise, and therefore performance and recovery (Horner et al., 2015). The majority of evidence suggests GER is delayed during strenuous exercise (Leiper et al., 2005, Leiper et al., 2001a, Neufer et al., 1989, Fordtran and Saltin, 1967) and shown to increase emptying rate (Neufer et al., 1986, Cammack et al., 1982), unchanged during moderate exercise (Feldman and Nixon, 1982), and in an early investigation, moderate exercise accelerated GER (Hellenbrandt and Tepper, 1934). The dynamic between exercise and GER is yet to be fully defined as the majority of studies have focused on improving performance within an athletic population and the implications of exercise-induced alterations in GER for weight management strategy is under examined (Horner et al., 2015).

Therefore, it is well documented that GER during exercise is effected by exercise intensity > 70% $\dot{V}O_{2Max}$ when consuming an carbohydrate solution (Costill and Saltin, 1974). This result was further supported by Leiper et al (2001a), as a

carbohydrate-electrolyte drink delayed GER supplementary during intermittent exercise to a greater extent than continuous exercise (66% $\dot{V}O_{2Max}$) or rest, showing an effect in mode of exercise. Leiper et al (2001b), additionally discovered a carbohydrate solution emptied from the stomach faster during walking than during a five-a-side football (USA: soccer) match, once again showing that the intermittent nature of exercise manipulates GER. One explanation is that walking is inherently lower intensity and the delay in GER that has been observed during exercise exceeding 70% $\dot{V}O_{2Max}$ has been attributed to a reduction in splanchnic blood flow, where walking may have less sympathetic drive (Evans et al., 2016, Brouns and Beckers, 1993). Support for this has been shown by a change in celiac artery blood flow (CABF) which supplies the stomach, pancreas, spleen, and liver with blood. Kashima et al (2017), revealed CABF was profoundly lower post-exercise, leading to a slower GER 5-min after exercise compared to 30-min, during which CABF had returned to baseline values.

Investigations examining how nutrients affect GER within the post-exercise period such as Clayton et al (2014), revealed a hypertonic 10% glucose-electrolyte drink emptied from the stomach at a slower rate compared with a hypotonic 2% glucose-electrolyte drink, when a volume of 150% of body mass lost (BML) was ingested ~30 after intermittent exercise. Evans et al (2018a), demonstrated that whey protein empties from the stomach at a slower rate than maltodextrin when consumed post-exercise. Although, in an additional investigation Evans et al (2016), provided participants with a 5% glucose solution 30-min after low-intensity and high-intensity intermittent exercise or rest found no differences in GER characteristics; where Kashima (2018), discovered timing of post-resistance exercise nutrient ingestion of a carbohydrate-protein solution delayed GER when consumed 5-min after exercise, but was unaffected after 30-min. Subsequently, the majority of literature to date which has measured the effects of GER post-exercise have predominantly used solutions which contain carbohydrate, protein or mixed carbohydrate-protein supplements. It could be considered that slower GER is detected when the meal, solution or liquid ingested provides a sufficient challenge to the gut (Chey et al., 2001), strongly suggesting that volume (meal, solution or liquid) is a major indicator of GER. As yet, minimal investigations have attempted to enhance how using a mixed macronutrient meal that

provides participants with a standardised energy content and volume might fluctuate GER after exercise, affecting nutrient delivery for recovery.

2.4.3. Measurement of Gastric Emptying

There have been a number of different methods used to assess gastric emptying, some of these methods are preferred within research or clinical settings, each method has advantages and disadvantages. Regardless of technique, standard terms and values are used to describe gastric emptying. Total emptying time, $T_{1/2}$. This is the time taken for half of the meal volume to empty (Hunt and Spurrell, 1951). T_{lag} is the time at which the lag phase ends, and the emptying begins (Liu et al., 2021)

Scintigraphy was the first method to be used to measure gastric emptying and was thought to be the gold standard in the 1960s (Griffith et al., 1966). This method involves ingestion of a meal labelled with a radioisotope (sodium chromate (200 µC in 5ml saline solution)) before imaging the abdominal gastric area with an external gamma camera (Olausson et al., 2013, Szarka and Camilleri, 2009b). This technique allows both liquid and solid gastric emptying to be assessed, although its application was restricted due to its interference with patient care and radiation exposure (Nguyen et al., 2013). This began the pioneering work to find a more appropriate method for assessing gastric emptying such as gastric aspiration, magnetic resonance imaging (MRI), ultrasound, stable isotope breath test, and recently developed wireless sports capsule technology (WMC)

A well-used method is the double sampling gastric aspiration technique. This method requires inserting/ swallowing of a tube into the stomach, after which a non-absorbable phenol red dye is consumed with the test meal to be analysed. This was first measured by Geroge (1968), and then modified and improved by Beckers et al, (1988). This method determines gastric secretion rate and volume, which then can be calculated to suggest total gastric content. Unlike the scintigraphy technique this method does not need to have a skilled operator using the gamma camera. However, one of the disadvantages is that limited participants have the ability to swallow the tube, which in turn reduces the study numbers and also limits test meals to liquids, but can be used to measure gastric emptying during exercise (Jeukendrup and Moseley, 2010, van Nieuwenhoven et al., 1999).

As methods within imaging technology have improved over recent years, other methods such as MRI and ultrasound have been used to assess gastric emptying. Although MRI is non-invasive, it does not allow accurate total gastric emptying to be measured but does allow all types of food and liquid to be ingested. Compared with other techniques MRI provides a large view of the stomach and digestive area enabling the structure of food to be viewed (Carbone et al., 2010) and validated against scintigraphy in healthy subjects, with the use of solid and liquid emptying rates showing strong correlation (Kunz et al., 1999). It is also possible to observe the natural digestion process, showing the food remaining in the digestive tract after absorption (Volkov et al., 2018). Despite being, regarded as the gold standard for determining gastric emptying processes it is expensive to buy and has a high cost to run. A further drawback is that patients are placed in a right sided semi-supine position as seated MRI are still uncommon (de Zwart and de Roos, 2010). The measurement of gastric emptying using real-time ultrasound and ultrasonography have been used by building up cross-sectional images of the stomach to produce a three-dimensional representation (Bateman and Whittingham, 1982). This technique is inexpensive, and the equipment is widely available (Szarka and Camilleri, 2009a, Szarka and Camilleri, 2009b). Nevertheless, to rebuild good quality images a skilled well trained operator is needed, and using ultrasound is time consuming (Darwiche et al., 1999).

New advances in technology have led to a nondigestible capsule (SmartPill) which records luminal pH, temperature, and pressure during GI transit providing a measure of gastric emptying time (Kuo et al., 2008). The SmartPill corporation uses a wireless transmitter capsule and a receiver which the subject wears, before being transferred to a computer for data analysis. The SmartPill technology receives information from the stomach, indicating changes in acid gastric pH to an alkaline duodenal pH associated with the capsule leaving the antrum through the pylorus into the duodenum, given an indication of the time taken for the capsule to leave the stomach not directly measuring gastric emptying (Kuo et al., 2004). This technique is relatively new, and whether it correlates with other methods requires further work, nevertheless is does show strong representation of normal and delayed gastric emptying (Maqbool et al., 2009).

Breath testing is another method for gastric emptying assessment which is performed by adding a substance with a labelled carbon atom (Carbon-13 [13C]). The ^{13}C substrate is added to meals where it is absorbed within the small intestine and metabolised in the liver before being exhaled as $^{13}CO_2$ in the breath (Waseem et al., 2009). The stable isotope breath test method is becoming an increasingly popular indirect method for measuring GER with both liquid and semi-solid meals (McIver et al., 2020, McIver et al., 2019, Evans et al., 2018a, McIver et al., 2018, Yau et al., 2017b, Yau et al., 2014). However, one limitation is that it cannot be directly comparable to the $T_{1/2}$ and T_{lag} from the phenol red method. Nevertheless, the breath test method is valid, non-invasive, safe, and reliable for a clinical and research setting (Braden, 2009, Ghoos et al., 1993). Please see section 3.8 General Methods for more information on how the stable isotope breath test method was used during this thesis to measure GER.

2.5. Gastrointestinal Hormones

The discovery of leptin in 1994 started the molecular era for obesity research (Zhang et al., 1994). Since this pioneering work, a number of other molecular hormones that regulate appetite, EI, and GER have been discovered (Murphy and Bloom, 2006). This section will focus on the 5 hormones measured within this thesis, with an extensive list of ~18 peptide hormones documented within Table 3, these hormones have important sensing and signalling roles derived from the gastrointestinal tract to support nutrient availability (Woodward et al., 2021, Murphy and Bloom, 2006). These peptide hormones which are secreted from enteroendocrine cells of the stomach and small intestine interact through the vagus nerve via the gut-brain axis stimulating the brainstem and the hypothalamus (Cork, 2018). In addition, other orangs such as the pancreas, liver, and adipose tissue also secrete hormones that send information from the peripheries via the central nervous system (CNS) in response to nutrient and EI (Suzuki et al., 2010), which directly communicate with the brain altering homeostatic and hedonic circuits (Woodward et al., 2021). GI peptide hormones can be divided into two categories, anorectic (appetite suppressing) and orexigenic (appetite stimulating) (Meneguetti et al., 2019). Since biomarkers may be neural or hormonal, it is important to consider the gastrointestinal tract as it is the body's largest endocrine organ (Karra and Batterham, 2010) and a large number of

these hormones have not yet been characterised or defined as anorectic or orexigenic (Ahlman and Nilsson, 2001). The primary gut hormones involved in regulating appetite, food intake and GER are discussed below.

2.5.1. Ghrelin

Ghrelin is a 28 amino acid peptide that is released from X/A-like cells of the fundus within the stomach, with much smaller amounts being synthesised in the intestine and the pancreas (King et al., 2013b). This peptide stimulates orexigenic neuropeptide Y (NPY) and agouti-related protein (AgRP) expressing neurons in the central nervous system, which co-express the ghrelin receptor, growth hormone secretagogue receptor (GHS-R) (Cowley et al., 2003). Ghrelin was identified as an endogenous ligand which stimulated growth hormone (GH) by binding to GHS-R via a novel independent pathway which had not previously been found before 1999, and was unknown to be important to metabolism (Kojima et al., 1999). This peptide can be found in two molecular forms; acylated and des-acylated ghrelin (Kojima and Kangawa, 2005). The acylated form of ghrelin is considered as the biologically active peptide responsible for its orexigenic and GH releasing action (Kojima et al., 1999). Acylated ghrelin is also considered as the most stable form when researching appetite and food intake, stimulation of ghrelin is usually in response to satiety and remains unique as the only known circulating peptide that stimulates appetite and food consumption before declining immediately after ingestion of food (King et al., 2013a, Broom et al., 2007, King et al., 2010b, King et al., 2010a). Recent research conducted using human participants has revealed a positive relationship between increased acylated ghrelin after moderate periods of food restriction. With ghrelin once more increasing within 1-h postprandially (McIver et al., 2018, Clayton et al., 2016). This increase in ghrelin in the hours after food is thought to be related to glucose homeostasis, as GHS-R are found to be expressed in pancreatic β-cells stopping insulin release via Ca^{2+} -mediated pathways (Alamri et al., 2016, Tong et al., 2010).

Moreover, ghrelin may also be linked to food behaviour such as taste sensation and reward (Overduin et al., 2012, Skibicka et al., 2012b, Skibicka et al., 2012a). Simon et al, (2017) used MRI to reveal that, when participants were in a satiated state, ghrelin concentrations were elevated at the same time as increased neural processing, potentially indicating ghrelin's impact on hedonic food intake. The secretion of ghrelin

by the stomach is dependent on meal volume, density, and calorie amount (Callahan et al., 2004), with meals high in carbohydrate decreasing ghrelin more than meals high in protein and fat (Erdmann et al., 2004, Kojima and Kangawa, 2005). Apart from ghrelin's role in short term energy balance, it is also relevant to long term energy stores, as evidence has emerged suggesting that ghrelin levels correlate inversely with adiposity and are affected by changes in body weight (Cummings, 2006).

In addition, several studies have been conducted using rodents. These have looked at pathways which engage in reward such as the mesolimbic, dopaminergic pathway which when ghrelin is administered to the ventral tegmental area (VTA) of the midbrain, ghrelin increases food intake, by boosting the willingness to work for food and arousing rodents in the anticipation of food intake and potential energy increase (Jerlhag et al., 2007, Naleid et al., 2005). Ghrelin's ability to increase food intake has been further shown when rodents were loaded with a high baseline intake of high-fat chow, before administration of an acute intracerebroventricular (ICV) ghrelin injection which caused increases in standard chow, regardless of the fact these rodents where already heavily fed (Bake et al., 2019, Le May et al., 2019). A similar response has been shown when rats' endogenous levels of ghrelin were naturally elevated during an overnight fast (Alvarez-Crespo et al., 2012). Increased perception of hunger and *ad libitum* EI has also been demonstrated in humans who were administered with an intravenous ghrelin infusion (Wren et al., 2001a).

In summary, the collective study within rodents and humans has shown ghrelin's role in hunger and meal initiation, with links to the reward and behaviour processes of food consumption. Ghrelin is now well recognised as the 'hunger hormone' although future research must take a holistic approach, as hormone regulation has the potential to override homeostatic regulations in the context of food intake.

2.5.2. Glucagon-Like Peptide-1 (GLP-1)

GLP-1 is a 30 amino acid peptide, derived from synthesizing a large precursor protein known as pre-proglucagon, with further processing a number of biologically active peptides are formed with the most common circulating form of glucagon-like peptide-$1^{7\text{-}36\text{amide}}$ (GLP-1) (Murphy and Bloom, 2006). This peptide is produced by

intestinal L cells located in the distal jejunum and ileum in response to food ingestion, with a key role in glucose homeostasis (Eissele et al., 1992). GLP-1 induces glucose-dependent insulin release making it an incretin hormone (Woodward et al., 2021). It stimulates insulin secretion by the β cells and reduces glucagon secretion by α cells in response to a meal, which causes a decrease in hepatic glucose production (Zouhal et al., 2019).

It has been shown that GLP-1 is attenuated in the postprandial period within obese individuals delaying satiety, leading to increased EI (Carroll et al., 2007, Adam and Westerterp-Plantenga, 2005, Verdich et al., 2001). GLP-1 exerts its stimuli via GLP-1 receptors (GLP-1R), these are expressed throughout the CNS and on peripheral tissues (Bullock et al., 1996). This has led to the development of novel development of GLP-1R agonists which inhibit food intake and stimulate satiety (Vilsbøll et al., 2012). Over the last decade, GLP-1-based therapies have been developed to treat diabetes using a pharmaceutical drug 'Liraglutide' (glucagon-like peptide-1 receptor agonist) (Ten Kulve et al., 2016). Results from studies in rodents have suggested that GLP-1R knockdown in the vagal afferents, increased meal size and duration (Krieger et al., 2016, Labouesse et al., 2012, Rüttimann et al., 2009), further supporting GLP-1 release is in part responsible for the feeling of satiety.

Within humans, higher fasting plasma GLP-1 concentrations are associated with lower carbohydrate intake (Basolo et al., 2019). Circulating levels of GLP-1 increasing 10-20 min after eating within healthy individuals and peak approximately 30 min following meal ingestion (Huda et al., 2006), although food composition can affect how fast circulating GLP-1 is present within the blood. Although, ingested carbohydrates produce a greater and more rapid secretion than lipids or proteins (Herrmann et al., 1995). It was subsequently that shown increasing dietary added sugar intake correlates with increased dorsal striatum reactivity to food cues (Bello et al., 2002, Colantuoni et al., 2002, Hajnal and Norgren, 2002). The dorsal striatum is related to motivation to engage in rewarding behaviour, suggesting the brain responds to the consumption of sugar like a drug (Volkow et al., 2002). Dorton et al, (2017) revealed following glucose consumption, individuals who consume greater habitual dietary added sugar have greater striatal responses to food cues and reduced GLP-1

response in the postprandial period. Elevated GLP-1 either prolongs the interval between meals or reduces the subsequent meal size (Feinle et al., 2002).

Additionally, changes in GLP-1 has also been shown to decrease nutrient absorption by slowing down GER (Deane et al., 2010). Hence, physiological action of endogenous GLP-1 is glucose dependent, and release is proportional to EI and might influence the reward system via changes in the mesolimbic system.

2.5.3. Peptide Tyrosin Tyrosin (PYY)

PYY is a 36 amino acid peptide that is a member of the PP fold peptide family (Tatemoto, 1982). PYY can freely cross the blood brain barrier (BBB), unlike its structural similarity peptides neuropeptide Y (NPY) and pancreatic polypeptide (PP) (Berglund et al., 2003). PYY is co-secreted with GLP-1 from L-cell of the intestinal mucosa (Lundberg et al., 1982), with increased expression of PYY along the intestine reaching its highest levels in the rectum (Adrian et al., 1985). Two main forms of PYY have been described, PYY $^{1-36}$ (the full-length peptide) and PYY $^{3-36}$, this form is generated through enzymatic cleavage by dipeptidyl peptidase IV (DPP-IV) of the PYY $^{1-36}$ at tyrosine and proline N-terminal (Meneguetti et al., 2019). The change in PYY structure occurs, with a modification in the Y receptor groups with PYY $^{1-36}$ having high binding affinity to Y1R, Y2R and Y5R (Cox, 2007). Thus, the biological activity of PYY^{1-36} is not abolished but co-activates Y2R and Y5R receptors to form PYY^{3-36} (Cox, 2007, Medeiros and Turner, 1994). Grandt et al, (1994) revealed fasted levels of PYY^{3-36} were lower than PYY^{1-36}, in contrast these values were inverted within the postprandial period. PYY^{3-36} has been recognised as the predominate circulating form in relation to appetite suppressant activity (Batterham et al., 2006, Chelikani et al., 2004).

Circulating concentrations of PYY^{3-36} are suppressed in the fasted state and increase within 30 min after consuming energy dense nutrient in humans (Martins et al., 2007) and rodents (Anini et al., 1999). The highest postprandial concentration of PYY^{3-36} is usually around 2-h after meal ingestion (le Roux et al., 2006). This increase can de dependent on the macronutrients within the meal, as PYY^{3-36} release is stimulated by nutrient intake in proportion to energy content (De Silva and Bloom, 2012, Huda et al., 2006). Meals high in fat have been found to stimulate PYY^{3-36} to a

greater extent than meals of similar energy content with carbohydrate and protein with human participants (Lomenick et al., 2009, Pironi et al., 1993, Adrian et al., 1985). Nevertheless, PYY^{3-36} release can occur through several other mechanism, such as the secretion of CCK, gastric acid, bile acids and fatty acids irrelevant to meal composition (McGowan and Bloom, 2004).

Investigations using obese individuals found PYY^{3-36} to be reduced in the postprandial period, which could lead to increased food intake and therefore a positive energy balance (Brownley et al., 2010, Zwirska-Korczala et al., 2007). Therefore, several investigations have used peripheral administration of PYY^{3-36} to reduces food intake and body weight in experimental animal and human trials. The anorectic effect of PYY^{3-36} has been thought to be mediated by Y2R, because they are attenuated by Y2R antagonists (Abbott et al., 2005). Batterham et al, (2002) discovered PYY^{3-36} infusion in Y2r-null mice did not cause an increase in food intake. Furthermore, in both lean and obese humans, intravenous infusion of PYY^{3-36} reduces food intake (Batterham et al., 2003a, Batterham et al., 2002), showing postprandial concentrations of PYY^{3-36} inhibit food intake for up to 12-h. The gut-hypothalamic pathway is possibly regulated through a Y2R-dependent mechanism in the ARC, which inhibit NPY/AgRP neurons, resulting in activation of the anorectic proopiomelanocortin (POMC) neurons, when considering postprandial PYY^{3-36} changes regarding feeding or infusion (Batterham et al., 2006). More recently, PYY^{3-36} levels did not correlated with striatal food-cue reactivity (Dorton et al., 2017). Suggesting, PYY^{3-36} is unlikely to be influenced by hedonic food intake, and therefore its treatment for the aetiology of obesity is uncertain (Murphy and Bloom, 2006).

2.5.4. Pancreatic Polypeptide (PP)

PP is a 36 amino acid peptide that belongs to the PP-fold peptide family (Berglund et al., 2003). PP is produced and secreted by F-cells predominantly found in the pancreatic islets of Langerhans and released into circulation after ingestion of food (Asakawa et al., 2003). PP is also secreted in distal areas of the GI tract, although awareness of PP activity within the colon regarding appetite and EI is still insufficient (Kim et al., 2014). Blood levels peak around 15-min after consuming food and remain elevated for 90-min (Kojima et al., 2007), as with GLP-1, circulating levels of PP increase postprandially in response to nutrient load and energy content, and may

remain elevated for up to 6-h (Track et al., 1980). The increase in PP secretion has further been established after consuming a breakfast meal, with substantial increases after exercise (brisk-walk) in fed and fasted conditions (McIver et al., 2018).

The food consumption and digestion activity of PP are mediated by Y4-R and Y5-R signalling through the parasympathetic vagus nerve (Field et al., 2010), despite Michel et al, (1998) suggesting PP has the ability to bind to all Y receptors (Y1-R to Y6R), although this theory is controversial. PP might inhibit intestinal peristalsis as it appears to constitute the part of feedback loop that controls gut motility and secretion related to the 'ileal brake effect' (Fujimiya and Inui, 2000). Within investigations involving human individuals, PP infusion has been found to inhibit gastric emptying when administrated peripherally (Field et al., 2010, Schmidt et al., 2005, Batterham et al., 2003b).

The major sites of action responsible for PP are believed to be, the brainstem (area postrema) and hypothalamus (paraventricular nucleus & ventromedial hypothalamic nucleus) (Asakawa et al., 2003, Katsuura et al., 2002). Studies in rodents and humans have arrived at different conclusions on the anorexic effect of PP. The administration route seems to perform a factor, with evidence suggesting different receptors are engaged (Huda et al., 2006). Therefore, administrating PP centrally increases food intake and gastric emptying within rodents (Asakawa et al., 2003, Kanatani et al., 2000) and peripheral administration has the opposite effect, reducing food intake and gastric emptying within rodents (Asakawa et al., 2003, Ueno et al., 1999, Whitcomb et al., 1997, Malaisse-Lagae et al., 1977) and in humans (Sam et al., 2015, Batterham et al., 2003b). The data presented in this section has shown the potential of PP in weight management and the regulatory processes of gut mobility.

2.5.5. Insulin

Insulin is a peptide hormone that is produced and secreted from ß-cells of the pancreatic islets of Langerhans (Fu et al., 2013). Circulating concentrations of insulin increase rapidly after a meal (Alsalim and Ahrén, 2019), potentially regulating appetite as it is key in the metabolism of carbohydrate, fat and protein making it an anabolic hormone (Dimitriadis et al., 2011). Although, most importantly insulin is well known to regulate blood glucose concentrations (Richter and Hargreaves, 2013).

The tissues most immediately related to plasma glucose changers are muscles, as insulin increasing glucose uptake and decrease gluconeogenesis within the liver and lipolysis inside adipose tissue (Pliquett et al., 2006).

Insulin is thought to play a role in regulating body weight through a direct central effect (Saad et al., 2002). An early animal model suggested insulin inhibits appetite at the CNS level as a ICV infusion of insulin reduces food intake and body weight of baboons (Woods et al., 1979). Insulin has also been suggested to promote satiety within human populations (Verdich et al., 2001, Holt et al., 1996), and furthermore regulates plasma ghrelin and modulates adipocyte leptin production (Saad et al., 2002, Saad et al., 1998). This is important as ghrelin stimulates appetite and promotes weight gain (Wren et al., 2001a, Wren et al., 2001b), whereas leptin inhibits food intake and supports weight loss (Friedman and Halaas, 1998). Insulin also circulates at levels proportional to body fat content (Schwartz et al., 2000) and insulin receptors are expressed within brain regions (hypothalamus, hippocampus and cortex) that are directly involved in EI (Hopkins and Williams, 1997).

Insulin is possibly more important for the regulation of energy balance, than appetite or GER (Berthoud et al., 2017). Therefore, changes in blood glucose concentrations remain the key mediator in a potential mechanism for insulin function in appetite control.

2.5.6. Other Hormones that Influence GER and Metabolic Pathways

CCK and Leptin that are documented within this section have not been measured within this thesis, although are important for metabolic pathways relevant to EI, weight management and regulating GER. Likewise, the role of physical activity in the management of obesity, might be regulated by changes in the concentrations of these hormones after acute exercise.

2.5.7. Cholecystokinin (CCK)

Multiple molecular forms of CCK can be found within the human body which range from 8-58 amino acids (Ritter et al., 1999). CCK is released postprandially in response to ingestion fat and protein (Lieverse et al., 1994), with carbohydrates only providing a weak stimulus (Liddle et al., 1985). Furthermore, highly acidic food enters the small intestine can also cause an increase of CCK in all macronutrient form, this increase in acidity within the duodenum also cause the release of other GI hormones (Zouhal et al., 2019).

A function of intestinal CCK is to activate organs of the alimentary canal for the arrival of macronutrients for optimal digestion and absorption (Ritter et al., 1999). It also contributes to gastrointestinal motility, exocrine pancreatic enzyme secretion, and contraction of the gallbladder (Zwirska-Korczala et al., 2007). Fasting results in a reduction in plasma CCK, whilst peripheral administration before the onset of a meal reduces meal size within humans (Stacher et al., 1982, Kissileff et al., 1981) and rodents (Crawley, 1985, Crawley and Kiss, 1985, Antin et al., 1975).

CCK is synthesised and released mainly from I-cells of the small intestine (Field et al., 2010), and the most widely investigated form of CCK in relation to appetite and food intake is CCK-8 (Dockray, 2009). CCK-8 is also synthesized and released as a neurotransmitter within the CNS binding to both CCK-1 receptors (CCK-1R) and CCK-2R (Barden et al., 1981). CCK-8 released postprandially seems to reduce food intake through CCK1-R on the vagal nerve (Murphy and Bloom, 2006). A CCK1-R antagonists have been reported to increase food intake in rodents (Edwards et al., 1986) and humans (Beglinger et al., 2001, Matzinger et al., 1999). Therefore, the short-term benefit of CCK for the regulating of satiety is well recognised, although the benefit for weight loss maybe more controversial as rodents that were repeatedly infused with CCK-8, reduced their meal size, but consumed food more regularly (Simmons et al., 1999, Asin et al., 1992), and increased net weight gain (West et al., 1984).

It is important to add, acute exercise seems to increase CCK within normal weight individuals and remains elevated for up to 2-h after exercise (Ströhle et al., 2006, Bailey et al., 2001, Sliwowski et al., 2001). This significant increase in CCK is

also associated with suppressed feelings of hunger. Castillo et al, (2004) found when using a similar hormonal receptor pathway CCK-1R, a CCK agonist (GI181771X) delayed GER in healthy volunteers. This might be a potential reason why GER is inhibited immediately after exercise, with CCK, GLP-1, and PYY all inhibiting GER after exercise (Dockray, 2009, Hellström et al., 2006).

2.5.8. Leptin

Leptin is a peptide hormone produced and secreted from adipose cells (white adipose tissue) (Münzberg and Morrison, 2015). Leptin, a product of the *ob* gene has several actions although most importantly helps to regulate energy metabolism (Schwartz et al., 2000). Circulating leptin is transported across the BBB and inhibits hunger by interacting with the hypothalamus, central and peripheral administration has been shown to reduce food intake following fasting (Farooqi et al., 1999).

Leptin stimulates neurons within the arcuate nucleus, that express POMC releasing α-melanocyte-stimulating hormone, while simultaneously neurons that express NPY are inhibited. Axons from both neurons are received from the paraventricular nucleus and lateral hypothalamic area, leading to activation of the melanocortin receptor 4 (MC4R) (Field et al., 2010, Farooqi et al., 2003). This pathway results in the reduction of food intake and increase the activation of energy expenditure (Morris and Rui, 2009).

Concentrations of leptin are strongly correlated with body mass accumulation from adipose tissue (Münzberg and Morrison, 2015). The release of leptin by adipose tissue is affected by numerous factors such as gender, age, exercise, and glucose uptake. Nevertheless, decreased levels of leptin can increase feeding behaviour (Farooqi et al., 2003) and therefore loss in body weight is associated with a rapid fall in plasma leptin levels and an increase in hunger (Chan et al., 2003).

Leptin is considered to exert a long-term regulatory role in appetite and food intake (Schwartz et al., 2000). Individuals that are obese can develop resistance to leptin, which disrupts the regulatory satiety effect of leptin and leads to uncontrolled food intake (Zouhal et al., 2019). On the other hand, exercise has been shown to affect leptin levels, with research that has investigated leptin concentrations after low-moderate exercise training periods have found decreased levels within 1-2 weeks

(Ackel-D'Elia et al., 2014, Martins et al., 2013, Unal et al., 2005, Koutsari et al., 2003, Gomez-Merino et al., 2002, Reseland et al., 2001). This is not the case after acute exercise bouts, as generally circulating leptin levels are not affected (Kyriazis et al., 2007, Jürimäe et al., 2006, Zoladz et al., 2005, Ferguson et al., 2004, Weltman et al., 2000, Torjman et al., 1999, Racette et al., 1997). Although, if acute exercise bouts are performed for >2-h (running, swimming, cycling, or rowing) circulating leptin is found to be increased (Jürimäe et al., 2009, Karamouzis et al., 2002, Zaccaria et al., 2002, Leal-Cerro et al., 1998, Landt et al., 1997). This increase in leptin after ultra-endurance events is possible to support metabolic pathways when continually exercising for long periods in a reduced energy state.

Table 3: Summary of Peptide Hormones and Neurotransmitters which Regulate Appetite, EI, and GER. Classified According to their Predominant Synthesis Site regarding, Nutrient Ingestion and Digestion.

Hormone	Sites of Synthesis*	Primary Receptor*	Site of Action* (*Gut-Brain Axis*)	Function on Appetite	Function on GER	References
Stomach						
Ghrelin	Stomach X/a-like cells, Small Intestine	GHS-R	Vagus nerve Solitary Nucleus Hypothalamus	Inhibit Satiety ↑ EI	Promotes Emptying	Simon et al., 2017(1) Wren et al., 2001(1,2) Levin et al., 2006(3)
Gastrin	Stomach & Duodenum (G-cells)	GPCRs (CCK-2R)	Brainstem (area postrema), Parietal cells (HCI production)	No Known effect (May Promote Satiety Through Gastric Motility)	Inhibits (Increased Intragastric Volume)	Danzer et al., 2004 Goetze et al., 2009(3)
Intestines						
GLP-1	Intestinal L-cells	GPCRs (GLP-1R)	Vagus nerve Brainstem Hypothalamus	Promotes Satiety ↓ EI	Strongly Inhibits (Ileal brake) ↑ Gastric Distension	Vilsbøll et al., 2012(1,2) Feinle et al., 2002 (1,2) Deane et al., 2010(3)
GLP-2	Intestinal L-cells, Brain	GPCRs (GLP-2R)	Hypothalamus (POMC Neurons, Dorsomedial Nucleus)¥	No Known effect (Promotes Nutrient Absorption)	↔ Unclear (Stimulating GI Motility)	Drucker., 2005(1-3) Cazzo et al., 2016(2) Lovshin et al., 2004¥
PYY_{3-36}	Intestinal L-cells	Y2-R, NPY2-R (Brain- ARC)	Vagus nerve Brainstem Hypothalamus	Promotes Satiety ↓ EI	Strongly Inhibits (Ileal Brake)	Dorton et al., 2017(1-2) Chen et al., 1996(3)

CCK	Duodenum I-cells	GPCRs (CCK-1R CCK-2R)	Vagus nerve Brainstem Hypothalamus	Promotes Satiety ↓ Food Intake (Acutely)	Inhibits	Moran., 1982[2, 3] Stacher et al., 1982[1]
GIP	Intestinal K-cells (Duodenum)	GPCRs (GIP-R)	Hypothalamus (ARC &VMH) Adipose Tissue[¥]	Ineffectually ↑ Satiety (↑ Insulin & Lipid storage)	Unaffected	Zhang et al., 2021[2] Asmar et al., 2010[1, 3] Miyawaki et al., 2002[¥]
Motilin	Duodenum M-cells	GPCRs (MLNR)	Vagus nerve	Inhibit Satiety ↔ Unknown EI	Promotes Emptying	Schmid et al., 1991[3] Sanger et al., 2013[1, 3]
Neurotensin	Small Intestine (N-cell), Brain (Immunoreactive-cell)	GPCRs (NTS_1)	Hypothalamus Midbrain (VTA &SNc)	Weight Status, Food Intake (Lipid Metabolism)	Gut Motility (Intestinal Inflammatory Mechanism)	Fredrickson et al., 2014[2] Boules et al., 2013[3] Kalafatakis et al., 2011[1]
OXM	Intestinal L-cells, (PC1) Brain	GPCRs (GLP-1R)	Vagus nerve Hypothalamus Medulla oblongata	Promotes Satiety (Suppresses Ghrelin)[¥] ↓ Food Intake	Inhibits Limits Gastric Acid Secretion	Schjoldage et al., 1989[1, 3] Cohen et al., 2003[¥]
Secretin	Duodenum S-cells, Brain	GPCRs (SCTR)	Hypothalamus (ARC &VMH), Adipose Tissue (Gut-BAT-Brain Axis)	Inversely-Promotes Satiety(↑ BATokines Stimulating POMC Neurons)	↔Unclear (↓ HCI & ↑ PB) Promotes Emptying (Non-Human) [(¥)]	Afroze et al., 2013[3] Mynatt et al., 2018[1] Jin et al., 1994[(¥)]

Pancreas

PP	Pancreatic Islets of Langerhans (F-cells), Distal GI (Colon)	Y4-R (All Y-R)¥	Vagus nerve Brainstem Hypothalamus	Promotes Satiety ↔ EI	Inhibits	Field et al., 2010 [1,3] Michel et al., 1998¥
Insulin	Pancreatic islets of Langerhans (β-cells)	RTKs (IR)	Hypothalamus (ARC)	Promotes Satiety	↔ Unclear	Verdich et al., 2001[1, 3]
Amylin	Pancreatic islets of Langerhans (β-cells)	GPCRs (AMY_{1-3})	Hypothalamus Brainstem (Area-Postrema)	Promotes Satiety (↓ Meal Size)	Inhibits (Preventing Overrating)	Hay et al., 2015[1, 3] Hinshaw et al., 2016[2]
GHIH	Pancreatic islet, Stomach D-cells, Gut-brain-Neurons	GPCRs (SSTR1-5)	Hypothalamus Pancreas (↓ Insulin & Glucagon)	↓ Universal off switch (↓ Gastrin, Secretin, Motilin, CCK, VIP, and GIP)	Inhibits (Slowing Down Digestive Process)	Kumar et al., 2020[1-2] Kim et al., 2014[3]
Glucagon	Pancreatic islets of Langerhans (α-cells) (PC2)	GCGRs (GCGR-B)	Hypothalamus (ARC) Brainstem Liver	Promotes Satiety Increase Lipolysis, Gluconeogenesis and Glycogenolysis¥	Inhibits	Hope et al., 2018¥ Patel et al., 1979[3]

Adipose Tissue						
Leptin	Fat cells (White Adipose)	Cytokine-R (LEP-R)	Hypothalamus (ARC)	Promotes Satiety	↔ Unclear	Ruiter et al., 2010[(2)]
Neurons						
VIP	Neurons Throughout GI; Pancreas (D2 cells);Adrenal Medulla (G cells)	GPCRs ($VPAC_1$ & $VPAC_2$)	Vagus nerve Hypothalamus (ARC)	Ineffectually Promotes Satiety ↓ Food Intake	Inhibits-Gut Motility (By Increasing; GLP-1, PYY, Leptin and Insulin)	Dickson et al., 2009[(3)] Vu et al., 2015 [(1-3)]

Abbreviations and symbols: GHS-R, growth hormone secretagogue receptor; EI, energy intake; GHIH, growth hormone-inhibiting hormone (somatostatin); GPCRs, G-protein-coupled receptors; POMC, proopiomelanocortin; OXM, oxyntomodulin; BBB, blood brain barrier; GI, gastrointestinal; NPY2-R, neuropeptide Y2 receptor; ARC, arcuate nucleus; VMH, ventromedial; VIP, vasoactive intestinal peptide hypothalamus; VTA, ventral tegmental area; SNc, substantia nigra; $VPAC_{1, 2}$, vasoactive intestinal polypeptide receptor; $PC_{1, 2}$, prohormone convertases; RTKs, receptor tyrosine kinases; IR, insulin receptor; PBS, pancreatic bicarbonate secretion; $SSTR_{1-5}$, somatostatin receptors; MLNR, motilin receptor; BATokines, brown adipokines.↑, increased; ↓, decreased; ↔, unchanged;.

*Site of synthesis, primary receptor, and site of action are adapted from; Huda et al., 2006; Field et al., 2010 & Meneguetti et al., 2019.

[1]Appetite references.

[2]EI references.

[3]GER references.

[¥]Relevant to that point only.

2.6. The Effect of Acute Exercise on GI Hormones and GER

The human body is a complex system in which peptides are secreted from the GI tract with the greatest attention within the literature focusing on ghrelin, PYY, and GLP-1, in response to nutrient ingestion after low, moderate and high intensity exercise (Crabtree and Blannin, 2015, Wasse et al., 2013a, King et al., 2010a, Martins et al., 2007a). The suppression in circulating concentrations of acylated ghrelin after aerobic exercise at an intensity > 60% $\dot{V}O_{2Max}$ have been well documented (Broom et al., 2017, Holliday and Blannin, 2017b, Holliday and Blannin, 2017a, Alajmi et al., 2016, Deighton et al., 2013a, King et al., 2011b, Vatansever-Ozen et al., 2011, Broom et al., 2009, Broom et al., 2007). In contrast GLP-1 and PYY increase during and post aerobic exercise (Kawano et al., 2013, Ueda et al., 2013, Larson-Meyer et al., 2012, Ueda et al., 2009b, Ueda et al., 2009a, Martins et al., 2007a). Although, these hormonal fluctuations shown post-exercise during moderate/high intensity > 60% $\dot{V}O_{2Max}$, have not always been observed following low-intensity exercise < 55% $\dot{V}O_{2Max}$, (McIver et al., 2018, King et al., 2010b, Unick et al., 2010), and therefore even if circulating GI hormones are affected by exercise, they usually return to resting control levels within 60 min (King et al., 2013b).

Nevertheless, most studies tend to investigate the effect of exercise on one or two GI hormones when many have been shown to influence appetite and EI, although minimal information has been gathered in relation to GER after exercise. Gastric emptying is understood to be regulated by a complex interaction of neuronal and hormonal input through the CNS (Horner et al., 2015). The secretion of hormones which control GER can depend on the chemical composition of chyme being delivered from the stomach to the duodenum (Liu et al., 2021). Taking CCK as an example, chyme rich in fat or protein cause CCK secretion from the upper small intestine, which stimulates pancreatic enzyme secretion and the contraction of the gallbladder releasing bile salts and in turn inhibit gastric emptying (Dockray, 2009, Fried et al., 1991, Takahashi et al., 1991). Furthermore, this processes also triggers feedback from the duodenum to slow down the gastric emptying and establish enterogastric reflex (Konturek et al., 1990, Konturek and Johnson, 1971). Therefore, other important

hormones in the regulation of GER are ghrelin, which acts to accelerate GER (Janssen et al., 2011, Levin et al., 2006). PYY and GLP-1 inhibit GER (Hunt et al., 2015, Camilleri, 2009, Hellström et al., 2006, Little et al., 2006, Chen et al., 1996), where elevated PP have been shown to reduce gut motility (Batterham et al., 2003b). Insulin has not been directly found to effect GER but does increase energy expenditure and glucose uptake (Chang et al., 2010, Deane et al., 2010, Fraser et al., 1990).

As previously mentioned, the process of gastric emptying is stimulated and affected by humoral activity and gut hormones involved in the regulation of appetite, food intake and energy balance also affect the stomachs ability to process chyme (Hellström et al., 2006). The effect of these hormones and several others (see Table 3) are known to influence gastric emptying are also manipulated by exercise. Therefore, comprehending how exercise interferes with hormone regulation which in turn might result in differences in GER needed further investigation.

2.7. Regulation of Appetite and Energy Intake

Appetite can be split into several different subsections with particular attention on hunger, which is defined as a strong desire or the need to consume food (Smith and Ferguson, 2008). Whereas satiety is defined as feeling full or satisfied, with meal-to-meal behaviour controlled by hunger and satiety sensations (Rui, 2013). Apart from homeostatic signals, food intake is strongly influenced by memory, food reminders and societal factors which encourage consumption of familiar food even when homeostatic requirements have been sustained (Kenny, 2011). This drive to consume food beyond homeostatic need is coordinated by the striatum region of the brain (hedonic system) and these reward-related neurocircuitry processes are complex (Woodward et al., 2021). Nevertheless, regions of the brain (ventral pallidum, ventral striatum and bed nucleus of the stria terminalis) which motivate behaviour and reward receive projections from or overlap with the hindbrain and hypothalamus which are critical for food intake and metabolism and therefore appetite response (Hetherington and Ranson, 1983, la Fleur, 2006).

During the cephalic phase, appetite control is regulated by the anticipation of nutrient ingestion, once food is consumed and enters the digestive system, in particular the stomach and the small intestine, enteroendocrine cells secret hormones which act

as signals to influence hunger and satiety (Rui, 2013). These hormones (see section 2.5. Gut Hormones), interact with G-protein-coupled receptors (GPCRs), transporters and ion channels on the cell membrane surfaces (Gribble and Reimann, 2019). These hormones act locally to influence nutrient absorption and metabolism, but also act directly in the brain to alter feeding behaviour (Hussain and Bloom, 2013). Furthermore, the GI tract is densely innervated by vagal sensory nerves (Cork, 2018), which directly communicate nutritional information from the gut to brainstem (Berthoud et al., 2017). This theory has been strengthened in recent years as the disruption of vagal signalling leads to larger meal consuming and an impaired ability to modulate food intake (Schwartz, 2000). How nutritional status of individual cells is transmitted to other cells, tissues, organs and the brain requires more comprehensive focus (Berthoud et al., 2017).

2.7.1. How can Exercise Change Appetite

Oven recent years, literature has been conducted on acute exercise to determine the effect this has on subjective feeling of appetite and subsequent food intake. The interest in appetite responses to exercise stems from the acknowledgement that physical activity might enhance energy expenditure (Donnelly et al., 2009) and any increase in appetite after exercise is likely to reverse the negative energy balance achieved by exercise. Therefore, initial investigations focused on optimising exercise protocols to control weight management, implied appetite and EI remained largely unchanged in the hours after exercise, documented within these reviews (Blundell and King, 1999, Blundell et al., 2003).

Alternatively, food restriction was found to provoke a rapid compensatory increase in appetite and food intake, which were not mimicked after exercise at equivalent energy deficit (Hubert et al., 1998, King et al., 2011a). Importantly, in both these investigations exercise intensity was performed at ~70% $\dot{V}O_{2Max}$ and high-intensity aerobic exercise has been consistently observed within the literature to suppress appetite (Broom et al., 2007, King et al., 2010a, Wasse et al., 2013b, King et al., 2013a, King et al., 2011a). Although, when exercise is performed at a much lower intensity < 55 $\dot{V}O_{2Max}$ suppression in appetite is not perceived (Unick et al., 2010, King et al., 2010b, Imbeault et al., 1997, Pomerleau et al., 2004).

This exercise-induced anorexia found after exercising > 60% $\dot{V}O_{2Max}$, is also consistent when different modes/types of exercise are used including running (Broom et al., 2007, Burns et al., 2007, King et al., 2010a), cycling (Becker et al., 2012, Ueda et al., 2009a, Martins et al., 2007a), swimming (King et al., 2011b), and resistance exercise (Laan et al., 2010, Broom et al., 2009). Suggesting, exercise intensity might perform a more critical stimulus in the metabolic regulation after exercise than mode/type of exercise which is supported by Wasse et al, (2013b) who did not find a difference in appetite between cycle and running performed at the same intensity.

In contrast, the exercise-induced suppression of appetite might be different between athletic and non-athletic populations as Holliday & Blannin, (2017c) observed no significant decrease in subjective feeling of appetite when endurance-trained males ($\dot{V}O_{2max}$ = 61.6 ± 6.0 mL/kg/min) completed a high-intensity aerobic exercise bout ~76% $\dot{V}O_{2max}$. This is consistent with other studies, which have conducted extended exercise bout using trained individuals (Deighton et al., 2013b, King et al., 2010b). Emerging research has suggested that habitual physical activity might be a fundamental indicator of how the human body responds in the hours after exercise regarding appetite and EI responses (Blundell et al., 2020, Paravidino et al., 2020, Beaulieu et al., 2016). Profoundly, any significant appetite variations discovered after most exercise conditions and environments usually returns to resting control values within 30-60 min (Deighton and Stensel, 2014).

Isolated exercise sessions elicit acute, brief cardiovascular, and metabolic responses such as blood lipids and glucose homeostasis changes (Devlin and Horton, 1985) Frequent repetition of these isolated sessions produces more permanent adaptions, known as the exercise training response (Thompson et al., 2001). These improvements have been found to target cardiorespiratory fitness, blood pressure, and body composition, which are all negatively correlated with the risk of chronic disease (Schubert et al., 2013). Although, this relationship between physical activity (PA) and total energy expenditure (TEE), in recent years has been challenged by the constrained energy hypothesis by Herman Pontzer (Pontzer, 2015). Energy expenditure does not increase with PA in a linear does response relationship among traditional Hadza hunter-gatherers living in northern Tanzania. Therefore, fat-free mass was the single strongest predictor of TEE among Hadza adults (Pontzer et al., 2015). Hadza adults

have a similar TEE compared with developed, industrial populations regardless to the fact they have greater daily walking distances and therefore increased PA levels. So, adults with high levels of habitual PA may adapt by reducing energy allocation to other metabolic pathways and systems within the human body, in order to maintain energy expenditure within a narrow range. Therefore, the role PA plays in the regulation of body weight through its effect on appetite, energy expenditure and energy intake may be more complicated than just disrupting the energy balance equation (Martins et al., 2008).

2.7.2. What Happens in The Postprandial Period?

We know several different events can affect appetite, but how the human body responds in the hours after food ingestion might be an interesting factor in key metabolic processes. Nevertheless, investigations that have monitored appetite in the immediate post-exercise period (0-120 min) have found minimal differences (Burns et al., 2007, Martins et al., 2007a, Becker et al., 2012, Gonzalez et al., 2013). The appetite suppression observed after exercise bouts conducted >70% $\dot{V}O_{2Max}$, does not seem to provoke changes in macronutrients or EI irrespective of the increased metabolic demands associated with high-intensity exercise compared to low-intensity exercise, with the majority of weight management research focusing on post exercise EI have found no significant modifications in the hours and days after exercise (2-24 hours) (McIver et al., 2020, McIver et al., 2019, King et al., 2013a, Hanlon et al., 2012, King et al., 2010a, Pomerleau et al., 2004, King et al., 1997).

It has been popular to provide an *ad libitum* meal 30-60 min after exercise conducted in a fasted state to assess EI. This method has been used after versus forms of exercise including running (Shorten et al., 2009, Kelly et al., 2012, Vatansever-Ozen et al., 2011, Balaguera-Cortes et al., 2011), walking (Tsofliou et al., 2003, Unick et al., 2010, King et al., 2010b), and cycling (Melby et al., 2002, Jokisch et al., 2012, Laan et al., 2010, Erdmann et al., 2007, King et al., 1994). However, investigations that have used *ad libitum* meal period can achieve energy replacement at ~1300 KCal, which could be seen as an unrealistic meal replacement and not an indication of compensatory effects post-exercise (King et al., 2013a).

2.7.3. Social Economy Influences on Energy Intake

Below are some quotes from the media related to post-exercise nutrition recommendations for the average user. Firstly, a quote from medical news today (Barhum, 2021).

"*Physical activity uses a lot of energy. It is difficult for the body to recover if energy levels are not replenished within 15-30 minutes after finishing a workout. Eating even a snack shortly after exercise can help to restore energy levels.*"

Secondly, a quote from Healthline (Semeco, 2016). These quotes are both very generalised, with no consideration of the adaptive response to exercise at different duration, intensity, or type.

"Eating the right nutrients soon after you exercise can help your body rebuild its glycogen stores and repair and regrow those muscle proteins faster. It is particularly important to eat carbs and protein after your workout"

It is fundamental to consider, in situations where exercise volume and duration are minimal, nutritional intervention other than the traditional main three meals breakfast, lunch, and dinner (Gonzalez, 2014) is not required except the replacement of minor fluid loss during exercise. Interestingly, more than 60 years ago, Mayer et al (1956) exposed the amount of food eaten was associated with the sedentary or active nature of the work being completed. Within jute mill workers high energy expenditure (EE) was closely associated with high levels of food intake. Suggesting the drive to eat reflects a need generated by metabolic EE. There is much more to be understood about the interaction between physical activity energy expenditure and the behaviour of consumers to select and eat food (Blundell et al., 2020). The biological drive to eat is closely balanced with cultural and socio-economical changes, resulting in an increase in the frequency of eating occurrences (Cutler et al., 2003), or the demand for continuous economic growth driving purchasing and consumption (Blundell, 2018). Such as the use and promotion of post-exercise recovery snakes which could affect EI in the hours after exercise. The large proportion of media hyped recovery aids are saturated with carbohydrate, protein and high in energy (KCal) such as:

- Sci-Mx protein flapjack chocolate and hazelnut (276 KCal)

- Grenade Carb Killa white chocolate Peanut Bar (242 KCal)
- Weetabix on the go chocolate drink (207 KCal)
- Lucozade original (186 KCal)

This list is not exhaustive. However, gives an example of commonly used 'between exercise period snacks' on most retail shelves. Unsurprisingly, together with a consumer driven environment and a nutritionally hyped market it is understandable how the accumulation of adipose tissue can spiral out of control. This might propose the use of whole mixed macronutrient meals in the period after exercise, advocating scheduled meal periods to avoid snacking might be an important consideration when designing exercise sessions for health promotion purposes. There are limitations in the literature related to measuring post-exercise EI as research has suggested that compensatory increase may expand further than the post 24-h after exercise as Rocha et al, (2013) has reported increases in food intake 48-h post-exercise. It therefore remains important to understand how exercise and physical activity status influences resting appetite and EI responses in the hours and days after exercise.

2.8. Fasting Before Exercise

Fasting is defined as restraining from consuming food/or energy dense material over a period of time (Longo and Panda, 2016). Fasting prior to exercise has gained substantial attention over recent years and has become increasingly popular when investigating breakfast consumption versus breakfast skippers in relation to overall appetite regulation and weight management (Betts et al., 2016). There are several fasting approaches used, although intermittent fasting is one of the most popular, also categorised as, "time-restricted feeding and alternate-day fasting" (Patterson et al., 2015). When commencing in any strategy related to fasting the main aim is to reduce calorie intake (Freedman et al., 2014), usually achieved by reducing the total number of hours available for eating, thereby controlling EI (Patterson et al., 2015). The majority of individuals accomplish an overnight fast regularly when sleeping for 8-10 h (Maughan et al., 2010) and if this overnight fast is extended and exercise is conducted, it is believed fat metabolism is increased resulting in increased weight loss. This question has raised significant attention as to why fasted exercise might therefore

be an effective weight loss strategy to improve health related metabolic and appetite markers.

During starvation, ketone bodies, NEFA, and glucose from liver glycogen and gluconeogenesis are the main energy sources (Cahill, 2006). It was originally thought that the glucose-FA cycle could explain why fat metabolism increase in fasted states (Randle et al., 1963). Fat metabolism is amplified when the availability of free fatty acids (FFA) are increased within circulation (Costill et al., 1977). These FFA's undergo β-oxidation in the mitochondria where they are converted to acetyl-CoA. An increase in acetyl-CoA can inhibit pyruvate dehydrogenase, which is responsible for the breakdown of pyruvate to acetyl-CoA from glycolysis (Jeukendrup, 2002). Additionally, increased formation of acetyl-CoA from FFA's causes a surge in citrate levels and inhibits phosphofructokinase, a rate limiting enzyme in glycolysis (Garland and Randle, 1964). This theory is one example, if an overnight fast is extended and exercise is conducted may further promote fat metabolism and oxidation (see section 2.9. Substrate Utilisation).

Additional research is required to appreciate whether regulating meal patterns in coordination with exercise will improve metabolic and appetite regulation in the postprandial period after exercising-fasted (La Bounty et al., 2011). Research has been conducted which demonstrated that breakfast consumption prior to exercise can drastically alter metabolic responses (Gonzalez, 2014, Gonzalez et al., 2013). Although, previous studies have found it may be more pertinent to omit breakfast if a negative fat balance is desired, with EI from breakfast and energy expenditure from exercise are not compensated for at a further meal period (lunch) (Gonzalez et al., 2013). Furthermore, Edinburgh et al, (2019) expanded on this research and reported no compensatory effects post-exercise for energy expenditure following fasted exercise, suggesting breakfast omission prior to exercise can produce a greater negative energy balance. This has also been reported by Bachman et al (2016), observed when healthy men perform 60 min of running after breakfast, their evening and 24-h EI are higher than if they exercise before breakfast. Further suggesting and supporting the theory endogenous carbohydrate stores (liver and muscle glycogen) may contribute to energy balance regulation after exercise (Flatt, 2001).

In addition, it is important to consider hormonal control when exercising, as GLP-1 concentrations increased after acute exercise in a fed state (Martins et al., 2007a), whether this increase was in relation to the food that was consumed or the exercise is an important consideration. For example GLP-1 increases when nutrients interact with intestinal L-cells which potentiates insulin secretion and a decreased food intake, and therefore perception of hunger (Suzuki et al., 2010). Performing endurance exercise in a fasted state increase the appearance of endogenous glucose and therefore will increase muscle glucose uptake after exercise (Goodyear et al., 1990). This might demonstrate that, when nutrients are ingested immediately post- exercise, the effect on acute post-prandial glucose may be predetermined on the nutritional status (fasted or fed) prior to exercise (Gonzalez et al., 2013).

Based on the current literature, there seem to be different benefits to conducting fasted and fed exercise although the mechanisms are still unclear. Nevertheless, the majority of research that focuses on gut hormone regulation after exercise has predominantly been conducted in a fasted state (Farah and Gill, 2013) and understanding diet/exercise strategies to control body fat/mass by exercising in a fasted state is becoming increasingly popular (Morton et al., 2010). Further studies are warranted to develop the research area of 'fasted exercise', with no investigation focusing on GER.

2.9. Substrate Utilisation

During aerobic exercise, carbohydrates and lipids are the primary substrates oxidised to facilitate oxidative processes and provide energy (Astorino and Schubert, 2018). Substrate utilisation can be affected by different exercise; duration, intensity, type (mode), and nutritional status (Jeukendrup and Wallis, 2005). At lower exercise intensities (~40% $\dot{V}O_{2max}$) fat oxidation provides energy to perform external work with the remaining energy demand being supplied by carbohydrate oxidation (Thompson et al., 1998, Romijn et al., 1993, Jones et al., 1980). As exercise intensity increases from low (25% $\dot{V}O_{2max}$) to moderate (60% $\dot{V}O_{2max}$) fat oxidation increases, whereas at high intensity (80% $\dot{V}O_{2max}$) fat oxidation declines and muscle/liver glycogen becomes the main fuel source (van Loon et al., 2001). During high intensity exercise (>70% $\dot{V}O_{2max}$) fat oxidation decreases, and carbohydrate oxidation increases in a

linear manner to allow for a change in energy demands during the exercise bout (Melanson et al., 2007, Kang et al., 2006). However, the way in which fat oxidation interacts to an increase in exercise intensity is not linear, in fact the amount of energy that is provided to the working muscle by fat peaks at ~ 65% $\dot{V}O_{2max}$ (Kang et al., 2006).

Several investigations have tried to explain the mechanisms how fuel selection within the human skeletal muscle is regulated although, it is well-established that plasma FFA concentrations do not change or decrease when the exercise intensity increases from moderate to high intensity (van Loon et al., 2001, Romijn et al., 1993), and robust evidence suggests plasma FFA availability within the blood is not the answer to why fat oxidation is limited during exercise above 65% $\dot{V}O_{2max}$ (Randle et al., 1963). Unsurprisingly, it is thought that there is a molecular change at the cellular level which causes a decrease in fat oxidation rates at high exercise intensities, such as a reduction in blood supply to adipose tissue which prevents the delivery of FFA to metabolic pathways (Hodgetts et al., 1991). A further theory suggests the transport of FFA into the mitochondria from long-chain fatty acids oxidation requires the carnitine palmitoyl transferase complex to be used as an energy source and enzymes in this process are down regulated as the buffering capability of the muscle is reduced (van Hall, 2015), whereas some medium-chain fatty acids can freely diffuse into the mitochondria unlike long-chain fatty acids (Achten and Jeukendrup, 2004, Romijn et al., 1993). This reduction in FFA therefore limit the capacity to produce ATP from oxidation of plasma FFA (Fielding et al., 2018). Nevertheless, we know fat oxidation has individual variability, and therefore within different populations a change in exercise intensity around the fat max zone may result in energy being unutilised in different amounts.

The nutritional state of an individual is an important determinant of substrate utilisation as fasting prior to exercise (> 6 h) heightens fat oxidation, whereas ingesting carbohydrate in the hours before an exercise bout does not (Coyle, 1995). This is primarily thought to be in relation to exogenous supply of glucose to body cells which decreases plasma fatty acid mobilisation. Furthermore, ingesting carbohydrate also causes an increase in plasma insulin concentration which inhibits the activation of adipose tissue triglyceride lipase, this in turn prevents intramuscular triacylglycerol

breakdown and reduces the availability of NEFA for oxidation (Spriet, 2014, Achten and Jeukendrup, 2004, Coyle and Coggan, 1984). Fasting prior to exercise stimulates hepatic glycogen breakdown to provide substrate for energy production (Maughan et al., 2010), although as glycogen availability diminishes causing a decrease in blood insulin concentrations this promotes once again energy to be predominantly provided by oxidation of plasma fatty acids (Aird et al., 2018).

Limited investigations have measured lipid oxidation rates in prolonged periods post exercise or after consuming food (Mulla et al., 2000). Therefore, the energy demands in the recovery period must be considered (Melanson et al., 2002), as any increase in fat oxidation during exercise may be mitigated by consuming food (carbohydrate) post-exercise. Overfeeding with carbohydrate has been shown to increase carbohydrate oxidation and energy expenditure 24 h post-trial, whereas overfeeding with fat did not lead to any further changes (Horton et al., 1995). Performing exercise at an intensity that increases fat oxidation 55-65% $\dot{V}O_{2max}$ (Achten et al., 2002) has been shown to improve insulin sensitivity (Venables et al., 2005). Therefore, research focusing on understanding the mechanisms behind how fat oxidation is adapted when exercise intensity is increased from low- high in the general population is required to clarify the acute and chronic adaptations to energy demands in the recovery period after exercise. How individuals metabolise substrate post-exercise may have critical long-term consequences for the way in which the human body stores and utilises body fat.

2.10. Postprandial Feeding

Circulating glucose concentrations represent the enhanced ability of the human body to switch between endogenous glucose appearance (from hepatic glycogenolysis and gluconeogenesis) and exogenous glucose appearance (via intestinal digestion), combined with glucose absorption into cells (Gonzalez, 2014). This carefully regulated homeostatic function enables energy to be provided, so the human body can function appropriately ensuring normal body function (Woodward et al., 2021). Therefore, the human body is dependent on the tight control of its blood glucose levels. This is accomplished by a network of hormones and neuropeptides released by organs (pancreas, liver, intestines, adipose, and muscle tissue) and interpreted and

disseminated by the brain to react and respond when necessary (Röder et al., 2016b). Fundamentally, this network is controlled and regulated by the pancreas, which secretes the blood sugar-lowering hormone insulin and its antagonist glucagon (Röder et al., 2016a). Insulin suppresses hepatic glucose output and stimulates muscle glucose uptake (Craig et al., 1961), by induing GLUT4 to translocate to the cell membrane surface increasing the absorption of glucose into the cell via facilitated diffusion (muscle and adipose tissues) (Navale and Paranjape, 2016, Jeukendrup, 2002). Muscle is extremely important with respect to postprandial glucose uptake, as at rest large amounts of glucose clearance is in response to the action of muscle tissue (Welsh et al., 2002).

Eating patterns usually result in the consumption of three meals per day (De Castro, 1997), given that the majority of people eat while still in the postprandial state from a previous meal, it is for that reason investigations need to better comprehend postprandial responses to sequential meal ingestion (Gonzalez, 2014). Nevertheless, an early investigation used sequential OGTTs when ingesting glucose repeatedly, they discovered the second-meal phenomenon (Hamman and Hirschmann, 1919). This theory suggests glucose tolerance is improved with the ingestion of sequential meals probably related to slower gastric emptying, increased insulin secretion which will inhibit endogenous glucose output from the liver resulting in enhanced glucose clearance by the muscle (Astbury et al., 2011, Jovanovic et al., 2009). Therefore, the pancreas is key in the regulation of macronutrients being digested and therefore controlling metabolism and energy homeostasis by releasing various digestive enzymes and pancreatic hormones at rest after food consumption or multiple meal ingestion (Cheng et al., 2010).

Besides the fact, most individuals identify that exercise is influential in the management of body composition, which stems from the acceptance that physical activity enhances weight loss via an increase in energy expenditure (EE) produced by a substantial change in substrate utilisation (Donnelly et al., 2009). Although, during high intensity endurance exercise carbohydrates provide energy in the form of muscle glycogen and plasma glucose (van Loon et al., 2001), as shown by Wee et al (2005) when exercising for 30 min at 70% $\dot{V}O_{2max}$ muscle glycogen were depleted by ~30%. This potentially suggests that postprandial glucose kinetics is driven to replenish

muscle and liver glycogen stores post-exercise. It is well established that exercise increases muscle glucose uptake independent of insulin (Richter and Hargreaves, 2013), which might be related to the fact that exercise training is the most potent stimulus to increase skeletal muscle GLUT4 expression (Richter and Hargreaves, 2013, Richter et al., 1985). With this in mind, elevated muscle glycogen concentrations from consuming food prior to exercise was thought to contribute to lower glucose uptake post exercise when compared to fasted exercise, although higher muscle glycogen before exercise actively increased the use of glycogen during exercise producing similar muscle glycogen concentrations at the end of exercise (Wee et al., 2005). Support of this notion has regularly been discovered after consuming food before exercise, which result in greater rates of carbohydrate utilisation during exercise (McIver et al., 2018, Clayton et al., 2015, Gonzalez et al., 2013).

It is important to consider that most populations consume food post-exercise and how the body responds in the postprandial period might provide insight into morbidity and mortality of chronic long-term illnesses (diabetes, heart disease and obesity). Improved glucose tolerance has been seen after consuming mixed-macronutrient meals (Astbury et al., 2011, Jovanovic et al., 2009), although limited research has considered whether a delayed gastric emptying and enhanced GLP-1 concentration supports insulin secretion leading to enhanced muscle glucose uptake and better regulation of blood glucose. In support of the above knowledge, it is becoming increasingly clear that adaptations initiated by exercise, can be strengthened by nutritional intervention, and consuming food in the hours after exercise is promoted to improve recovery (Thomas et al., 2016). One of the most frequently recommended strategies to stimulate post-exercise adaptations by accelerating the remodelling process is immediate protein feeding (Ranchordas et al., 2017). It has been documented by a number of recent reviews (Phillips, 2014a, Phillips, 2014b), in the absence of protein post-exercise, net protein synthesis is lowered leading to a reduced protein balance within the muscle. There is also evidence, low-carbohydrate availability can promote specific adaptations in the muscle and blood glucose is the primary energy fuel for the human body following carbohydrate ingestion. According to the glucostatic theory, low blood glucose concentration is associated with elevated food intake (Mayer, 1955).

In summary, several factors can affect how the human body responds in the postprandial period such as pre-nutritional intervention (fed or fasted) and exercise characteristics (mode, intensity, and timing). Hence the mechanisms which control glucose tolerance following exercise may involve enhanced intestinal absorption and reduced insulin sensitivity after exercise but may be counteracted by exercise-induced muscle damage by the working muscle and elevated NEFA accumulation within the non-exercised muscle. More recently, following exercise, glucose tolerance was worsened by prior meal ingestion (Gonzalez et al., 2013). Therefore, within this thesis all investigations were conducted in the fasted state, so comparisons can be made between different exercise conditions within the postprandial period.

2.11. Summary

Regular physical activity is an important component of overall health with reductions in chronic disease risk factors associated with as little as 3% change in body weight (Donnelly et al., 2009). Therefore, regularly exercising can help to create an energy deficit that may disturb homeostasis and hormone levels which regulate substrate utilisation and unwanted accumulation of substrates within tissues and cells of the human body. Despite a large amount of research focusing on appetite control by endeavouring to emphasise the relationship between energy expenditure from physical activity and EI from food consumption (Blundell et al., 2003), it is still apparent, exactly how exercise influences EI and appetite regulation is quite controversial and is constantly evolving (Schubert et al., 2013). Nevertheless, vast amounts of data have demonstrated that the fundamental aspect of the GI system is to digest and absorb nutrients, although very little is known about the extent GER performs in the regulation of appetite and metabolic health. It is unclear whether an increase or decrease in the ability of the stomach to empty its contents (GER) is responsible for the metabolic adjustments seen in the postprandial period, as GER has been suggested as a mediator of energy recovery. The absence of any studies connecting the modifications in GER to alterations in food intake and appetite regulation in the immediate hours after exercise is clear, warranting further investigation. Furthermore, concentrating on a healthy untrained population is also required to foster the research relevant to the general population. This thesis will give further insight into this developing theory and area of research.

CHAPTER 3.

GENERAL METHODS

3.1. Ethical Approval

This Chapter describes the materials and methods used within each of the experimental Chapters within this thesis. Any methodologies that were used only in a specific study were described within the section of the methodology of that specific Chapter. All experimental Chapters followed all procedures and protocols adhered to the guidelines of the World Medical Association Declaration of Helsinki version (2013) and all investigations were approved by the Faculty of Science and Engineering Research Ethics and Governance Committee prior to commencement (Approval letters provided in Appendix A to D).

3.2. Participants

Healthy male participants were recruited for each experimental trial within this thesis. 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 regulating hormones, and EI (Lissner et al., 1988, Wade and Jones, 2004, Brennan et al., 2009, Brennan et al., 2011). Participants were aged between 18y to 40 y, this narrow age range was chosen as food intake diminishes with age as changes to gut function; dyspepsia hypochlorhydria, and quicker filling of the distal gastric antrum all leading to reduced appetite (Nieuwenhuizen et al., 2010, Nigam and Knight, 2017). Furthermore, physiological changes such as decreased secretion of ghrelin have also been noted with an elderly group compared to a young control (Di Francesco et al., 2008) and Di Francesco et al (2005) also revealed longer gastric emptying time with an elderly group (77 ± 3 years old) compared with younger controls (32 ± 8 years old). Participants had a body mass index (BMI) <29.9 kg/m^2 and had no history of respiratory, cardiovascular or chronic gastrointestinal disease, and were not taking regular prescription medication as assessed by a medical screening

questionnaire (see Appendix E). Furthermore, participants were non-smokers, not dieting, vegan or lactose intolerant (Chapter 6 & Chapter 7 only) and free from musculoskeletal injury.

During recruitment, participants approached the principal investigator via email or by telephone after browsing a requirement poster located around MMU buildings (lecture theatres, laboratories, social spaces and the library), social media pages (e.g. Twitter) and MMU TV's located in John Dalton. Details outlining the study were provided, so that participants could make an informed decision whether to participate before attending a familiarisation trial. Potential participants were encouraged to read the information before making a decision in private, so that they did not feel pressurised into agreeing to participate. All participants were made fully aware of their right to withdraw from the study at any time before being informed of the experimental details both verbally and in writing prior to providing their written informed consent.

3.3. Clinical Trial Registry

Clinical trial registry helps to reduce selective reporting of results and ensures an unbiased and complete evidence base. Both Chapter 6 and Chapter 7 within this thesis were prospectively clinically registered with ISRCTN or ClinicalTrials.gov. Details and reference numbers are provided in the relevant chapters.

3.4. Familiarisation Visits

All participants conducted a familiarisation visit at least 7 days prior to their first experimental trial in each study within this thesis. During this visit, participants were provided with a written handout, which outlined the nature of the study and any risks and discomforts associated with taking part before completing a health screening questionnaire. Participants were familiarised with the gastric emptying breath sampling technique and the appetite visual analogue scale (VAS) questionnaire both detailed below (see section 3.8. Gastric Emptying Measurement; and 3.9. Appetite Assessment) to be used during the experimental trials before commencing anthropometric measures of height, weight, body composition and blood pressure were made (see section 3.4.2. Anthropometry Measurements). Following this, all participants completed an incremental exercise test to exhaustion (described below-

see section 3.4.1. Peak Oxygen Uptake Tests). Before leaving participants were provided with food scales (Salter, ARC 1066 Electronic Kitchen scale Range 1g to 3 kg, Tonbridge, UK) and a physical activity and food diary to be completed prior to their first visit (example; Appendix H).

3.4.1. Peak Oxygen Uptake Tests

For the study reported in Chapter 4, peak oxygen uptake ($\dot{V}O_{2peak}$) was determined using an incremental exercise test. $\dot{V}O_{2peak}$ was measured on a cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). Oxygen consumption ($\dot{V}O_2$) and carbon dioxide output ($\dot{V}CO_2$) measurements were taken by indirect calorimetry and expired air was collected using a Douglas bag (HaB International Ltd, Warwickshire, UK). Heart rate (HR) (Polar FS2c, Kemple, Finland) was recorded along with ratings of perceived exertion (RPE; Borg, 1982). Before the incremental exercise test commenced a 3 min resting expired gas sample was measured on the cycle ergometer. After the resting gas sample was collected, participants started cycling at a power output of 50 watts (W) with a cadence maintained throughout of 70 revolutions/min (rpm). Workload increased by 50 W every 3 min until participants began to show signs of fatigue (assessed through HR and RPE). Once reached, workload was increased by 20 W every minute until volitional exhaustion. $\dot{V}O_{2peak}$ was calculated by averaging the highest oxygen volume consumed over the final 1 min period before exhaustion.

For the study reported in Chapter 5, $\dot{V}O_{2peak}$ was measured on a cycle ergometer. HR was measured continuously and RPE at regular 2 min intervals. Expired air was continuously collected using a breath-by-breath gas analyser (Metalyzer 3b, Cortex, Leipzig, Germany). The protocol initially commenced with a 5 min warm-up with workload set at 0 W after which workload was increased to 50 W. Participants cycled at a cadence of 70 rpm throughout. Workload was then increased by increments of 50 W every 3 min until respiratory exchange ratio (calculated as VCO_2/VO_2) was greater than 1.0 for at least 1 min. From this point onwards, increments of 20 W were applied every minute until volitional exhaustion. $\dot{V}O_{2peak}$ was calculated by averaging the oxygen volume consumed over the final 1 min period.

For the studies reported in Chapter 6 and Chapter 7, $\dot{V}O_{2peak}$ was assessed through the performance of a continuous, incremental protocol that initially commenced with a 5 min warm-up with workload set on 0 W followed by increments of 1 W every 2 s until volitional exhaustion using a cycle ergometer (Lode Corival CPET, Cranlea Human Performace Ltd, Birmingham). Expired gas (Metalyzer 3b, Cortex, Leipzig, Germany) and HR were continuously collected using a breath-by-breath gas analyser with RPE being obtained every 2 min. $\dot{V}O_{2peak}$ was calculated by using the rolling mean of 10 breaths and work rate. The highest mean value of the rolling 10-breath $\dot{V}O_2$ values was accepted as $\dot{V}O_{2peak}$.

3.4.2. Anthropometry Measurements

3.4.2.1. Height

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Holtain Limited, Crymych, Dyfed, Great Britain). Participants removed footwear and stood flat footed with their heels against a back plate standing vertically facing away from the stadiometer.

3.4.2.2. Body Mass

Body mass was recorded to the nearest 0.01kg using electronic scales (GFK 150; Adam Equipment Co. Ltd., Milton Keynes, UK). Participants removed their shoes and socks and only wore minimal clothing (shorts, t-shirt and underwear) during body mass measurements.

3.4.2.3. Body Mass Index

Body mass index (BMI) was calculated using the formula kg/m^2. This was performed by dividing body weight (kg) by the square of height (m) (Hoffman, 2006). BMI standards were used to control the population within this thesis, BMI Classification (Table 4). Participants with a BMI >29.9 Kg/m^2 were excluded from the study after the familiarisation visit.

Table 4. Classification of Weight by Body Mass Index (BMI)

Category	BMI (Kg/m^2)
Underweight	<18.5
Normal	18.5-24.9
Overweight	25.0-29.9*
Obese	30.0-34.9

* Cut off range for all studies within this thesis (Hoffman., 2006)

3.4.2.4. Body Composition

Body fat percentage was approximated using a bioelectrical impedance analysis (Omron BF306; Kyoto, Japan). Data was entered into the device (height, body mass, sex and age) before participants were asked to remove their shoes and socks and laid down flat on a bed face up. Electrodes were placed on the participants right hand and foot and they were asked to keep as still as possible for 30s whilst a small electrical current was passed through their body between the electrodes.

3.4.2.5. Blood Pressure

Participants were seated for ~15 min before blood pressure was measured using a non-invasive digital sphygmomanometer (Omron M2, Kyoto, Japan). Normal blood pressure was assumed around 120/80 mmHg.

3.5. Exercise Intensity

Off-line analysis was used to calculate exercise intensity that would elicit the required percentage of maximal oxygen uptake to be used during main trials. This analysis was calculated independently for each experiment. Details are provided in the relevant research chapters and within Table 5.

Table 5. Classification of Exercise Intensity for this Thesis

Study	Intervention	WR	Intensity
Chapter 4	CON cycle for 60 min	40 % $\dot{V}O_{2Peak}$ 70 % $\dot{V}O_{2Peak}$	LOW HIGH
Chapter 5	CON cycle for 60 min INT cycling for 60 min [20x (1 min cycle / 2 min rest)]	40 % $\dot{V}O_{2Peak}$ 60 % $\dot{V}O_{2Peak}$	LOW MOD
Chapter 6	CON cycle for 60 min CON cycles @ 2 x 30 min	70 % $\dot{V}O_{2Peak}$	HIGH
Chapter 7	CON cycle for 30 min INT cycling for 30 min [10 x (1 min cycle / 2 min rest)]	50 % $\dot{V}O_{2Peak}$ 100 % $\dot{V}O_{2Peak}$ (PPO)	MOD VERY HIGH

WR = work rate of percentage of maximal oxygen uptake, CON = continuous, MOD = moderate, INT = intermittent, PPO = peak power output

3.6. Meal Details

Food was provided to participants in all four experimental Chapters within this thesis. Within Chapter 4, a standardised semi-solid meal consisted of one ~400g can of Heinz classic chicken and sweetcorn soup. The meal provided 242 KCal (1006 kJ) and the macronutrient content of the meal was 11.8 g fat, 8.2 g protein, 25.2 g carbohydrates, 0.8g fibre and 2.2 g salt according to manufactures details (Appendix G). The amount provided was determined by the manufacturer's recommendation of an average serving and provided each participant with ~10% of their reference intake of an average male adult 2500 KCal (Public Health England, 2016).

Chapter 5 also entailed one meal period in which a standardised semi-solid meal consisted of two cans (~800 g) of Heinz classic vegetable soup heated in a microwave. The meal provided 376 KCal/1584 kJ and the macronutrient content was 6.4 g fat, 8.8 g protein, 66.4 g carbohydrates, 7.2 g fiber and 4.8 g salt according to manufactures details (Appendix G). The amount (~400 to 800 g) and overall EI (242 to 376 KCal) of the meal was increased from Chapter 4 to Chapter 5 respectively. The

rationale for this increase was firstly to provide a vegetarian choice of soup so a more diverse population could be recruited and secondly to better understand if increase in meal volume would influence GER.

Food was provided to participants in Chapter 6 through two meal periods. The total energy utilisation during exercise and the remaining periods of rest between exercises was calculated as estimated trial energy expenditure (ETEE). This equation is documented in section 3.6.1. Meal one (breakfast) consisted of a glass of semi-skimmed cow's milk which equalled approximately 30% of ETEE. The volume of milk that was provided to participants was determined by using manufacturer's details (Appendix F). Participants were also provided with water so the total volume during meal period one was standardised to 500 mL to prevent differences in gastric distension. The second meal (Lunch) consisted of a bowl of Heinz classic vegetable soup which provide participants with the remaining 70% of ETEE. The weight of soup was determined using manufacturer's details (Appendix G). Water was added to the soup in order for the final weight to be standardised at 1000g.

Chapter 7 consisted of providing participants with food over two meal periods. The first of which was breakfast (meal-one) consisted of 30g of Kellogg's Special K original cereal with 125 mL of semi-skimmed cow's milk, a Sainsbury's butter croissant (51 ± 4 g) and a 200 mL of Sainsbury's pure orange juice. This provided a total of 434 KCal (1,816 kJ) and the macronutrient content was 13 g fat, 67g carbohydrate and 12.5g protein according to manufacturer's details (Appendix F). The second meal (lunch) was identical to the lunch meal provided within Chapter 5 (see details above). The amount of food chosen for the breakfast meal was based on the recommended serving size of approximately 400 Kcal for a man (Public Health England, 2018). Furthermore Betts et al (2016) state, that generally most investigations providing a breakfast meal are typically above 400 KCal (1673.6 KJ).

3.6.1. Calculating Estimated Trial Energy Expenditure

Daily estimated calorie requirements was calculated in Chapter 6 using the Mifflin-St Jeor formula (Mifflin et al., 1990) (Equation 1).This predictive equation determined resting energy expenditure (REE) using multiple-regression analyses to

derive relationships between REE and weight, height and age within healthy men. The calculated REE predicts 24-h energy expenditure (24-EE, in Kcal/d). Equation 2 establishes trial resting energy expenditure (TR-EE). This is determined by dividing REE by the number of hours within a day (24 h), before multiplying by six the number of resting hours within each trial day (Td). All participants completed 60 min of exercise at a continuous intensity at 70% $\dot{V}O_{2Peak}$. Equation 3 determined exercise expenditure in KJ (EE-KJ) by multiplying work rate (W) by exercise duration in seconds, before divided the answer by one thousand. Then EE-KJ were converted to physical activity energy expenditure in KCal (PA).

Equation 4 was used to add physical activity energy expenditure (PA) and trial resting energy expenditure (TR-EE), which equalled estimated trial energy expenditure (ETEE).

Equation 1

$$REE = (\big((10 \times weight\ [kg]) + 6.25\big) \times (height\ [cm] - 5)) \times (age\ [y] + 5))$$

Equation 2 $$TR\ EE = ((REE \div 24) \times 6\ [Td])$$

Equation 3

$$EE\ KJ = ((W \times 3600) \div 1000)$$
$$PA = (EE\ KJ \times 0.2390057361)$$

Equation 4 $$ETEE = (PA + TR\ EE)$$

3.7. Pre-Trial Standardisation

In the 24 h preceding each experimental trial, participants were asked to refrain from alcohol and caffeine consumption as well as strenuous physical activity. Any activity that was performed was documented in a 24 h activity log. Each participant was asked to record and weigh all food and drink consumed until 2200 h and then asked to replicate this prior to the next experimental visit (s). Participants fasted

overnight for a minimum of 9 h with the exception of *ad libitum* water intake. All experimental trials were performed between 0700 h to 0900 h to allow an overnight fasted baseline blood samples to be collected in the morning as metabolic and gut hormone response were being measured.

To ensure that participants were adhering to the standardised dietary procedures, the research team contacted each participant via email or telephone 48 h before each experimental trial. This was to ensure standardisation and consistency of macronutrient intake and metabolic status in the 24 h leading up to each trial.

Approximately 90 min prior to the arrival at the laboratory participants were asked to consume 500 mL of water. This was to ensure euhydration upon arrival and a consistent level of hydration status before any blood sample was collected.

All experimental Chapters within this thesis involved multiple experimental trials and were conducted in a single blind randomised-crossover fashion. The order of trials within each experimental study were randomised for each participant to eliminate order effects occurring or bias from the investigator. Allocation of participant numbers was also randomly assigned to increase the randomisation sequence. Randomisation for all studies was conducted using a research randomizer website, which is a free resource for researchers to generate random numbers or assign participants to experimental conditions (Urbaniak and Plous, 1997). Due to the nature of the experiments conducted in this thesis, blinding of trials for both investigator and participant was not possible. The investigator was not aware of the condition and/or the sequence of trials that participants were going to follow before recruitment. Both participants and researcher were not made aware of the condition they were undertaking until they were setting up the cycle ergometer for the commencement of exercise. Participants were blinded from the order of the exercise for the whole experiment, except for the final experimental trial as participants could guess which trial they were going to be doing by deduction.

3.8. Gastric Emptying Measurement

Gastric emptying was assessed in all four experimental Chapters within this thesis using the ^{13}C breath test method. This method does not expose participants to

large doses of radiation and, is a reliable, non-invasive and accurate measurement of gastric emptying (Braden, 2009) when compared with other methods such as scintigraphy (Ghoos et al., 1993). Isotope breath tests are usually measured using ^{14}C octanoic acid (Ghoos et al., 1993, Maes et al., 1994, Galmiche et al., 1998) however Braden et al., (1995) identified ^{13}C sodium acetate was as effective at measuring gastric emptying when ingesting a liquid or semi-solid meal. The test meals provided to measure gastric emptying rate was a semi-solid meal containing100 mg of [^{13}c] Sodium acetate (1-13C, 99%; Cambridge Isotope Laboratories Inc., Andover MA, USA). This isotope absorbs freely in the proximal small intestine, before being transported to the liver and metabolised to $^{13}CO_2$ which is then exhaled from the lungs in the breath (Wölnerhanssen et al., 2016). Prior to ingestion of the semi-solid test meal (described in more detail within each research method section of each Chapter), a basal end-expiratory breath sample was collected into a 100 mL foil bag by exhalation through a one-way valve mouthpiece (Wagner Analyzen-Technik, Bremen, Germany) and further test samples were collected every 15 min for a total of 120 min following the ingestion of the meal. Bags were sealed with a plastic stopper and stored in a dark and temperate environment, temperature (19 ˚C to 21˚C) ready for analysis within 2 weeks from sample collection.

Previous studies have used a range of time frames to measure gastric emptying, depending on the composition of the nutrient/meal being consumed. Liquid emptying has been assessed over 1 h (Yau et al., 2014, Yau et al., 2017b). While semi-solid (Braden et al., 1995) and solid meal (Galmiche et al., 1998) ingestion have been assessed over a 4 h period. However, a 2 h sampling period was deemed sufficient as data collected from our laboratory with seven participants showed that the results from sampling every 10 min for a period of 2 h period did not show the parameter time of maximum emptying rate (T_{lag}) to increase over 90 min post-ingestion (Evans et al., 2018b).

Breath samples were determined by non-dispersive infrared spectroscopy using an isotope ratio mass spectrophotometer (IRIS Dynamic, Kibion, Germany) for the ratio of $^{13}CO_2$:$^{12}CO_2$. 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 ($T_{1/2}$) and time of maximum emptying rate (T_{lag}) was calculated

using the manufacturer's integrated software evaluation based on the equations of (Ghoos et al., 1993). This software also calculated recovery of ^{13}C tracer in breath over time, by using the CO_2 production of the participants, which was assumed to be 300 mmol/h multiplied by the body surface area. This body surface area was calculated using the Haycock et al., (1978) weight height formula. This value would allow percentage accumulative dose over time to be documented.

A previous study documented that a sampling period of a least 4 hours is required to allow the full recovery of the ingested food (Sanaka & Nakada, 2010). Although, using this duration for sample collection may burden participants or patients involved. Alternatively, liquid meals have been found to empty somewhat faster, showing rapid emptying rates particularly if the macronutrient content of the test solution is low (Camps et al., 2016). Therefore, lower calorie or semi-solid meals which are less dense may empty a lot quicker, making a shorter breath sampling period more adequate to detect differences (Bluemel et al., 2015). A basel end-expiratory breath sample was collected pre-meal ingestion then at 15 min intervals post -meal ingestion for a total of 2h following food ingestion. 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). Previous studies have also used a sampling period of 60 min for liquid emptying rate (Jeukendrup and Moseley, 2010, Yau et al., 2014, Yau et al., 2017b). Breath samples were analysed for the ratio of $^{13}C0_2$: $^{12}C02$. The difference in the ratio $^{13}C0_2$: $^{12}C0_2$ from baseline breath to post-ingestion breath samples are expressed as delta over baseline (DOB). Half emptying time ($T_{1/2}$) and time of maximum emptying rate (T_{lag}) were calculated using the manufacturer's integrated software evaluation based on the equation of Ghoos et al (1993). DOB would be considered to be the most important result for GER within the studies presented in this thesis. $T_{1/2}$ and T_{lag} results should be used with caution if DOB results were not significantly different.

The measurement of gastric emptying was an essential outcome for each experiment conducted within this thesis, for this reason each participant was shown by the researcher how to perform an end-expiratory breath sample during their familiarisation visit. The purpose of introducing this technique was to make sure each participant was competent with provided the breath sample before their first

experimental visit. On experimental trial days the researcher demonstrated how to perform the end-expiratory breath sample again before participants completing their first sample at baseline. To make sure each breath sample was valid, the researcher reminded the participants how to conduct the measurement before each sample was performed.

3.9. Appetite Assessment

Appetite was assessed using a visual analogue scale (VAS). This method of measurement attempts to quantify sensations that are not directly or easily measured. Most VAS use a straight horizontal line with a fixed length. A 100 mm visual analogue scale was chosen to measure: ratings of hunger, fullness, prospective food consumption (PFC), satisfaction, nausea and bloat in the studies presented in this thesis. These measurements were collected at baseline, post-exercise, pre-food consumption and at regular intervals during recovery; 15 min (Chapter 3), 30 min (Chapter 4 & Chapter 5) and 1 h (Chapter 6). Each VAS was composed of questions which asked “how hungry do you feel?” “How full do you feel?” “How much do you think you can eat?” “How satisfied do you feel?” “How nauseous do you feel” and “How bloated do you feel. Each anchor defined the extreme limits of the parameter being measured. A copy of this VAS used is in Appendix I. These scales are often used in clinical research to measure the intensity or frequency of a particular symptom and the choice or terms defined on this assessment was based on research conducted by (Flint et al., 2000).

The measurement of appetite was an important outcome, and for this reason each participant was shown a VAS during their familiarization visit. The purpose of introducing this scale was to explain how each question was relevant to the assessment of appetite and each question had to be completed correctly with a vertical straight line on each horizontal line. Each participant was also informed that during experimental trials that they would be provided with multiple VAS and the order of the questions and the anchors would not change. Participants were shown a VAS again before completing their first scale at baseline. After this the researcher did not intervene at any point when the participants completed each VAS, apart from checking each

question had been completed. This was to prevent the researcher influencing the outcome of the result.

3.10. Expired Gas Samples

Expired gas was collected for the study reported in Chapter 4. Gas samples were collected via Douglas bags and samples were analysed for $\dot{V}O_2$ and $\dot{V}CO_2$ production using a paramagnetic oxygen analyser and an infa-red carbon dioxide analyser (Series 1400, Servomex, East Sussex, UK). Analysers were initially calibrated using known concentrations of nitrogen and an oxygen and carbon dioxide mixture. The volume of expired air was measured with a dry gas meter (Harvard Ltd., Kent, UK) and corrected to standard pressure and temperature (Edale Thermistor, Cambridge, UK), before $\dot{V}O_{2peak}$ determined (Varley, 2014).

Each participant inserted a sterile mouthpiece into their mouth for a 2 min period during exercise and 5 min during rest. Expired air was only collected for analysis during the final 1 min during exercise and 3 min during rest so $\dot{V}O_2$ could be calculated in 15 min durations at 15, 30, 45, and 60 min. This allowed workload during the main trials to be monitored.

3.11. Substrate Utilisation

Expired air was collected using a breath-by-breath gas analyser (Metalyzer 3b, Cortex, Leipzig, Germany) during Chapter 5, Chapter 6, and Chapter 7, for the calculation of substrate utilisation at baseline and at further regular intervals throughout all trials. During the rest period participants wore the expired air mask for 15 min in a controlled semi-supine position on a bed and continuously throughout exercise only within Chapter 6.

Average $\dot{V}O_2$ and $\dot{V}CO_2$ measurements from the last 5 -min were used to calculate fat and carbohydrate (CHO) oxidation rates using stoichiometric equations (Péronnet and Massicotte, 1991). Whole-body rates of CHO and fat oxidation (g/min) were calculated from respiratory gas samples that were collected using non-protein respiratory exchange ratio (RER) equations, which are based on the assumption that $\dot{V}O_2$ and $\dot{V}CO_2$ accurately reflect tissue O_2 consumption and CO_2 production (Leckey et al., 2018);

$$CHO\ oxidation\ (g/min) = 4.585\ VCO_2\ (L/min) - 3.226\ VO_2\ (L/min)$$

$$Fat\ oxidation\ (g/min) = 1.695\ VCO_2\ (L/min) - 1.701\ VO_2\ (L/min)$$

3.12. Blood Sampling and Analysis

Blood samples were collected in the studies reported in Chapter 4, Chapter 6 and Chapter 7 of this thesis. All blood samples were collected with the participant in a semi-supine position. A 20-gauge cannula (Venflon: Becton Dickinson, Plymouth, UK) was inserted into an antecubital vein and an extension (Vygon, Ecouen, France) attached. Before a blood sample for analysis was drawn, 4 -5 milliliters (mL) sample was drawn to clear the catheter extension and disposed of. Next 5 mL of blood was collected using a syringe (Terumo 10mL sterile syringe, Bunzl Heathcare, UK) and dispensed into serum vacutainers (Gold top, Becton Dickinson, Plymouth, UK). The cannula was kept patent by flushing with nonheparinized saline (0.9% sodium chloride; Becton Dickinson, USA) after every blood sample was taken to prevent blood clotting within the cannula.

Upon collection of blood samples, 50 microliters (µL) of Pefabloc (Roche Diagnostics Limited, Burgess Hill, UK) and 50µL Dipeptidyl peptidase IV inhibitor (DPP-IV), (Merck Millipore Limited, UK) was immediately added to the vacutainers to prevent the degradation of acylated ghrelin and active GLP-1. Each compound was prepared in accordance with the manufacturer's instructions using 10 µL/mL of whole blood for both inhibitors.

Blood samples were kept on ice until they were centrifuged (Sigma 3-16KL, Germany) at 1500 × g for 15 min at 4 ˚C. Serum was then extracted from the

vacutainers using a Gilson pipette (Gilson single P1000, Fisher Scientific Ltd, UK) and blue pipet tip-ends (Thermo Scientific, ART Barrier Pipet Tip P1000, Fisher Scientific Ltd, UK), before aliquoting the serum into three separate 1.5 mL Eppendorf tubes (Flat-top snap cap microcentrifuge tubes, Fisher Scientific Ltd, UK) labeled (*a, b and c*). Serum was then immediately stored at -80 ˚C. During biochemical analysis, only one Eppendorf tube was removed for each sample to prevent defrosting samples multiple times. Each batch was left to rise to room temperature naturally over 60 – 120 min.

3.12.1. Gut Hormones

Gut hormones were determined using a multiplex analyser (Luminex 200, Luminex Corporation, Austin, TX, USA). This machine analysed fluorescently labelled microsphere beads. Equipment settings were set for a minimum of 50 beads per event and a sample size of 50 µL. The probe height was adjusted between every plate and gate parameters were set to 8,000-15,000 count (CT) which was sufficient to measure circulating concentrations of total peptide YY (PYY), pancreatic polypeptide (PP), insulin, active GLP-1 (both GLP-1^{7-36} and GLP-1^{7-37}) and acylated ghrelin using human gut hormone Merck-Millipore kits (Milliplex MAP, Merck Millipore Ltd, UK). This method allowed simultaneous quantification of all gut hormones on their respective immunoassay plate in a 96 well format. This procedure included an overnight incubation period at 4 ˚C for 17 h, in order to improve the sensitivity of the assays. Manufacturer kit sensitivities of each analyte are shown in Table 6 and coefficient of variance results are presented in each individual Chapter. Each plate contained a background, seven standards, a high and low quality control with the remaining wells filled with serum samples in duplicate.

3.12.2. Metabolites

Biochemical markers were determined from serum samples using a clinical chemistry analyser (Randox Daytona, Randox Laboratories, Crumlin, UK) that operates a photometric technique allowing dye that is produced during reagent reactions between the target markers within the serum to be measured using an enzymatic end point method. Glucose (GOD-PAP), total cholesterol (CHOD-PAP),

triglycerides (GPO-PAP) and non-esterified fatty acids (NEFA) (ACOD-POD), concentrations were determined in duplicate and an average value was calculated. Manufacturer kit sensitivities of each analyte are shown in Table 6 and coefficient of variance results are presented in each individual Chapter.

Glucose was determined by enzymatic oxidation of glucose, by glucose oxidase to form gluconic acid and peroxide. The hydrogen peroxide, phenol and 4-aminophenazone are catalyzed by peroxidase to form quinoneimine and measured at a wavelength of 505 nanometers (nm).

Similarly, total cholesterol was determined when cholesterol esterase is added to hydrolyse cholesterol esters into cholesterol and fatty acids, then oxidation by cholesterol oxidase to give cholestene-3-one and peroxide. This peroxide reacts with 4-aminoantipyrine and hydroxybenzoate, catalyzed by peroxidase to form quinoneimine, which were measured at a wavelength of 600 nm.

Triglycerides were determined by an enzymatic hydrolysis by lipases into glycerol and fatty acids, followed by glycerol and ATP forming glycerol-3-phosphate and ADP catalysed by glycerol-kinase. Glycerol-3-phosphate and oxygen is then hydrolysed to form dihydroxyacetone, phosphate and peroxide, which converts 4-aminophenazone and 4-chlorophenol in the presence of peroxidase forms the marker quinoneimine, which were measured at a wavelength of 546 nm.

NEFA and triglycerides should not be tested in the same run nor serum samples that are heparinised, as this stimulates lipoprotein lipase activity, causing the release of NEFA from triglycerides. Therefore, NEFA samples were analysed separately. NEFA were measured in a 2-stage reaction, in which NEFAs were converted to thioesters of coenzyme A by acyl-CoA synthetase in the presence of ATP, magnesium ions and CoA. Stage 2, acyl-CoA is oxidized by acyl-CoA oxidase to produce hydrogen peroxide, which converts 3-methyl-N-ethyl-N-(β-hydroxy-ethy)-aniline and 4-aminoantipyrine in the presence of peroxidase forms the marker quinoneimine, which were detected at 550 nm (Stokol and Nydam, 2006).

3.12.3. I-FABP$_2$

Circulatory intestinal fatty-acid binding protein-2 (I-FABP$_2$) concentration was measured using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) (EHF-ABP2 Human FABP2 Intestinal ELISA, Thermo Scientific, Frederick, USA) in Chapter 6. Manufacturer kit sensitivities of the analyte are shown in Table 6, and the coefficient of variance results are presented in Chapter 6. Assay plates were analysed using a 96 well microplate reader set for absorbance (Synergy HTX Multi-mode microplate reader, BioTek, Winooski, VT, USA). The ELISA plate reader was set at 450 nm and 550 nm. The 550 nm values were subtracted from the 450nm values to correct for optical imperfections in the microplate. A standard curve generated by absorbency readings were used to calculate concentrations. Samples were run in duplicate and an average value calculated.

Table 6. Manufacturer Sensitivity Characteristics and Percentage (%) CVs of Analyses Measured within Blood Samples.

Analyses	Minimum Detectable Concentration	Intra-Assay %CV	Inter-Assay %CV
Gut Hormones (pg/mL) *			
PYY	28	10	15
PP	2	10	15
Insulin	87	10	15
Active GLP-1	1.2	10	15
Acylated ghrelin	13	10	15
Metabolise (mmol/L) #			
Glucose	0.200	1.96	1.96
Total cholesterol	0.865	1.67	1.00
Triglycerides	0.134	1.55	2.58
NEFA	0.040	0.42	1.00
Intestinal (ng/mL) ǂ			
I-FABP$_2$	0.025	10	12

*Sensitivity characteristics are those provided by the manufacturer. *Intra-assay percentage CV is calculated from 8 reportable results at 2 concentrations. Inter-assay percentage CV across 2 different concentrations of analyses across 6 different assays (Merck Millipore, UK). # Information provided by the manufacturer (Randox laboratories, UK). Intra-assay percentage CV is calculated from 20 results for each analyte. ǂ information provided by the manufacturer (Thermo Scientific, USA) Intra-assay percentage CV is calculated from 3 reportable results at 2 concentrations. Inter-assay percentage CV across 2 different concentrations of analyses across 3 different assays.*

3.13. Reliability Measurements

Coefficients of variation (CVs) is widely used to quantify precision of biological measures and inter and intra-assay CV are used to control for potential analytic error (Hanneman et al., 2011). Using CVs is well accepted as a more useful index than standard deviation (SD), because SD can be proportional to the sample

values and can increase or decrease systematically over time depending on the participant sample being used (Connett and Lee, 1990). Where applicable (i.e. blood metabolites, gut hormones and I-FABP$_2$), samples from each participant were assayed in the same plate or run to minimise inter-assay variation. Within this thesis, a large number of samples were tested, and for this reason samples were run on multiple assay plates, or multiple batch runs on a clinical chemistry analyser. Plates and batches were run with their own calibration for the standard curve and known concentration controls were used at a high and low value. The inter-assay CV is an expression of plate-to-plate consistency that is calculated from the mean values for the high and low controls on each plate (Salimetrics, 2018). Corresponding intra-assay CVs were determined from all duplicate samples. The % CV for each sample were calculated by (CV % = SD/mean × 100). The average of the individual CV is reported as the intra-assay CV (Boss et al., 2015). The intra-assay and inter-assay CVs are reported within Biochemical analysis section off Chapter 4, Chapter 6 and Chapter 7.

3.14. Capillary Blood Sample

Capillary blood sampling was only utilised in Chapter 5. Within Chapter 5 all blood samples were collected using the capillary method, with the participant in a seated position. A capillary blood was taken from the fingertip in order to provide a small (25 µL) blood sample for the measurement of glucose. Prior to the puncture being made the participants finger was cleaned using an isopropyl 70% alcohol swab (UHS, Enfield, UK) before making an approximately 3-mm puncture using a 23 gauge single use safety lancet (Unistik-3, Owen Mumford, Oxford, UK). The lancet was then disposed of directly into a sharps bin before blood was collected in microvettes (Hemocue Glucose 201$^+$ Microcuvettes, Ângelholm, Sweden) containing anticoagulant EDTA, lithium heparin. Blood was analysed immediately using a desk top plasma glucose analyser (Hemocue Glucose 201$^+$ analyser, Ângelholm, Sweden). Blood glucose results were obtained and documented in ~60 s as mmol/L.

3.15. Wellbeing Assessment

Wellbeing was assessed only during Chapter 7, using an in-house questionnaire. The wellbeing questionnaire was designed using a numeric rating scale (NRS). In recent years this method of obtaining valuable information in a fast,

undemanding manner to quantify the participants feelings in an easy to process way has become a popular method (Heidari et al., 2019, Saw et al., 2016). These questionnaires have predominately been used by swimmers (Hooper et al., 1995) and professional football players (Abbott et al., 2019a). By depicting the player's perception of his or her stress level and provides information on the training load both from a physiological and psychological standpoint when multiple exercise bouts are performed on the same day or over a long training week.

Most NRS use a straight horizontal line with a fixed length with evenly spaced numbers starting at zero and ending at ten. For the experimental trials a scale between one to seven was chosen to measure ratings of muscle soreness, fatigue, mood, stress and sleep. Each question was added together and the sum of scores for the five questions was used for data analysis. These measurements were collected at baseline (0 min), post-first exercise (30 min), pre-second exercise (255 min), post-second exercise (285 min), end-trial (450 min) and 24 h post trial.

The choice or terms defined on the wellbeing assessment were based on previous research (Abbott et al., 2019a, Hooper et al., 1995). Each wellbeing assessment was composed of questions asking "How sore do your muscles feel?" "How fatigued do you feel?" "How is your mood?" "How stressed do you feel?" and "How sleepy do you feel". Each anchor defined the extreme limits of the parameter being measured. A version of this questionnaire is presented in Appendix J.

Participants were shown a wellbeing questionnaire during their familiarisation visit and shown how to complete the questionnaire correctly by circling the number that best identified their feelings on the scale. The questionnaire was also provided in colour; point one (purple) through to point seven (red). This was so participants could visually understand the differences between the wellbeing questionnaire and the appetite VAS provided at the same time. Participants were also informed that during main trials they would be provided with multiple wellbeing questionnaires and the order of the questions and the anchors would not change.

On experimental trial days, the wellbeing questionnaire was shown again before completing baseline measurements. The researcher did not intervene at any point when the participants completed each questionnaire, apart from checking each question had

been completed. This was to prevent the researcher influencing the outcome of the result. The final wellbeing questionnaire was given to participants to be completed 24 h after they had finished each main trial. To ensure that participants completed their final wellbeing questionnaire, the research team contacted participants via telephone.

3.16. Dietary Assessment

Participant's 24 h post-trial food and drink consumption was assessed using a weighed dietary record during Chapter 5, Chapter 6, and Chapter 7; for the calculation of total calorie (KCal) content, macronutrient content from the total KCal (carbohydrate, fat, protein and fiber), and total water intake.

Participants recorded and weighed all food and drink consumed from the point the cannula was removed (Chapter 6 and Chapter 7) or final capillary blood sample was taken (Chapter 5). After which, participants were free to leave the laboratory, consume food, and drink for a further 24 h. Each participant was informed of the time period during which they had to record their diet. For instance, if the participant finished their trial at1300 h they would record and weigh their diet through to 1300 h the next day for 24 h. Food records were analysed using the weight documented for each food or ingredient by using manufacturer's values provided when possible, or by using DietPlan dietary analysis (Software 6, Forestfield software limited, Horsham, West Susses, UK).

3.17. Water Consumption

Cold water (~6 ˚C) was provided to the participants in all four experimental trials within this thesis. The nature and design of each study resulted in water being provided at different intervals and periods. Participants completed a number of different strenuous exercise bouts (low, moderate and high) in each study. For this reason, it was integral for participants to be able to consume water during or after exercise. Within each study, participants consumed water provided before the start of the gastric emptying measurement phase. This was to prevent the fluid intake manipulating the gastric emptying outcome.

Water was provided *ad libitum* during the first 60 min of exercise or resting control period during Chapter 4. Each participant had access to water *ad libitum*. If

participants finished the water provided, further water was available. This water bottle was weighed pre and post and the weight was recorded for further analysis.

Water was provided in a standardised amount (125 mL) within Chapter 5 during exercise period (15, 30, 45 and 60 min), resulting in a total of 500 mL being consumed during the 60 min exercise bout. This meant participants did not consume any further water after exercise in both Chapters 4 and Chapter 5, leaving a 30 min gap between water ingestion and consumption of the semi-solid meal.

Within Chapters 6 and Chapter 7, 500 mL of water was provided to participants during their first post-exercise recovery phase, 60 min after breakfast in a standardised amount (125 mL). All water that was provided was consumed before participants started their second bout of exercise. No more water was provided to participants until the end of the trial, apart from the semi-solid lunch meal post second exercise bout.

3.18. Heart Rate and RPE

Heart rate (HR) and RPE were measured during exercise in all four research chapters within this thesis. An HR strap was placed around the participants upper torso and was measured continuously throughout all exercise periods to monitor exercise intensity. With the nature of each study, HR was documented at different intervals and periods depending on the exercise mode and study design.

Rating perceived exertion (RPE), is a simple objective measure used during exercise trials. The scale allows participants to rate their individual rating of exercise intensity, formed by assessing the body's physical signs, HR and breathing rate. A 15-point scale starting at 6 (Very, Very light) to 20 (exhaustion) was used to measure RPE during this thesis (Borg, 1973). Participants were introduced to the scale before they started exercise and were told, 'there is no right or wrong answer'. The number that was chosen by the participant gives the researcher an idea of how they were feeling.

3.19. Statistical Methods

The statistics used during this thesis are reported in the methodological section of each Chapter. P values of < 0.05 were considered statistically significant. All statistical analysis was performed with Statistical Package for the Social Sciences (SPSS) version 24 (IBM, New York, NY, USA).

3.19.1. Area Under Curve (AUC)

The methodological approach to an AUC calculation was undertaken using the trapezoidal rule. This uses a numerical integration method to approximate the integral or the area under a curve. The integration of (a, b) from a functional form is divided into equal pieces, called a trapezoid (see Equation 5). Each subinterval is added together resulting in a total (Yeh, 2002). One of the benefits of using AUC is time series data are integrated to form a single value characterising the overall response of an area. This practice can provide a valid estimate of the overall exposure to the parameter of interest (Narang et al., 2020) .Within this thesis a number of AUC calculations have been performed within Chapter 4, Chapter 5, Chapter 6 and Chapter 7 and documented as the product of concentration and time (e.g $mmol \cdot L^{-1} \cdot 120\ min^{-1}$).

Equation 5 $$A = \frac{a + b}{2} h$$

3.19.2. Post-Statistics Effect Size

Post-statistical effect size was determined for all results documented in this thesis (Chapter 4, Chapter 5, Chapter 6, and Chapter 7). For all analyses of variance (ANOVA), effect size was calculated as partial eta squared (η^2_p) with the effect size being interpreted at small (0.01-0.06), medium (0.06-0.14) or large (≥ 0.14). For all pairwise comparisons and paired sample t-tests, effect size was calculated as Cohen's (d) and 95% confidence intervals (CI) were shown. The effect sizes can also be interpreted as trivial (< 0.20), small (0.20–0.49), moderate (0.50–0.79) or large (≥ 0.80) (Cohen, 1992).

Chapter 4.

The Effect of Exercise Intensity on Gastric Emptying Rate, Appetite, and Gut Derived Hormone Responses after Consuming a Standardised Semi-Solid Meal in Healthy Men[1, 2]

[1] Some of the data from this study contained within this chapter was orally presented "Mattin LR, Yau AMW, James LJ, Evans GH. (2016). Is appetite and gastric emptying effected by different exercise intensities after consuming a standardised calorie semi-solid meal. *19th-21st December 2016, ISENC International sports + Exercise Nutrition Conference, Newcastle.*

[2] The data from this study contained within this chapter has been accepted for publication in "Mattin LR, Yau AMW, McIver VJ, James LJ, Evans GH. (2018). The Effect of Exercise Intensity on Gastric Emptying Rate, Appetite and Gut Derived Hormone Responses after Consuming a Standardised Semi-Solid Meal in Healthy Males. Nutrients. 10(6): 787. https://www.mdpi.com/2072-6643/10/6/787 "

4.1 Introduction

Exercising at different intensity has become increasingly popular over recent years, with the belief that high-intensity (Broom et al., 2007, King et al., 2010a, Wasse et al., 2013b, King et al., 2013a, King et al., 2011a) but not low intensity (Unick et al., 2010, King et al., 2010b, Imbeault et al., 1997, Pomerleau et al., 2004) aerobic exercise suppresses appetite. Strenuous exercise >60% of $\dot{V}O_{2max}$ has been consistently shown to suppress appetite for up to 30 min after an exercise bout and this small delay in appetite does not influence EI regardless of the increased metabolic response of the exercise (Thompson et al., 1988, Ueda et al., 2009a, Becker et al., 2012). Although, it has been reported that changes in appetite do not always result in decreased food intake, but instead an increase (Martins et al., 2007a, Martins et al., 2007b, Shorten et al., 2009). Therefore, optimising physical activity to increase energy expenditure without increasing subsequent EI may be critical for preventing a positive energy balance in the hours after a bout of exercise which might lead to overall weight gain (King et al., 2008, Deighton and Stensel, 2014).

The literature that have measured appetite hormones in relation to changes in exercise intensity, has designated one or two peptide hormones to examine, with ghrelin, GLP-1 and PYY being deemed the most important (Broom et al., 2007, King et al., 2010a, King et al., 2011a, Crabtree and Blannin, 2015). Martins et al (2007a), observed that cycling at a moderate intensity for 60 min increased PYY, GLP-1 and decreased ghrelin. Which strengthens the idea that ghrelin remains unique as the only known orexigenic hormone (Hazell et al., 2016). The digestive system is complex with many more peptide hormones that are secreted for the gastrointestinal tract in response to nutrient ingestion after low to moderate intensity exercise (Wasse et al., 2013a, Martins et al., 2007a). Therefore, expanding this critical area of research by measuring a wider number of appetite hormones within the same study is required to increase understanding of perception of hunger and satiety.

Interestingly, limited research has focused on how GER might influence appetite regulation. To date it is known that GER is influenced by some gut-derived hormones, as ghrelin increases GER (Levin et al., 2006, Falkén et al., 2013) while PYY and GLP-1 have been shown to decrease GER (Witte et al., 2009, Edholm et al.,

2010). The regulation of both appetite and gastrointestinal motility appears to be intrinsically linked, as the rate of gastric emptying determines the time of gastric distention, which is known to be a satiety signal (Horner et al., 2011). Therefore, GER may be an important, understudied factor in appetite control and metabolic health, as GER has been suggested as a rate-limiting step in the delivery of nutrients to the small intestine after exercise (Geliebter, 1988). This delay in nutrients being absorbed in the small intestine is largely dependent on the GER process. One potential mechanism of interest after feeding is how GER and a selection of gut derived peptide hormones (ghrelin, GLP-1, insulin, PP and PYY) may influence subjective feelings of hunger during recovery.

The majority of studies that have focused on using exercise as a long-term programme for weight loss have exposed little change in post exercise EI (Schubert et al., 2013), but these investigations typically provide *ad libitum* meals as a method of assessing EI (Imbeault et al., 1997, King et al., 1997, Wasse et al., 2012, King et al., 2010a). Controversially, providing an *ad libitum* meal after exercise could be seen as unrealistic as energy within these ingestion periods can be as high as ~ 5500 kJ (King et al., 2013a). No studies have attempted to determine whether a standardised meal consumed 30 min after exercise affects appetite regulation in healthy untrained males.

To date, no investigation has examined gastrointestinal responses after exercise, compared to a non-exercise control trial following the consumption of a standardised semi-solid meal. Therefore, it was hypothesised that exercise, regardless of intensity would delay GER, compared to a no-exercise control and consequently lead to a delay in appetite. The null hypothesis is, therefore, no differences between control and exercise conditions. Hence, the aim of this investigation was to compare the effect of exercise intensity and the response to a standardized semi-solid meal on 1) Gastric emptying rate, 2) Circulating gut-derived hormone response, 3) Changes is blood metabolite response, and 4) participant feelings of appetite.

4.2 Methods

4.2.1. Participants

Twelve healthy males were recruited from central Manchester, UK (Table 7). Verbal and written explanations of the experimental procedures were given before the start of any trial and written informed consent to participate was obtained. The nature of the study design did not allow for a power-calculation to be performed. However, previous research within this area had commonly used twelve participants (Alajmi et al., 2016, Holliday and Blannin, 2017c, King et al., 2011a, Martins et al., 2007a); eleven (King et al., 1997, Wasse et al., 2013b, Crabtree and Blannin, 2015); Ueda *et al* (2009a) used ten participants and Broom et al, (2007) used nine. From this data, we decided to recruit twelve participants, as this was the higher participant number from the range of journals reviewed.

Table 7: S-1, Baseline Participant's Characteristics [1]

	Males (*n*=12)
Age, (y)	26 ± 5
Height, (cm)	176 ± 0.10
Weight, (Kg)	80.6 ± 12.7
BMI, (kg/m²)	25.5 ± 3.5
Body fat, (%)	18.9 ± 8.1
Systolic BP, (mmHg)	134 ± 8
Diastolic BP, (mmHg)	74 ± 11
VO2max, (ml/kg/min)	42 ± 6
70% VO2max, (W)	165 ± 31
40% VO2max, (W)	78 ± 23

[1] Values are means ± SD

4.2.2. Experimental Trials

Participants reported to the laboratory on three occasions, each separated by a minimum of 7 days. In addition to the pre-trial familiarisation outlined in general methods. Each participant was also required to standardise their diet and physical activity before each trial (see section 3.7. Pre-trial standardisation). Upon arrival at the

laboratory, participants were asked to completely empty their bladder before pre-trial body mass was obtained. Following this, participants rested for 15 min in a semi-supine position whilst a cannula was inserted into the antecubital vein to enable venous blood collection. A baseline (0-min), blood sample was obtained, and a VAS appetite questionnaire was completed using the procedure outlines in general methods.

Participants then completed a 60 min period of rest (CONTROL) or cycle exercise at 40% (LOW) or 70% (HIGH) $\dot{V}O_{2peak}$. HR and RPE was recorded at 0, 15, 30, 45 and 60 min and expired air was collected and analysed for $\dot{V}O_2$ every 15 min during the exercise or rest 15, 30, 45 and 60 min. During the first 60-min of the experimental trials, participants had *ad libitum* access to water. The volume of water consumed was measured by weighing the drink bottles pre and post-exercise (Adam Equipment Co Ltd., PGL 303, Milton Keynes, UK). After completion of the exercise bout, a further blood sample was collected and a VAS was completed (60 min; post-exercise), before participants were given 30 min to shower and change their clothes if necessary.

Following collection of a further blood sample and completion of a VAS pre-meal (90 min), participants were fed a standardised meal consisting of ~400g of chicken and sweetcorn soup (see section 3.6. Meal Details). Whereby participants were given a maximum of 15 min to consume the standardised meal and were given verbal instructions to consume it as quickly as they were able. The time taken to eat this meal was recorded. Participants remained in a semi-supine position throughout the remaining 2 h sampling procedure. Additional blood samples were obtained every 30 min (135, 165, 195 and 225 min). GER and appetite were assessed during the trial as described in general methods every 15 min post-meal ingestion (120, 135, 150, 165, 180, 195, 210 and 225 min). Following all sample collections, the cannula was removed and participants were free to leave the laboratory. A schematic diagram of the experimental protocol is presented in (Figure 2)

4.2.3. Exercise Intensity

Exercise intensity was calculated independently for each participant within this Chapter. Using the results from the peak oxygen uptake test which participants completed during their familiarisation visit (see section 3.4.1). This information

allowed $\dot{V}O_2$ (ml/kg/min) to be calculated by multiplying $\dot{V}O_2$ (l/min) by one thousand and dividing by body mass (Kg). $\dot{V}O_2$ (ml/kg/min) was then plotted on the Y axis against exercise intensity (W) on the X axis. Workload was then calculated using the liner trend line equation to evoke a work rate equivalent to 40% $\dot{V}O_{2Peak}$ (Low intensity) and 70% $\dot{V}O_{2Peak}$ (High intensity).

4.2.4. Biochemical Analysis

Blood sample analysis is described in general methods. Gut hormone analysis was performed on 168 duplicate samples. Corresponding intra-assay CVs for active ghrelin, GLP-1, insulin, PP and PYY were 14%, 18%, 17%, 14% and 13%, respectively. Inter-assay CVs were determined from concentrations across four different assays for active ghrelin, GLP-1, insulin, PP and PYY were 24%, 26%, 19.5%, 10.75 and 16%, respectively.

Metabolite analysis was performed on 168 duplicate samples. Corresponding intra-assay CVs for glucose, triglycerides, cholesterol and NEFA were 1%, 1%, 0.9% and 4.2%, respectively. Inter-assay CVs were determined from concentrations across three verification runs for glucose, triglycerides, cholesterol and NEFA were 3.4%, 1.9%, 0.9% and 7.3%, respectively.

4.2.5. Statistical Analysis

All data are presented as mean ± standard deviation (SD) unless otherwise stated. Data was tested for normality of distribution using the Shapiro-Wilk test. Differences in standardisation measurements, Pre-EI, *ad libitum* fluid intake, gastric emptying T_{lag}, $T_{1/2}$, and all AUC results were analysed using one-way repeated measures analysis of variance (ANOVA). Two-way repeated ANOVA were used to examine differences in HR, gastric emptying DOB values, gut hormones concentrations, metabolites concentrations and appetite VAS scores. Sphericity for repeated measures was assessed and Greenhouse-Geisser epsilon < 0.75 and the Huynh-Feldt correction adopted for less severe differences to correct for violations. Significant main effects were followed by paired students' t-Test or one-way repeated ANOVA with Bonferroni adjusted pairwise comparisons as appropriate. Effect size was documented as partial eta squared (η^2_p) or Cohen's (d) and 95% confidence intervals (CI) (see section 3.19.2. Post-Statistics Effect Size).

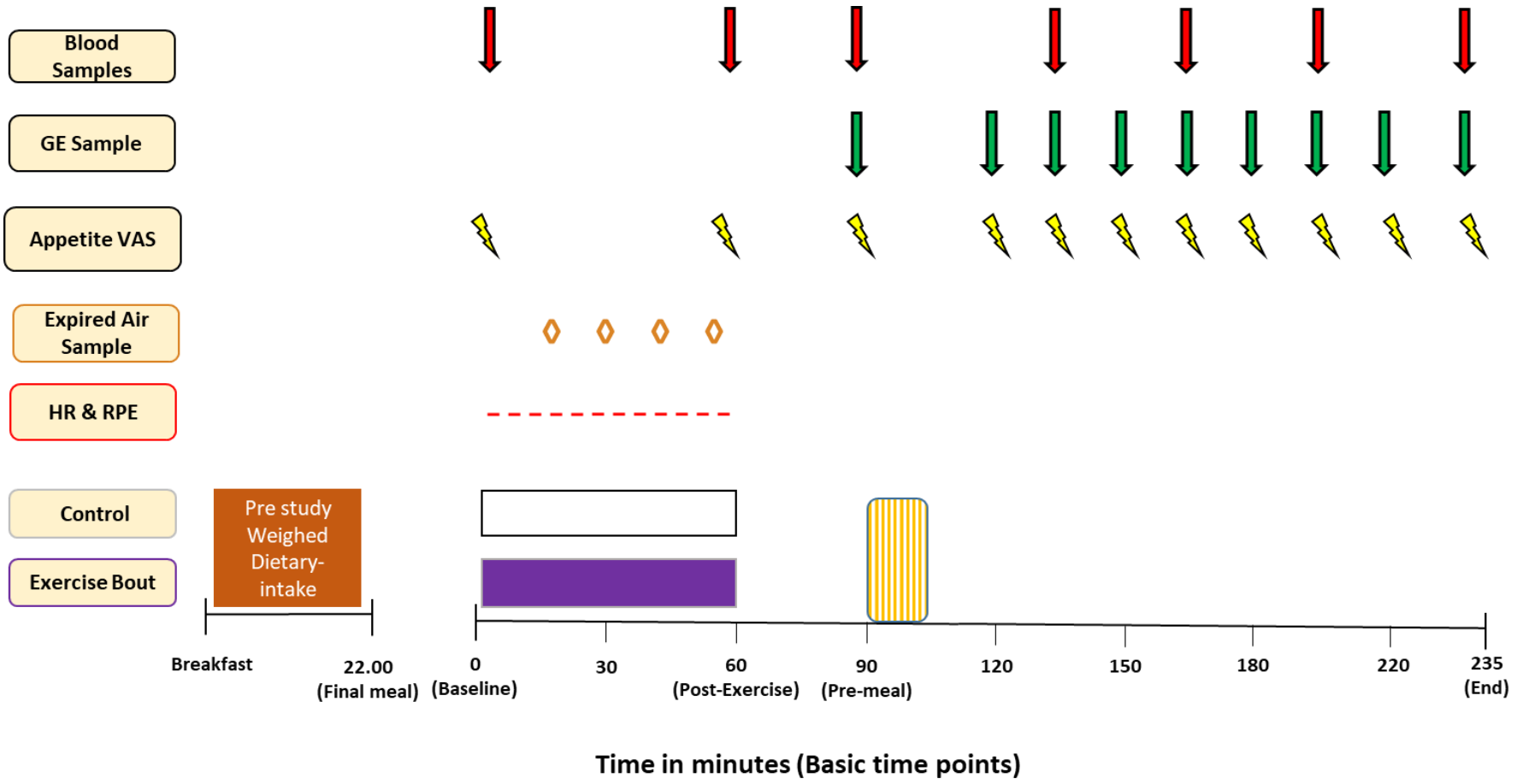

Figure 2: Schematic diagram of the experimental trial protocol S-1. Yellow lined rectangle represents 15 min standardised semi-solid meal period. HR, heart rate. RPE, rating of perceived exertion. GE, gastric emptying. VAS, visual analogy scale. Control, (CON). Exercise Bout, (LOW, HIGH).

4.3 Results

4.3.1 Standardisation Measurements

Pre-trial body mass was not significantly different between trials (P = 0.621, η^2_p = 0.042). In addition, environmental temperature during exercise (P = 0.182, η^2_p = 0.143) and recovery (P = 0.460, η^2_p = 0.068) was similar between trials. VO_2 was significantly different between trials (P < 0.001, η^2_p = 0.968). Therefore, this resulted in the percent VO_2 participants worked at also being significantly different (P < 0.001, η^2_p =0.989). Water consumption during the 1 h exercise was significantly greater during the HIGH than CON trial (P = 0.008, d = 1.14, 95% CI=-148.80 - 162.39 g). The weight of the soup consumed was not significantly different between trials (P = 0.150, η^2_p = 0.158). Differences in time to eat the soup between trials approached significance (P = 0.063, η^2_p = 0.058) (Table ***8***)

Table 8: S1, Standardisation Measurements During Laboratory Visit

	CON	LOW	High	*P=Value*
Pre-trial measurements				
Body Mass (KG)	80.4 ± 12.6	80.6 ± 12.7	80.7 ±12.5	0.621
Exercise measurements				
V02 (ml/kg/min)	4 ± 1	17 ± 3	30 ± 5	<0.001
V02 (%)	9 ± 2	41 ± 4	70 ± 4	<0.001
Environment				
Temperature during exercise (°C)	20.5 ± 0.4	20.2 ± 0.3	20.3 ± 0.5	0.182
Temperature during recovery (°C)	23.4 ± 1.2	23.7 ± 1.2	23.1 ± 1	0.460
Semi-solid meal				
Weight of soup consumed (g)	397 ± 3	395 ± 2	396 ± 2	0.150
Time to eat soup (S)	307 ± 113	303 ± 82	327 ± 73	0.063
Fluid Consumption (mL)	317 ± 285	476 ± 303	617 ± 264	0.008

Data are means ± SD. Values are significant *P*<0.005

4.3.2. Pre-Trial Diet Analysis

Pre-trial energy intake was not different between trials (CON, 2363 ± 720 Kcal; HIGH, 2399 ± 666 Kcal; LOW, 2384 ± 706 Kcal; *P* = 0.968, η^2_p = *0.003*) (Figure 3). This led to a similar proportion of energy from carbohydrates (P = 0.670,

$\eta^2_p = 0.105$), fats (P = 0.339, $\eta^2_p = 0.093$), protein (P = 0.294, $\eta^2_p = 0.030$) and fibre (P = 0.875, $\eta^2_p = 0.019$) being consumed. In addition, fluid consumption before was also not significantly different between trials ($P = 0.196$, $\eta^2_p = 0.088$, Table 9).

Table 9: Pre-Trial Macronutrient Content (KCal) from total Energy Intake

	CON	LOW	HIGH	P =Value
Carbohydrate	1051 ± 424	1095 ± 397	996 ± 379	0.670
Protein	447 ± 204	461 ± 198	494 ± 228	0.294
Fat	823 ± 313	787 ± 301	867 ± 338	0.339
Fibre	42 ± 23	41 ±20	41 ± 27	0.875
Water (g)	2042 ± 288	2170 ± 954	2176 ± 1046	0.196

Data are means ± SD. Values are significant at ($P < 0.005$)

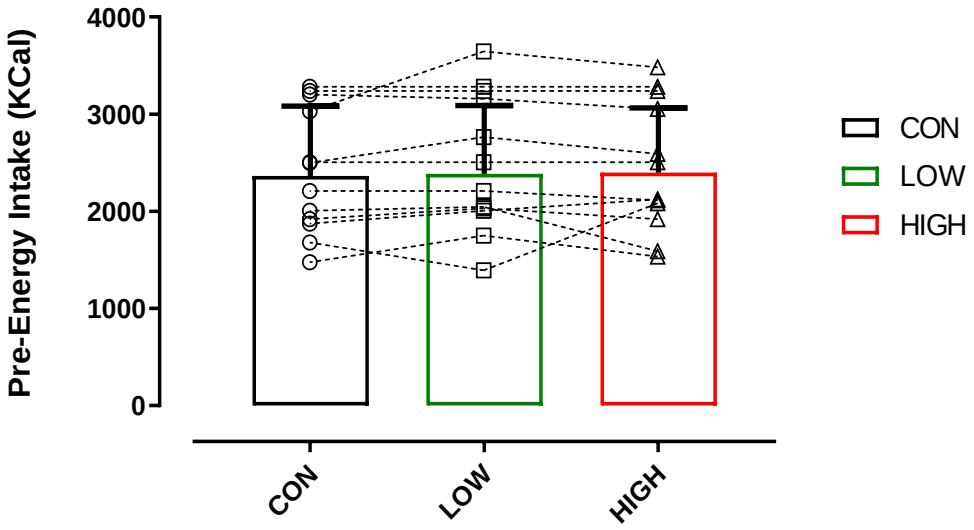

Figure 3: Representative of mean for pre-trial energy intake (KCal), with vertical error bars representing SDs (n = 12). Individual data is documented as (○) CON, (□) Low and (Δ) HIGH. There was no main effect observed (p > 0.05) examined by a one-way repeated-measured ANOVA.

4.3.3. Heart Rate (HR) and RPE

Two-factor ANOVA demonstrated main effects of time ($p < 0.001$, $\eta^2_p = 0.951$), trial ($p < 0.001$, $\eta^2_p = 0.914$) and interaction effects for HR ($p < 0.001$, $\eta^2_p = 0.887$) (Figure 4A). Post hoc tests revealed. RPE was not significantly different at baseline ($P = 0.331$, $\eta^2_p = 0.096$); HIGH (6 ± 0 bpm), Low (7 ± 1 bpm) and CON (6 ± 0 bpm). 15 min ($P < 0.001$, $\eta^2_p = 0.874$); HIGH (13 ± 1 bpm), Low (9 ± 2 bpm) and CON (6 ± 0 bpm). 30 min ($P < 0.001$, $\eta^2_p = 0.906$); HIGH (15 ± 2 bpm), Low (10 ± 2 bpm) and CON (6 ± 0 bpm). 45 min ($P < 0.001$, $\eta^2_p = 0.919$); HIGH (16 ± 2 bpm), Low (11 ± 2 bpm) and CON (6± 0 bpm). 60 min ($P < 0.001$, $\eta^2_p = 0.931$); HIGH (17 ± 2 bpm), Low (11 ± 2 bpm) and CON (6 ± 0 bpm).

Two-factor ANOVA demonstrated main effects of time ($p < 0.001$, $\eta^2_p = 0.924$), trial ($p < 0.001$, $\eta^2_p = 0.955$) and interaction effects for HR ($p < 0.001$, $\eta^2_p = 0.923$) (Figure 4B). Post hoc tests revealed. HR was not significantly different at baseline ($P = 0.876$, $\eta^2_p = 0.019$); HIGH (63 ± 9 bpm), Low (64 ± 7 bpm) and CON (66 ± 8 bpm). 15 min ($P < 0.001$, $\eta^2_p = 0.949$); HIGH (149 ± 19 bpm), Low (113 ± 14 bpm) and CON (69 ± 10 bpm). 30 min ($P < 0.001$, $\eta^2_p = 0.951$); HIGH (156 ± 19 bpm), Low (113 ± 15 bpm) and CON (65 ± 8 bpm). 45 min ($P < 0.001$, $\eta^2_p = 0.954$); HIGH (158 ± 19 bpm), Low (112 ± 15 bpm) and CON (66 ± 8 bpm). 60 min ($P < 0.001$, $\eta^2_p = 0.942$); HIGH (160 ± 18 bpm), Low (114 ± 17 bpm) and CON (69 ± 11 bpm).

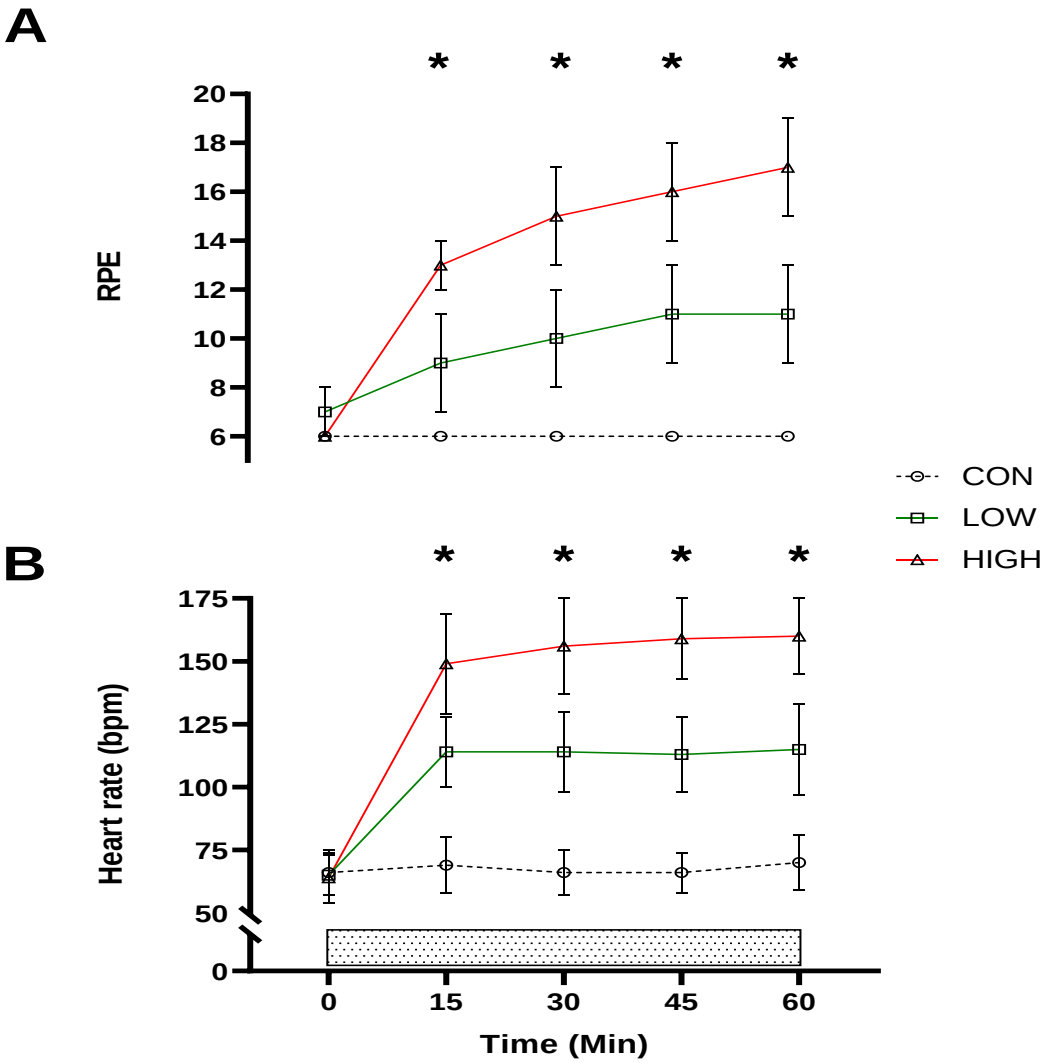

Figure 4: Representative of mean RPE (A) and HR (bpm) (B), with vertical error bars display SDs (n = 12). *Unfilled rectangle* with black spots indicates 60-min exercise period. There was an effect of time, trial and interaction found for RPE and HR (P < 0.001), examined by 2-way repeated-measures ANOVA * Indicating that trials were significantly different from each other. RPE; rate of perceved exertion, bpm; beats per minute, HR; heart rate.

4.3.4 Gastric Emptying

The time taken to empty half of the soup from the stomach (T_{half}) amounted to 89 ± 13 min, 82 ± 8 min and 94 ± 31 min on CON, LOW and HIGH, respectively (*P* = 0.247, η^2_p = *0.121*). The time of maximal emptying rate (T_{lag}) amounted to 63 ± 13 min, 56 ± 10 min and 60 ± 16 min for CON, LOW and HIGH, respectively (P = 0.235, η^2_p = *0.125*) (Figure 5). No statistical difference in mean DOB-AUC over 120 min post meal ingestion was demonstrated (*P* = 0.848, η^2_p=*0.015*) (Figure 7A) nor

cumulative dose of percent $^{13}CO_2$, as final values at 120 min were similar (CON, 24 ± 3 $^{13}CO_2$ %; HIGH, 24 ± 4 $^{13}CO_2$ %; LOW, 24 ± 4 $^{13}CO_2$ %; *P* = 0.134, η^2_p = *0.222*) (Figure 6)

Two factor ANOVA demonstrated no main effect of trial (P = 0.853, η^2_p = *0.014*), a main effect of time (P < 0.001, η^2_p = *0.929*) and no interaction effect (P = 0.162, η^2_p = *0.135*) for DOB values (Figure 7B). DOB values were elevated (P < 0.05) from pre-meal values from 15 minutes after soup ingestion until the end of each trial.

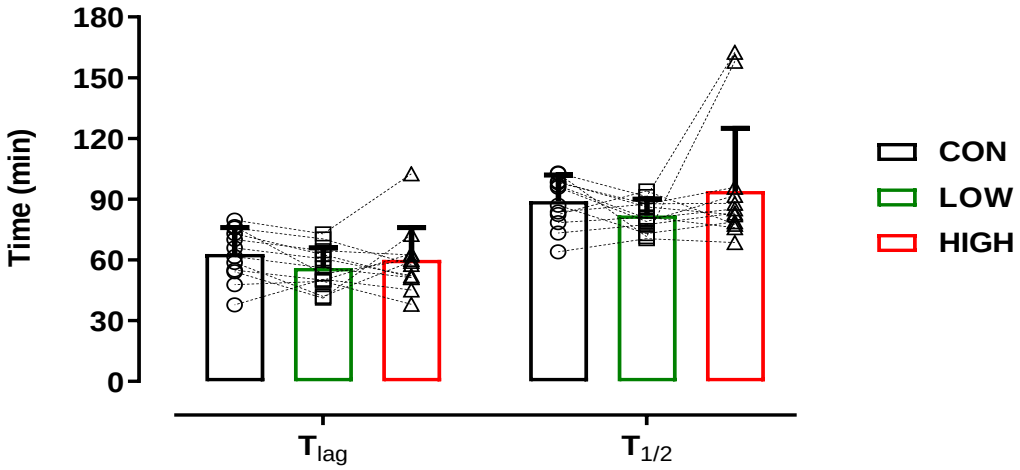

Figure 5: T_{lag} and $T_{1/2}$ are represented as mean, with vertical error bars display SDs (n = 12). Individual data is represented as (○) CON, (□) Low and (Δ) HIGH. There were no main effects observed for T_{lag} and $T_{1/2}$ (p < 0.05) examined by a one-way repeated-measured ANOVA. T_{lag}, Time of maximal emptying rate; $T_{1/2}$, Half emptying time.

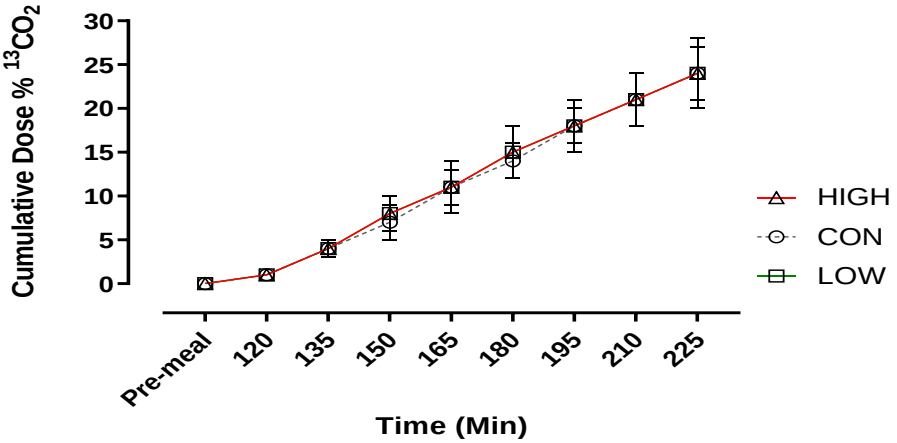

Figure 6: Representative of mean for cumulative dose of % $^{13}CO_2$, with vertical error bars display SDs (n = 12). There were no main effect of time (P < 0.001) however no trial and interaction effect were found for dose % $^{13}CO^2$ (P > 0.05), examined by 2-way repeated-measures ANOVA.

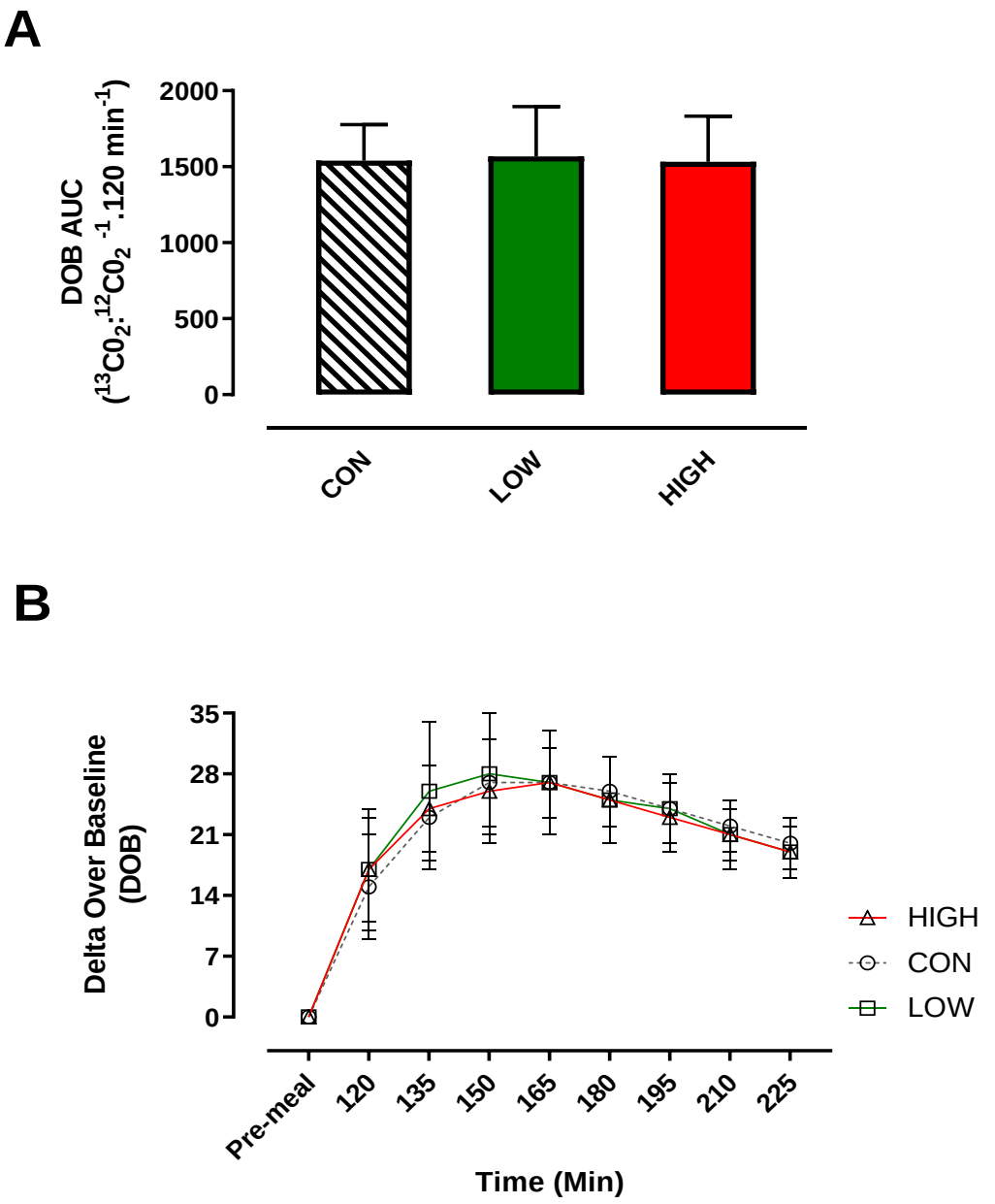

Figure 7: Representative of mean DOB AUC (0-120 min^{-1}) (A) and DOB respose (B), with vertical error bars display SDs (n = 12). There were no main effects for DOB AUC ($P > 0.05$), examined by repeated-measures ANOVA. There was a main effect of time ($P < 0.001$), however no trial, nor interaction effect were found for DOB ($P > 0.05$), examined by 2-way repeated-measures ANOVA. DOB, delta over baseline; AUC, Area under curve.

4.3.5 Appetite

A repeated measures ANOVA demonstrated no main effects for AUC; hunger (*P* = 0.453, η^2_p = *0.060;* Figure 8A), PFC (*P* = 0.070, η^2_p = *0.221;* Figure 10A), Satisfaction (*P* = 0.431, η^2_p = *0.067;* Figure 11A) and Bloat (*P* = 0.199, η^2_p = *0.136;* Figure 12A). However, there was a main effect for Fullness (*P* = 0.041, η^2_p = *0.253;* Figure 9*). A p*ost hoc test revealed LOW was significantly larger than HIGH (3150 ± 2091 vs 2555 ± 1828 mm 225 min^{-1}; P = 0.023, d = 0.32, 95% CI = -1182 - 1034 mm 225 min^{-1}). Furthermore, there was also a main effect for nausea (*P* = 0.023, η^2_p = *0.289;* Figure 13) *A p*ost hoc test revealed HIGH was significantly larger than CON (1939 ± 2359 vs 1106 ± 1575 mm 225 min^{-1}; P = 0.020, d = 0.43, 95% CI = -1333 - 898 mm 225 min^{-1}).

Two-factor ANOVA demonstrated main effects of time for hunger (p < 0.001, η^2_p = *0.507*: Figure 8B), fullness (p < 0.001, η^2_p = *0.509;* Figure 9B), PFC (p = 0.008, η^2_p = *0.343:* Figure 10B), and satisfaction (p < 0.001, , η^2_p = *0.124;* Figure 11B), but not for nausea (p = 0.074, η^2_p = *0.201;* Figure 13B) and bloating (p = 0.218, η^2_p = *0.478:* Figure 12B). Hunger and PFC decreased, whilst fullness and satisfaction increased after eating the semi solid meal. There were no trial or interaction effects for hunger (p = 0.339, η^2_p = *0.098*; p = 0.190, η^2_p = *0.123*), PFC (p = 0.058, η^2_p = *0.240*; p = 0.087, η^2_p = *0.155*), satisfaction (p = 0.248, η^2_p = *0.102*; p = 0.650, η^2_p = *0.074*), nausea (p = 0.104, η^2_p = *0.637*; p = 0.637, η^2_p = *0.054*) or bloating (p = 0.302, η^2_p = *0.120*; p = 0.456, η^2_p = *0.058*). There was no interaction effect for fullness (p = 0.456, η^2_p = *0.079*), but there was a main effect of trial (p = 0.025, η^2_p = *0.287*); however, post hoc tests revealed no between-trial differences.

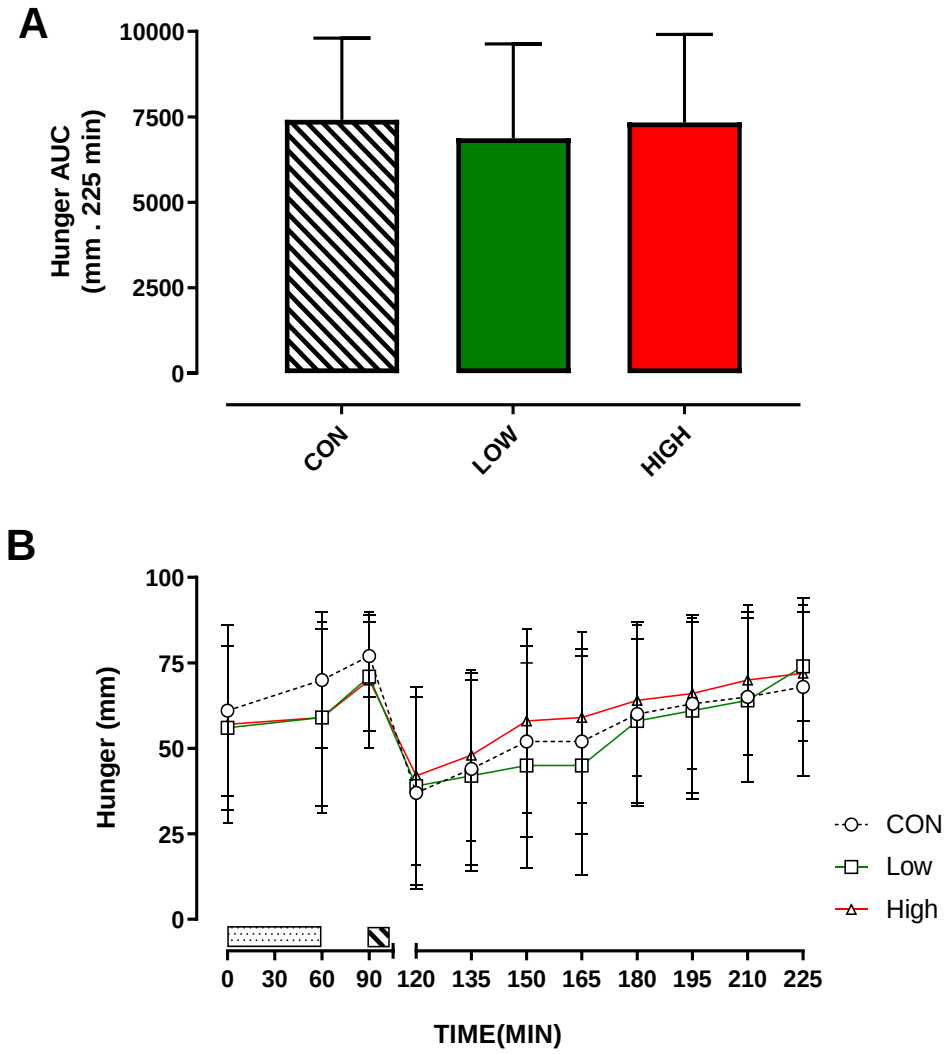

Figure 8: Representative of mean hunger AUC (0-225 min) (A) and hunger VAS response (B), with vertical error bars display SDs (n = 12). *Unfilled rectangle* with black spots indicates 60-min exercise period; *Hashed rectangle* indicates ingestion of a semi-solid meal. There were no main effects for hunger AUC ($P > 0.05$), examined by repeated-measures ANOVA. A main effect of time ($P < 0.001$), However no trial, nor interaction effect werre found for hunger ($P > 0.05$), examined by 2-way repeated-measures ANOVA. AUC, area under curve. VAS, visual analogue scale.

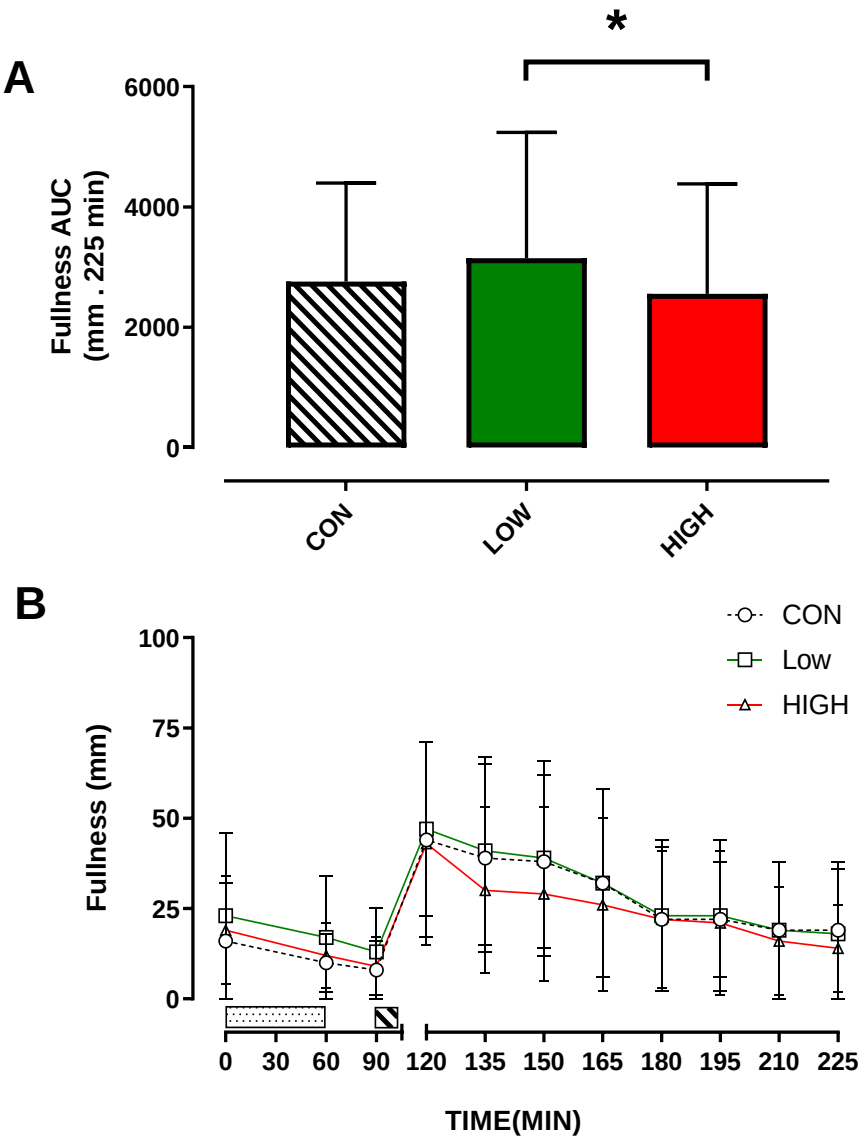

Figure 9: Representative of mean fullness AUC (0-225 min) (A) and fullness VAS response (B), with vertical error bars display SDs (n = 12). *Unfilled rectangle* with black spots indicates 60-min exercise period; *Hashed rectangle* indicates ingestion of a semi-solid meal. There was a main effect for fullness AUC ($P < 0.05$), examined by repeated-measures ANOVA. A main effect of time ($P < 0.001$). However, no trial, nor interaction effect were found for fullness ($P > 0.05$), examined by 2-way repeated-measures ANOVA. * Indicates LOW was significantly different than HIGH. Determined by Bonferroni adjusted paired t test ($P = 0.023$). AUC, area under curve. VAS, visual analogue scale.

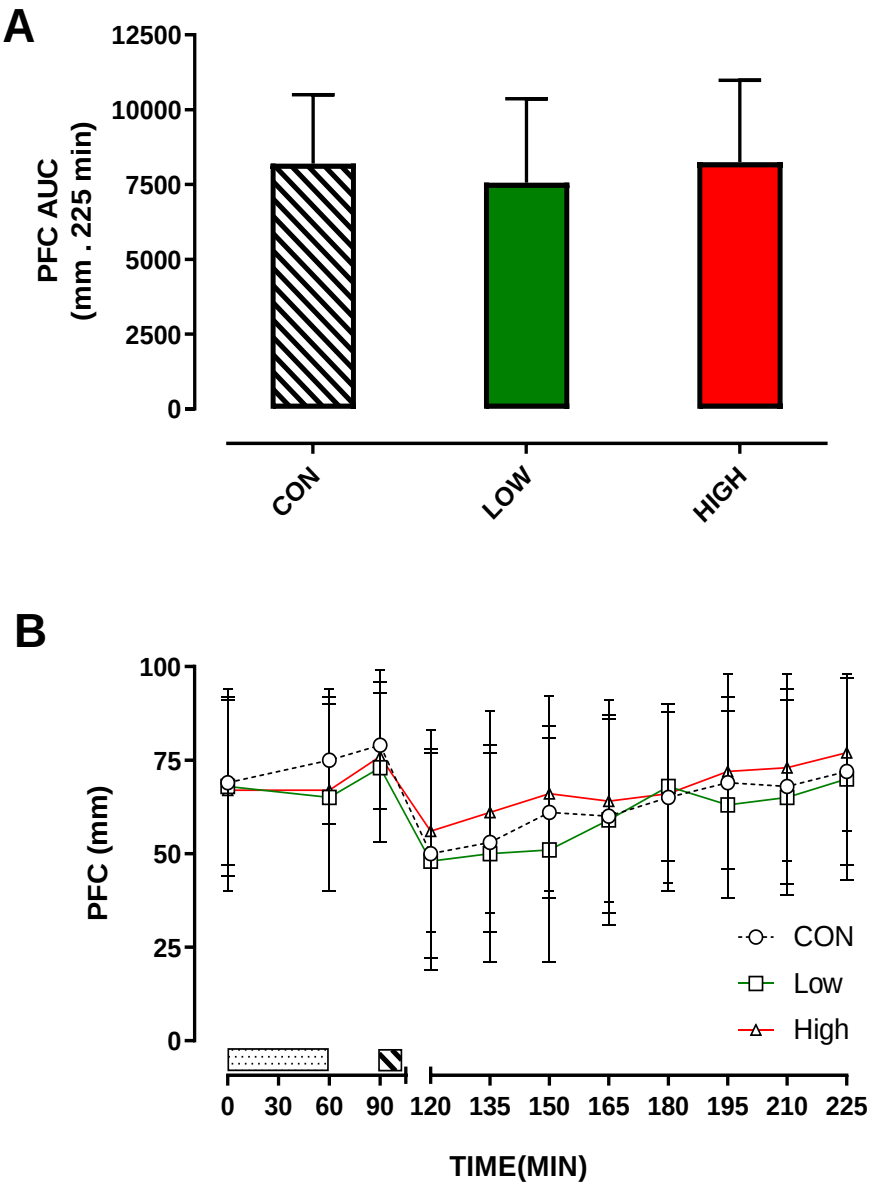

Figure *10*: Representative of mean PFC AUC (0-225 min) (A) and PFC VAS response (B), with vertical error bars display SDs (n = 12). *Unfilled rectangle* with black spots indicates 60-min exercise period; *Hashed rectangle* indicates ingestion of a semi-solid meal. There was no main effect for PFC AUC ($P > 0.05$), examined by repeated-measures ANOVA. A main effect of time ($P < 0.001$). However, no trial nor interaction effect was found for PFC ($P > 0.05$), examined by 2-way repeated-measures ANOVA. AUC, area under curve. VAS, visual analogue scale.PFC, prospective food consumption.

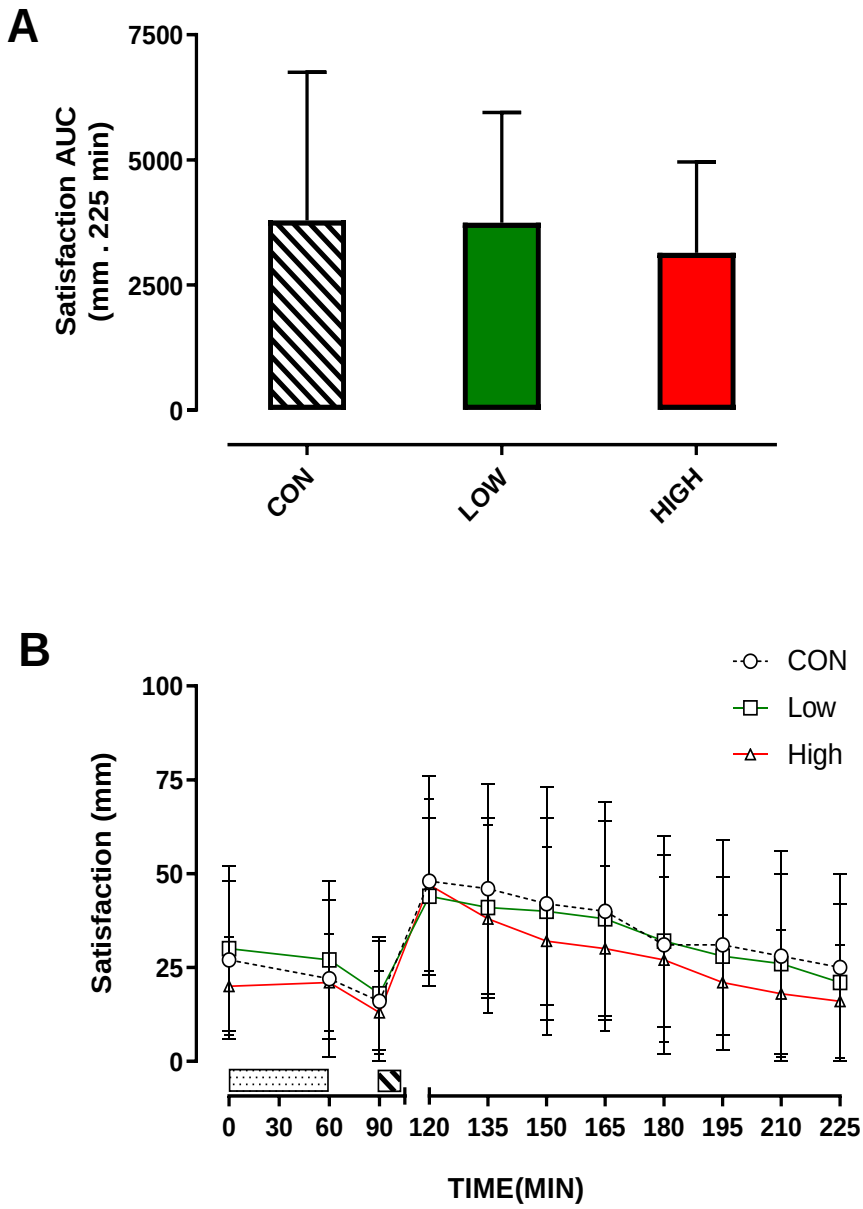

Figure 11: Representative of mean satisfaction AUC (0-225 min) (A) and satisfaction VAS response (B), with vertical error bars display SDs (n = 12). *Unfilled rectangle* with black spots indicates 60-min exercise period; *Hashed rectangle* indicates ingestion of a semi-solid meal. There were no main effect for satisfaction AUC ($P > 0.05$), examined by repeated-measures ANOVA. A main effect of time ($P < 0.001$). However, no trial nor interaction effect was found for satisfaction ($P > 0.05$), examined by 2-way repeated-measures ANOVA. AUC, area under curve. VAS, visual analogue scale.

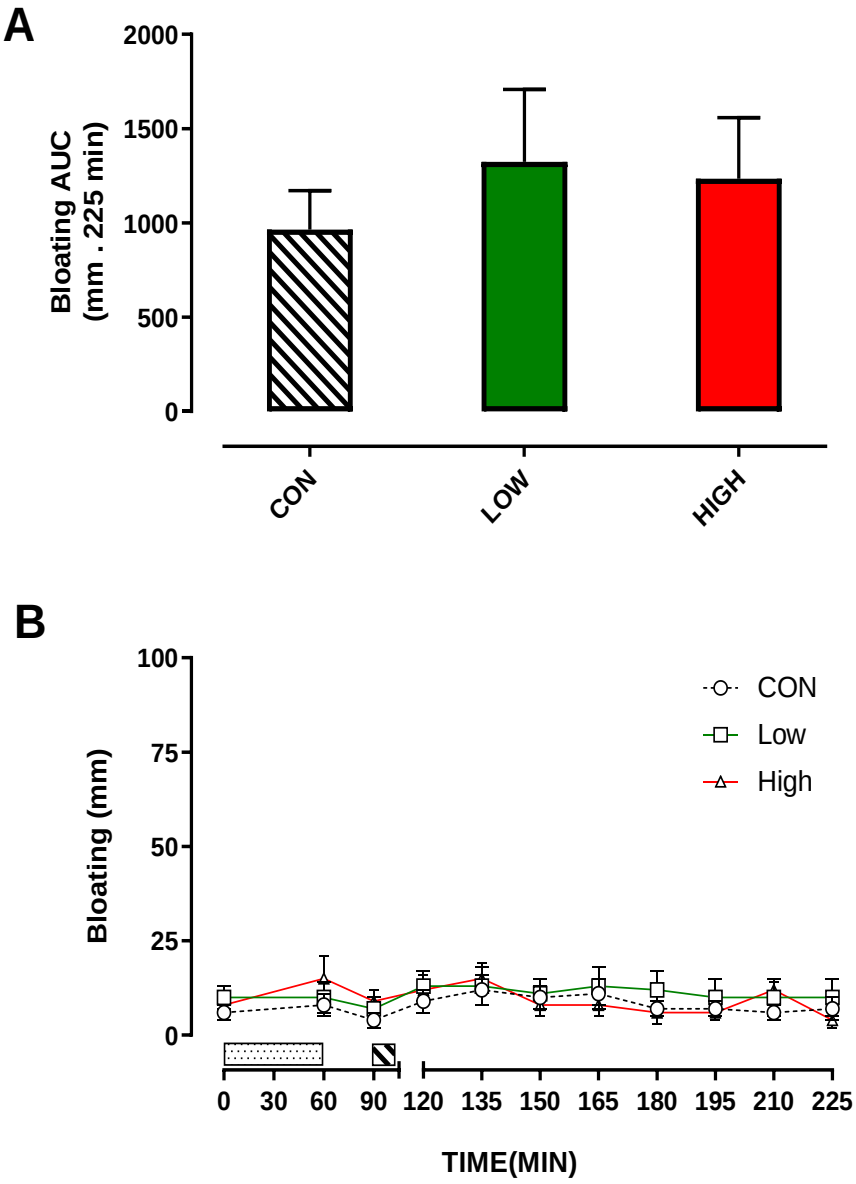

Figure 12: Representative of mean bloat AUC (o-225 min) (A) and bloat VAS response (B), with vertical error bars display SDs (n = 12). *Unfilled rectangle* with black spots indicates 60-min exercise period; *Hashed rectangle* indicates ingestion of a semi-solid meal. There was no main effect for bloat AUC ($P > 0.05$), examined by repeated-measures ANOVA. A main effect of time ($P < 0.001$). However, no trial nor interaction effect was found for bloat ($P > 0.05$), examined by 2-way repeated-measures ANOVA. AUC, area under curve. VAS, visual analogue scale.

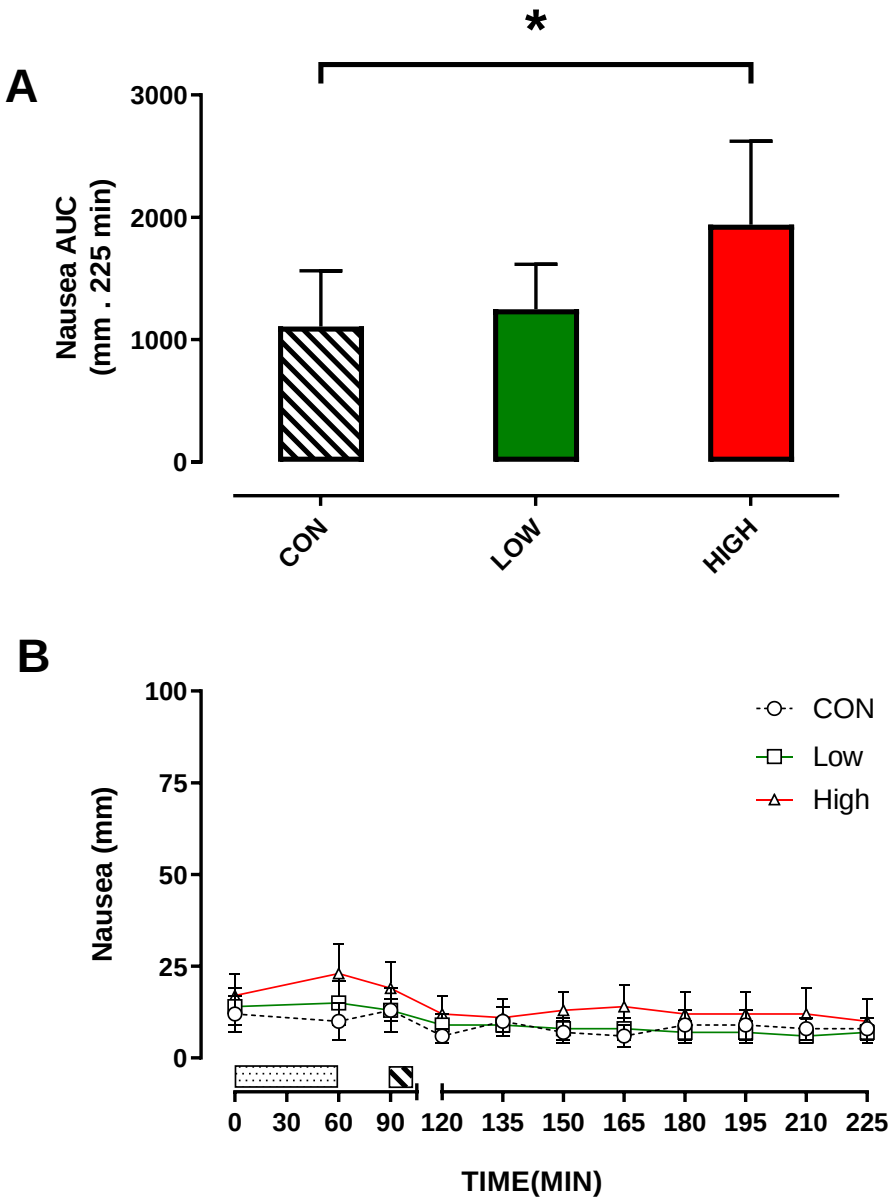

Figure 13: Representative of mean nausea AUC (0-225 min) (A) and nausea VAS response (B), with vertical error bars display SDs (n = 12). *Unfilled rectangle* with black spots indicates 60-min exercise period; *Hashed rectangle* indicates ingestion of a semi-solid meal. There was a main effect for nausea AUC ($P < 0.05$), examined by repeated-measures ANOVA. A main effect of time ($P < 0.001$). However, no trial nor interaction effect was found for nausea ($P > 0.05$), examined by 2-way repeated-measures ANOVA. * Indicates CON was significantly different than HIGH. Determined by Bonferroni adjusted paired t test ($P = 0.020$) AUC, area under curve. VAS, visual analogue scale.

4.3.6. Blood Metabolites

4.3.6.1. Glucose

Glucose responded differently immediately post-exercise as HIGH increased from baseline (5.2 ± 0.4 vs 5.4 ± 0.1 mmol.L) and LOW (5.1 ± 0.3 vs 5.0 ± 0.4 mmol.L) and CON (5.1 ± 0.4 vs 4.9 ± 0.3 mmol.L) decreased from baseline. Glucose increased in all three trials after lunch; HIGH (7 ± 1 mmol.L), LOW (6.9 ± 0.8 mmol.L) and CON (6.8 ± 1 mmol.L). However, these changes in glucose did not lead to differences in the 2h recover period post-lunch. A two-way repeated ANOVA showed an effect of time ($P < 0.001$, $\eta^2_p = 0.727$), but not for trial ($P = 0.426$, $\eta^2_p = 0.106$) nor interaction effect ($P = 0.215$, $\eta^2_p = 0.188$) (Figure *14*B).

4.3.6.2. Triglycerides

Triglyceride responded differently immediately post-exercise as HIGH (1.42 ± 1.22 vs 1.50 ± 1.01 mmol.L) and CON (1.18 ± 0.70 vs 1.20 ± 0.66 mmol.L) increased from baseline, and LOW decreased (1.24 ± 0.81 vs 1.15 ± 0.61 mmol.L). Although, triglyceride remained elevated within the HIGH trial this did not lead to differences in the 2h recover period post-lunch. A two-way repeated ANOVA showed no effect of time ($P = 0.107$, $\eta^2_p = 0.295$), trial ($P = 0.197$, $\eta^2_p = 0.218$) nor interaction ($P = 0.342$, $\eta^2_p = 0.140$) (Figure *15*B).

4.3.6.3. Cholesterol

Cholesterol increased in all three trials from baseline to immediately post-exercise; HIGH (4.06 ± 0.71 vs 4.41 ± 0.86 mmol.L), LOW (4.08 ± 0.93 vs 4.29 ± 0.89 mmol.L) and CON (4.17 ± 0.79 vs 4.31 ± 0.77 mmol.L). Furthermore, cholesterol then decreased in all three trials pre-meal and further decreased 30 min after food ingestion, where cholesterol remained stable throughout the 2 h recovery period. A two-way repeated ANOVA showed an effect of time ($P < 0.001$, $\eta^2_p = 0.817$), but not for trial ($P = 0.414$, $\eta^2_p = 0.114$) nor interaction effect ($P = 0.093$, $\eta^2_p = 0.251$) (Figure *16*B).

4.3.6.4. NEFA

NEFA increased from baseline to immediately post-exercise in HIGH (0.47 ± 0.15 vs 0.90 ± 0.19 mmol.L) and LOW trials (0.50 ± 0.13 vs 0.68 ± 0.26 mmol.L) but decreased in CON (0.59 ± 0.20 vs 0.54 ± 0.24 mmol.L). Post-meal, NEFA decreased in all three trial. However, HIGH remained elevated over LOW and CON throughout the 2 h recovery period. A two-way repeated ANOVA showed an effect of time ($P < 0.001$, $\eta^2_p = 0.763$), but not for trial ($P = 0.114$, $\eta^2_p = 0.278$) nor interaction effect ($P = 0.103$, $\eta^2_p = 0.232$) (Figure *17*B).

4.3.6.5. Metabolites AUC

A repeated measures ANOVA demonstrated there was a main effect for, NEFA AUC ($P = 0.037$, $\eta^2_p = 0.376$; Figure 17A). A post hoc test revealed HIGH showed significantly more NEFA than CON (68.5 ± 22.9 vs 43 ± 11.5 mmol.L 225 min^{-1}; P = 0.50, d = 1.60, 95% CI = -14.36 - 9.47 mmol.L 225 min^{-1}). However, no main effects for; glucose ($P = 0.316$, $\eta^2_p = 0.152$; Figure 14A), triglyceride ($P = 0.158$, $\eta^2_p = 0.232$; Figure 15A) and cholesterol ($P = 0.553$, $\eta^2_p = 0.081$; Figure 16A).

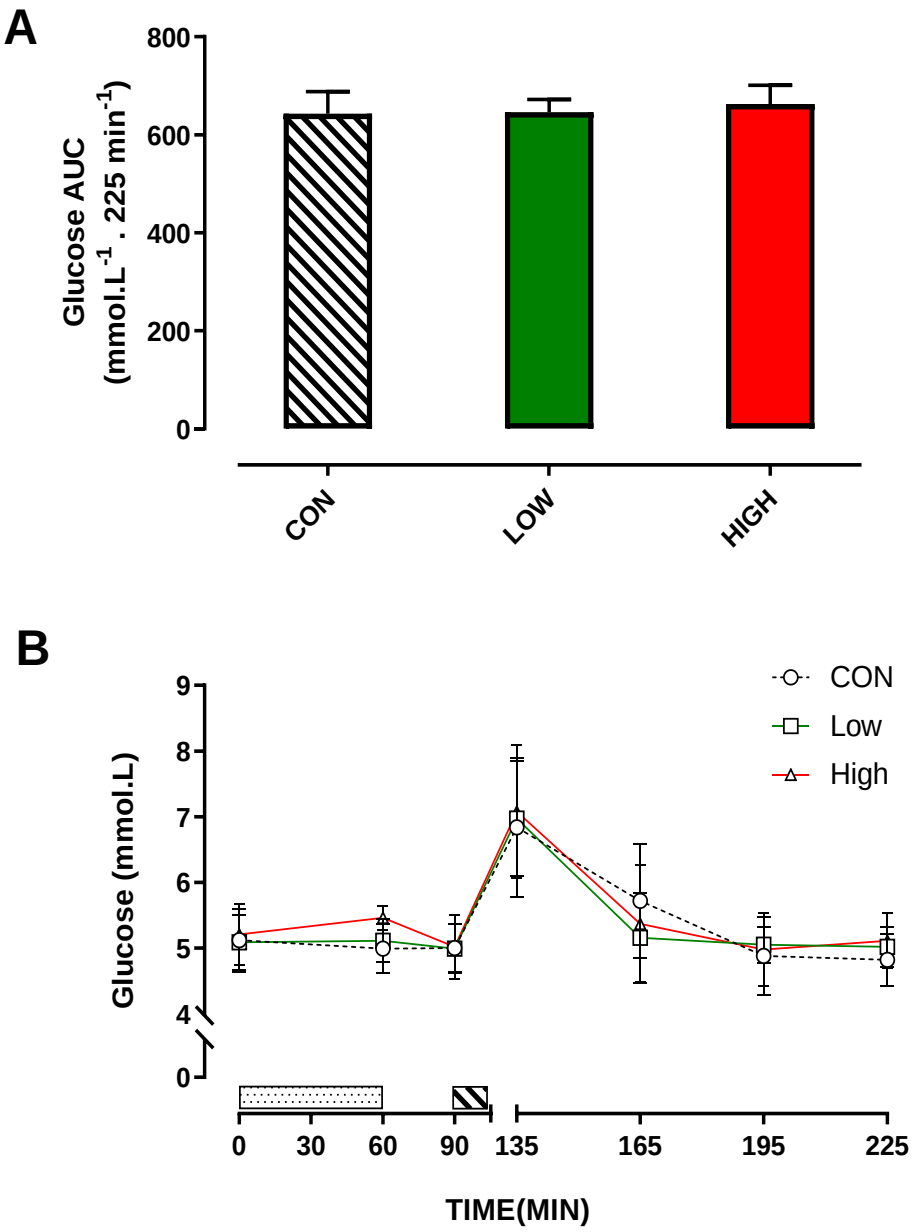

Figure 14: Representative of mean serum concentration of glucose AUC (0-225 min^{-1}) (A) and glucose response (B), with vertical error bars display (n = 8). *Unfilled rectangle* with black spots indicates 60-min exercise period; *Hashed rectangle* indicates ingestion of a semi-solid meal. There was no main effect for glucose AUC ($P > 0.05$), examined by repeated-measures ANOVA. A main effect of time ($P < 0.001$). However, no trial nor interaction effect were found for glucose ($P > 0.05$), examined by 2-way repeated-measures ANOVA. AUC; area under curve.

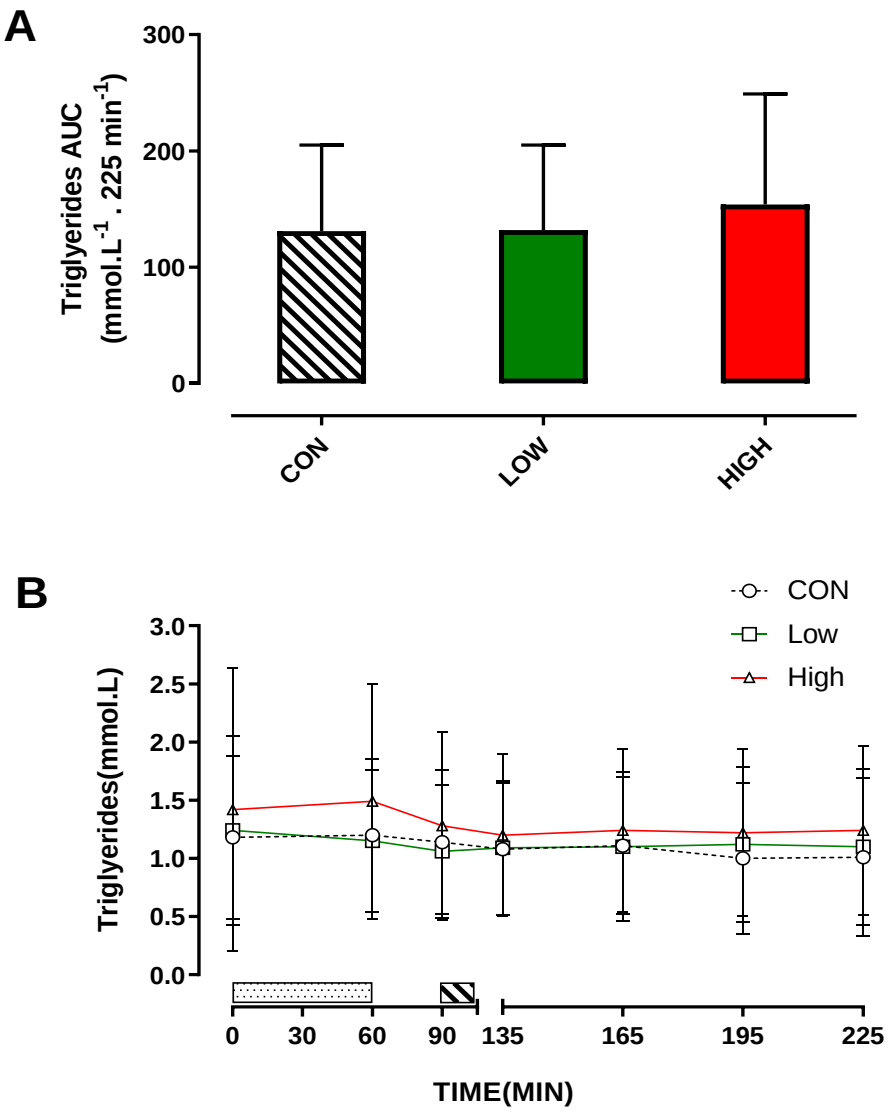

Figure 15: Representative of mean serum concentration of triglycerides AUC (0-225 min^{-1}) (A) and triglycerides response (B), with vertical error bars display (n = 8). *Unfilled rectangle* with black spots indicates 60-min exercise period; *Hashed rectangle* indicates ingestion of a semi-solid meal. There was no main effect for triglycerides AUC ($P > 0.05$), examined by repeated-measures ANOVA. There were no main effect of time, trial nor interaction effect found for triglycerides ($P > 0.05$), examined by 2-way repeated-measures ANOVA. AUC; area under curve.

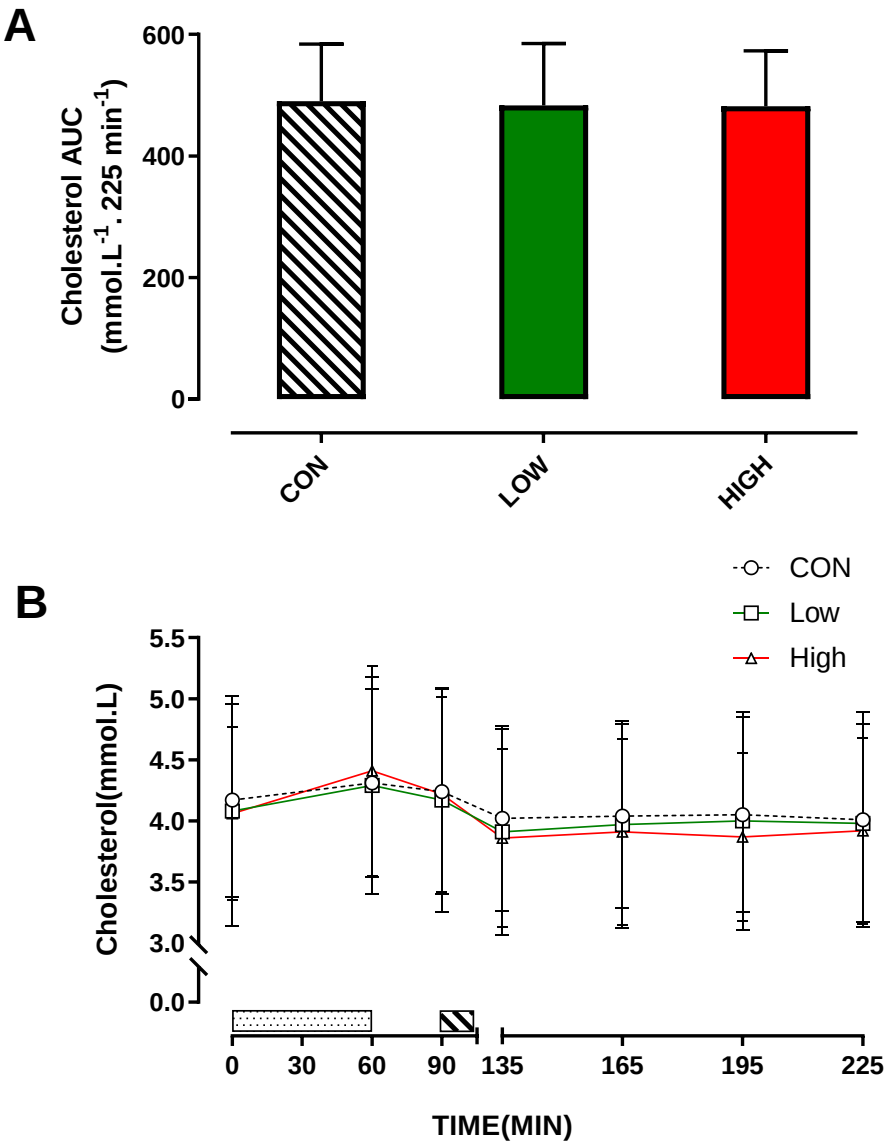

Figure 16: Representative of mean serum concentration of cholesterol AUC (0-225 min^{-1}) (A) and cholesterol response (B), with vertical error bars display (n = 8). *Unfilled rectangle* with black spots indicates 60-min exercise period; *Hashed rectangle* indicates ingestion of a semi-solid meal. There was no main effect for cholesterol AUC ($P > 0.05$), examined by repeated-measures ANOVA. A main effect of time ($P < 0.001$). However, no trial nor interaction effect found for cholesterol ($P > 0.05$), examined by 2-way repeated-measures ANOVA. AUC; area under curve.

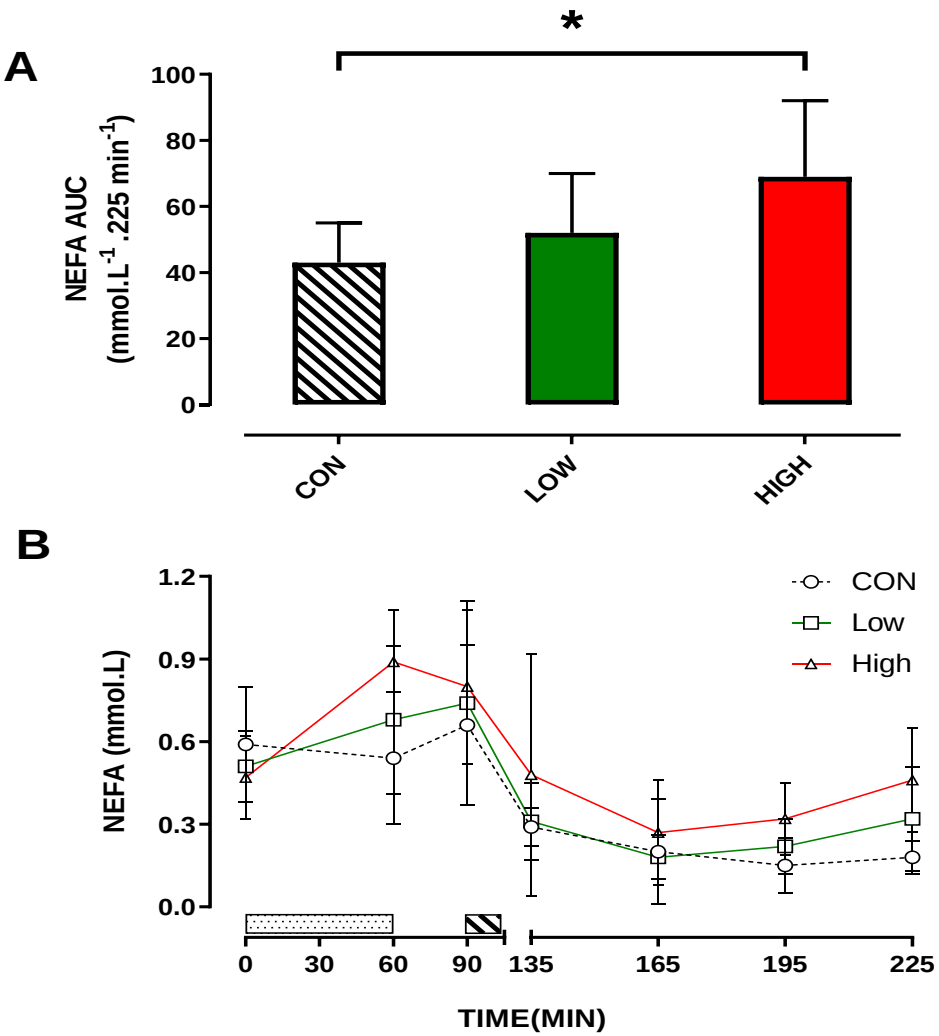

Figure 17: Representative of mean serum concentration of NEFA AUC (0-225 min^{-1}) (A) and NEFA response (B), with vertical error bars display (n = 8). *Unfilled rectangle* with black spots indicates 60-min exercise period; *Hashed rectangle* indicates ingestion of a semi-solid meal. There was a main effect for NEFA AUC ($P < 0.05$), examined by repeated-measures ANOVA. A main effect of time ($P < 0.001$). However, no trial nor interaction effect was found for NEFA ($P > 0.05$), examined by 2-way repeated-measures ANOVA. * Indicates CON was significantly different than HIGH. Determined by Bonferroni adjusted paired t test ($P = 0.037$). AUC; area under curve.

4.3.7 Gut Hormones

4.3.7.1. Ghrelin

Active ghrelin responded differently immediately post-exercise as LOW increased from baseline (222 ± 108 vs 174 ± 52 pg.mL) and HIGH decreased from baseline (100 ± 38 vs 174 ± 63 pg.mL). However, these changes in active ghrelin did not lead to differences in the 2h recovery period post-lunch. A two-way repeated ANOVA showed no effect of trial (P = 0.080, η^2_p = *0.335*), time (P = 0.249, η^2_p = *0.180*) or interaction (P = 0.090, η^2_p = *0.259;*Figure 18B).

4.3.7.2. Glucagon-like Peptide-1 (GLP-1)

GLP-1 responded similarly in all three trials pre-meal. Although, post-meal GLP-1 was elevated in the LOW trial 14.56 ± 12.42 pg.mL at 135 min and 11.44 ± 9.53 pg.mL 165 min, compared to the CON (10.48 ± 10.01; 6.28 ± 6.12 pg.mL) and HIGH (10.56 ± 10.28; 8.30 ± 5.36 pg.mL). However, a two-way repeated ANOVA showed an effect of time (P = 0.018, η^2_p = *0.390*), but not for trial (P = 0.629, η^2_p = *0.060*) nor interaction effect (P = 0.392, η^2_p = *0.130*; Figure 19B).

4.3.7.3. Insulin

Insulin responded similarly in all three trials pre-meal. Nevertheless, post-meal insulin did increase higher during the LOW trial (2048 ± 1192 pg.mL) 30 min after consuming food, compared to CON (1647 ± 389 pg.mL) and HIGH (1649 ± 1096 pg.mL). However, a two-way repeated ANOVA showed an effect of time (P < 0.001, η^2_p = *0.829*), but not for trial (P = 0.604, η^2_p = *0.050*) nor interaction effect (P = 0.422, η^2_p = *0.119;*Figure 20B).

4.3.7.4. Pancreatic Polypeptide (PP)

PP increased from baseline in both exercise trials immediately post-exercise; LOW (47.7 ± 30.1 vs 78.8 ± 61.4 pg.mL), HIGH (31.4 ± 30 vs 94.4 ± 93.8 pg.mL) and decreased in the CON (53.5 ± 31.3 vs 51.3 ± 30.8 pg.mL). Although, pre-meal, HIGH continued to be elevated, before all three trials increased post-meal ingestion. Nevertheless, HIGH remained elevated over LOW and CON at 30 and 60 min post-meal. A two-way repeated ANOVA showed an effect of time (P < 0.001, η^2_p = *0.619*),

but not for trial (P = 0.377, η^2_p = *0.116*) nor interaction (P = 0.153, η^2_p = *0.210*; Figure 21B).

4.3.7.5. Peptide YY (PYY)

PYY responded similarly, both immediately post-exercise and pre-meal. However, there was a marginal small increase in PYY 30 min after consuming a meal in all three trials. This slight elevation remained until the end of the 2 h recovery period. A two-way repeated ANOVA showed no effect of time (P = 0.361, η^2_p = *0.126*), trial (P = 0.992, η^2_p = *0.001*) nor interaction (P = 0.422, η^2_p = *0.119:*Figure 22B).

4.3.7.6. Hormone AUC

A repeated measures ANOVA demonstrated there was a main effect for, active ghrelin AUC (*P* = 0.015, η^2_p = *0.451;* Figure *18*A*).* A post hoc test revealed Low specified significantly more circulating active ghrelin than HIGH (3150 ± 2091 vs 2555 ± 1828 pg.mL^{-1}. 225 min^{-1}; P = 0.006, d = 1.60, 95% CI = -3149 - 2099 pg.mL^{-1} . 225 min^{-1}). However, no main effects for; GLP-1 (*P* = 0.414, η^2_p *=0.118;* Figure 19A), insulin (*P* = 0.796, η^2_p = *0.032;* Figure 20A), PP (*P* = 0.320, η^2_p = *0.150;* Figure 21A) or PYY AUC (*P* = 0.917, η^2_p *=0.012;* Figure 22 A*).*

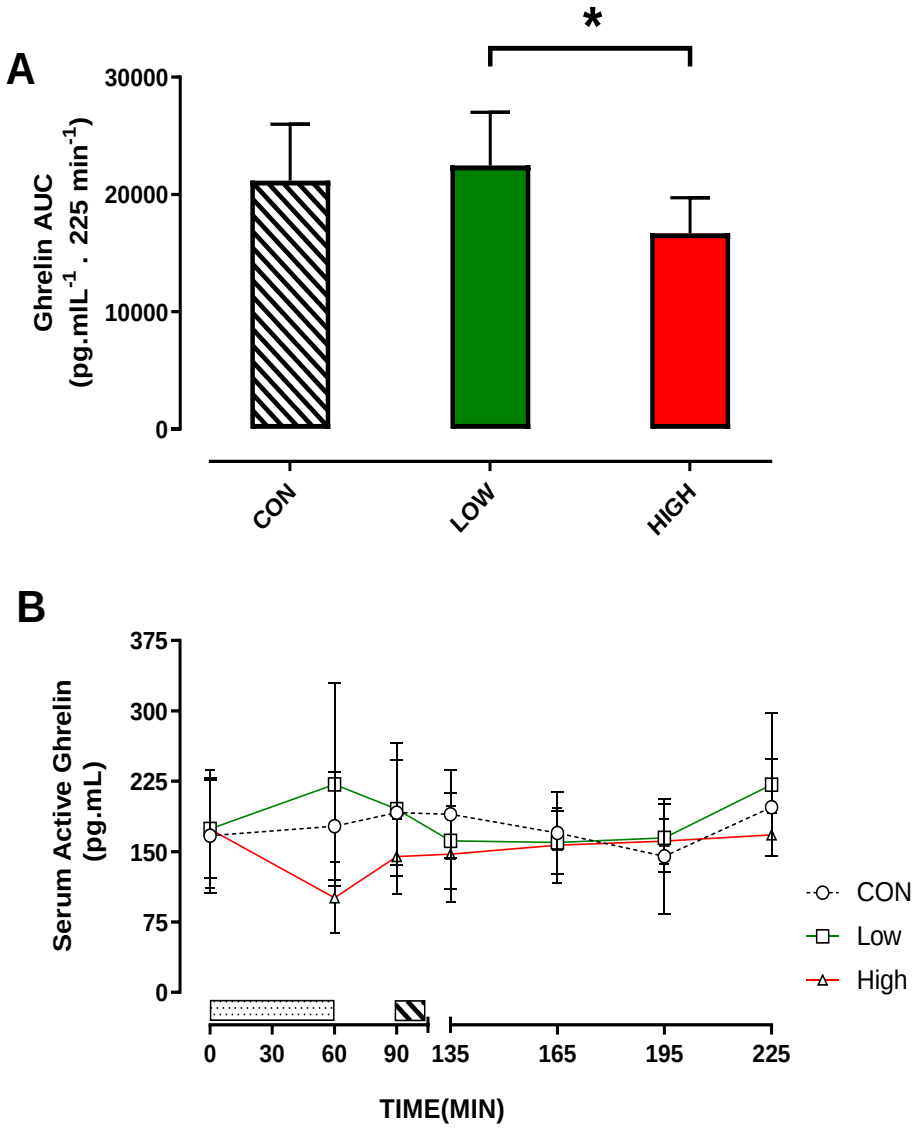

Figure 18: Representative of mean serum concentration of active ghrelin AUC (0-225 min^{-1}) (A) and active ghrelin response (B), with vertical error bars display SDs (n = 8). *Unfilled rectangle* with black spots indicates 60-min exercise period; *Hashed rectangle* indicates ingestion of a semi-solid meal. There was a main effect for active ghrelin AUC ($P < 0.05$), examined by repeated-measures ANOVA. There were no effect of time, trial nor interaction effect found for active ghrelin ($P > 0.05$), examined by 2-way repeated-measures ANOVA. * Indicates LOW was significantly different than HIGH. Determined by Bonferroni adjusted paired t test ($P = 0.015$). AUC; area under curve.

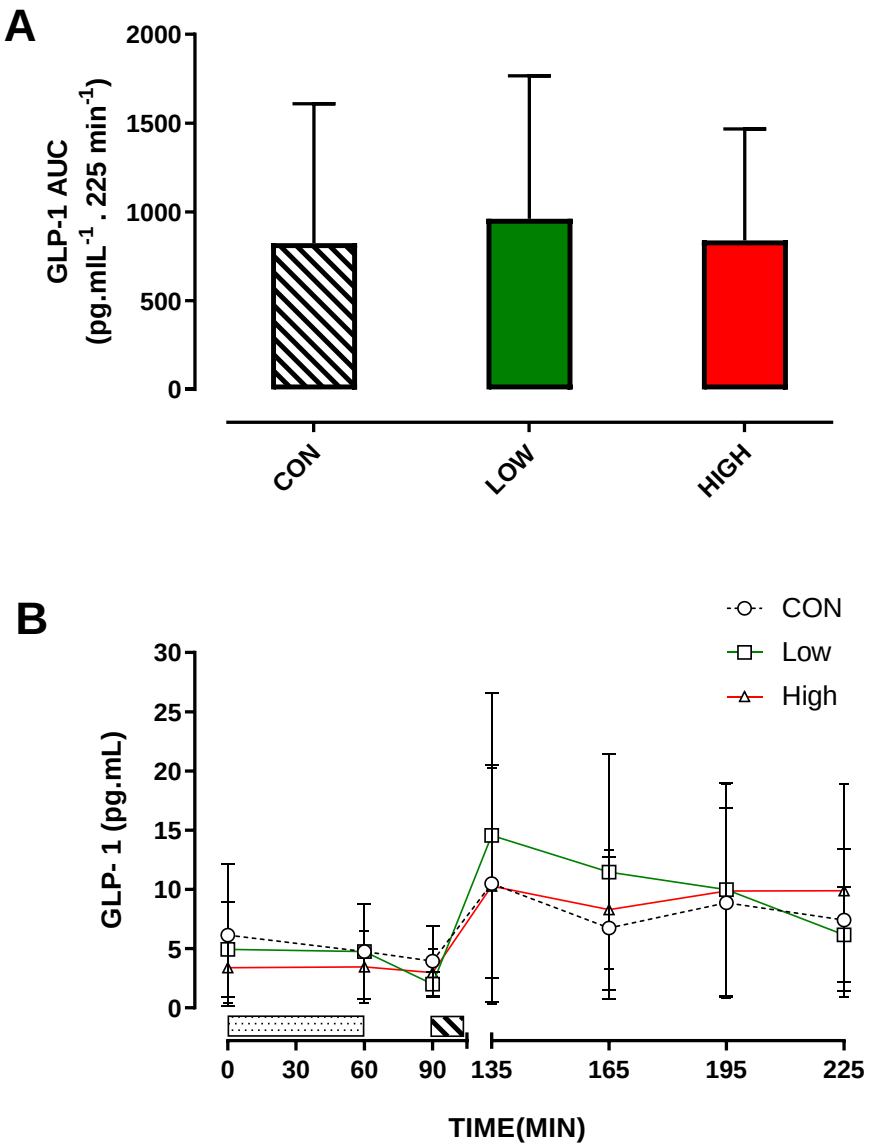

Figure 19: Representative of mean serum concentration of GLP-1 AUC (0-225 min^{-1}) (A) and GLP-1 response (B), with vertical error bars display SDs (n = 8). *Unfilled rectangle* with black spots indicates 60-min exercise period; *Hashed rectangle* indicates ingestion of a semi-solid meal. There was no main effect for GLP-1 AUC ($P > 0.05$), examined by repeated-measures ANOVA. There was a main effect of time ($P < 0.001$). However, no trial nor interaction effect was found for GLP-1 ($P > 0.05$), examined by 2-way repeated-measures ANOVA AUC; area under curve, GLP-1; glucagon-like peptide-1.

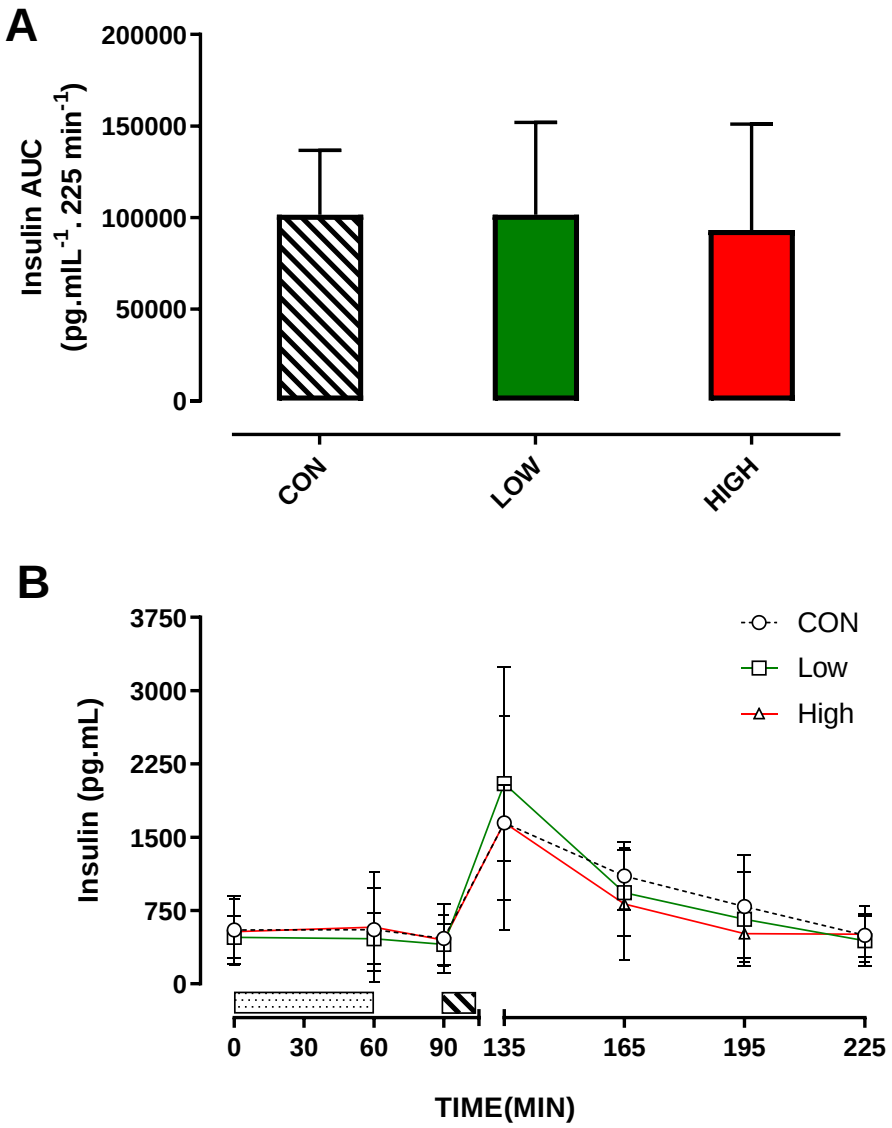

Figure 20: Representative of mean serum concentration of insulin AUC (0-225 min^{-1}) (A) and insulin response (B), with vertical error bars display SDs (n = 8). *Unfilled rectangle* with black spots indicates 60-min exercise period; *Hashed rectangle* indicates ingestion of a semi-solid meal. There was no main effect for insulin AUC ($P > 0.05$), examined by repeated-measures ANOVA. There was a main effect of time ($P < 0.001$). However, no trial nor interaction effect was found for insulin ($P > 0.05$), examined by 2-way repeated-measures ANOVA. AUC; area under curve.

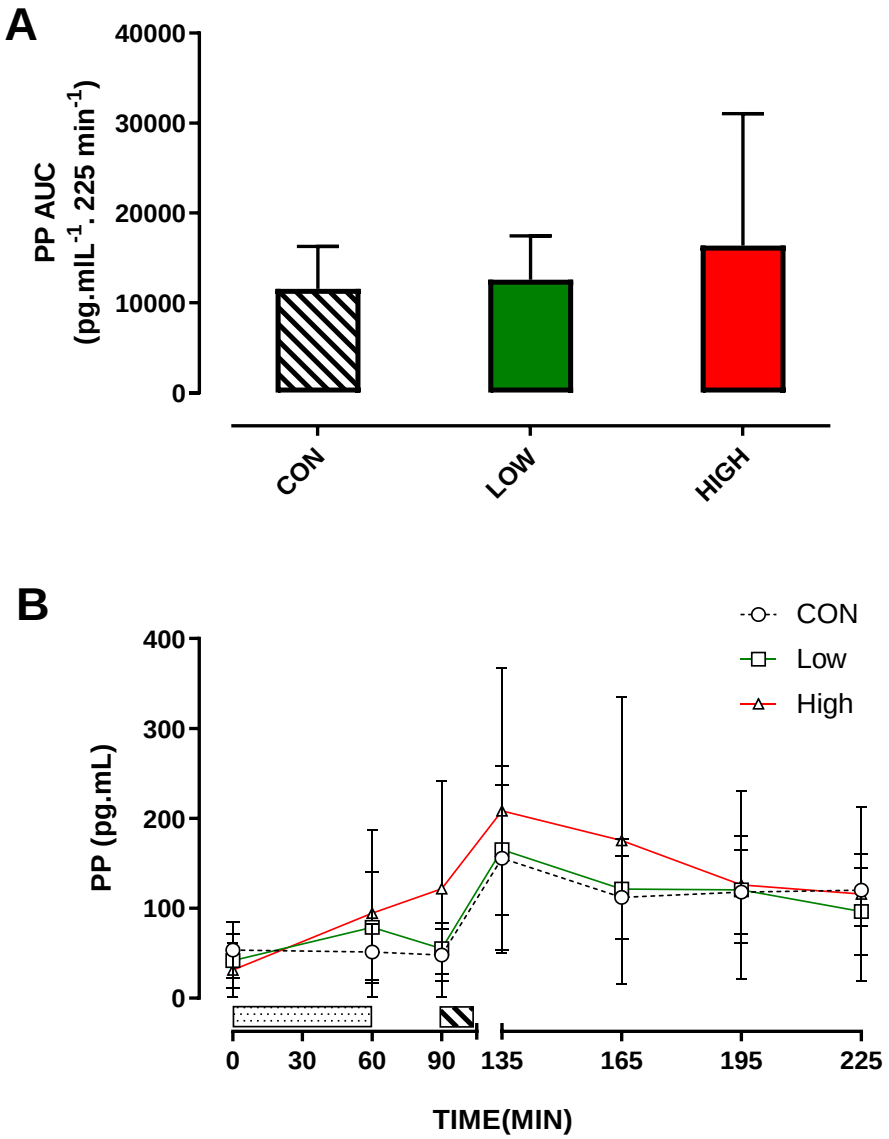

Figure 21: Representative of mean serum concentration of PP AUC (0-225 min^{-1}) (A) and PP response (B), with vertical error bars display SDs (n = 8). *Unfilled rectangle* with black spots indicates 60-min exercise period; *Hashed rectangle* indicates ingestion of a semi-solid meal. There was no main effects for PP AUC ($P > 0.05$), examined by repeated-measures ANOVA. There was a main effect of time ($P < 0.001$). However, no trial nor interaction effect was found for PP ($P > 0.05$), examined by 2-way repeated-measures ANOVA. AUC; area under curve, PP: pancreatic polypeptide.

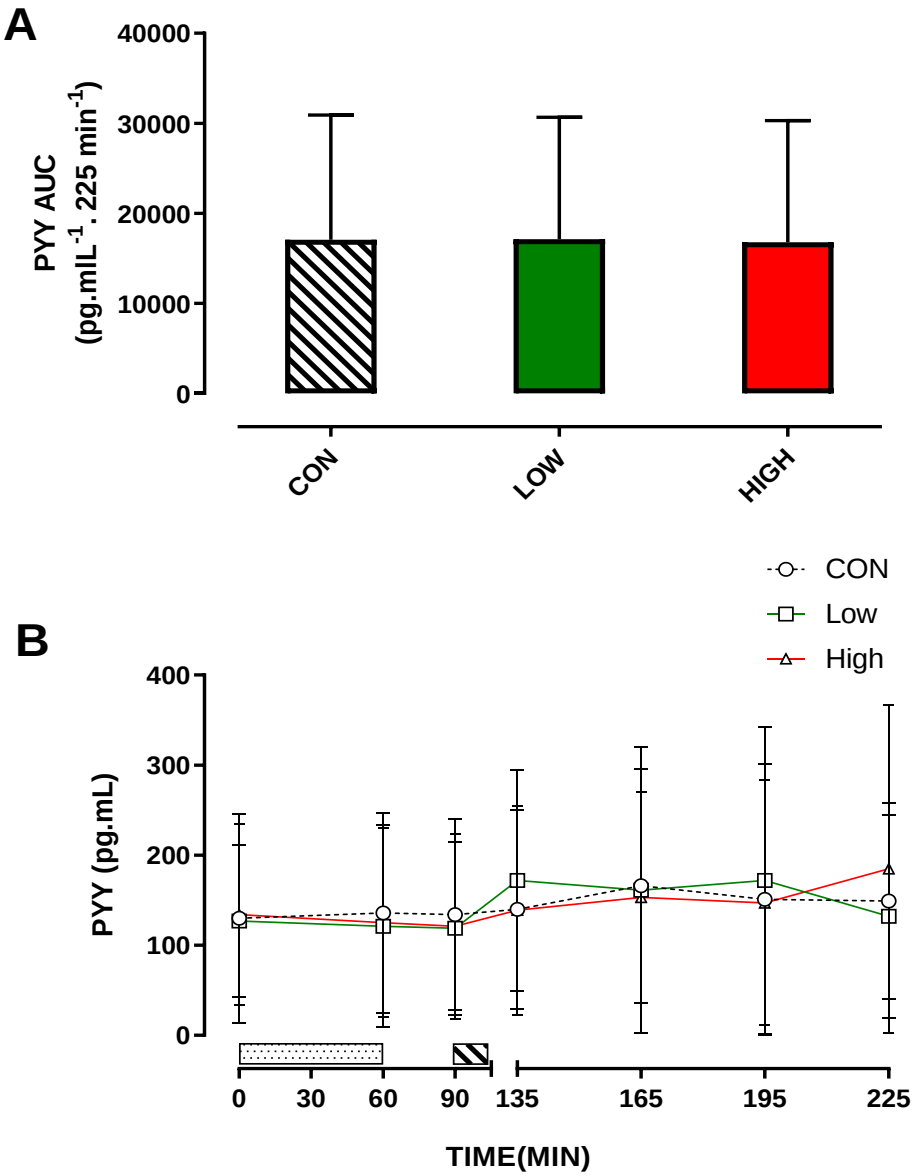

Figure 22: Representative of mean serum concentration of PYY AUC (0-225 min^{-1}) (A) and PYY response (B), with vertical error bars display SDs (n = 8). *Unfilled rectangle* with black spots indicates 60-min exercise period; *Hashed rectangle* indicates ingestion of a semi-solid meal. There was no main effect for PYY AUC ($P > 0.05$), examined by repeated-measures ANOVA. There was no main effect of time, trial nor interaction effect found for PYY ($P > 0.05$), examined by 2-way repeated-measures ANOVA. AUC; area under curve, PYY; Peptide YY.

4.4. Discussion

The aim of the present study was to assess the effect of exercise intensity on subjective appetite, gastric emptying, blood metabolites and appetite-regulating hormones in healthy males. The main findings were that exercise intensity had little effect on GER, appetite, nor gastrointestinal hormone response despite some minor deviations at some time points. This suggests that exercise intensity is unlikely to have a significant effect on EI in the period after exercise despite the increased energy expenditure that occurs at higher exercise intensities.

To date, this is the first study to examine GER of a standardised semi-solid meal and the responses of numerous appetite-regulating hormones after continuous aerobic exercise at different intensities. Previous studies have reported exercise intensity >60% $\dot{V}O_{2max}$ results in suppression of appetite in untrained individuals (Martins et al., 2007a, Becker et al., 2012, Ueda et al., 2009a, King et al., 2010a). In the present study, appetite was marginally suppressed immediately post-exercise in the HIGH and LOW trials, yet no significant difference was observed at subsequent time points post-exercise or post-meal.

Appetite suppression immediately post-exercise has been observed in non-athletic populations (Martins et al., 2007a, King et al., 2010a) but this suppression has not always been reported. Holliday et al, (2017c) reported that in endurance-trained males ($\dot{V}O_{2max}$ = 61.6 ± 6.0 mL/kg/min), no significant reductions in subjective appetite were seen when participants completed a bout of high intensity aerobic exercise. Consistent with these findings, King et al, (2010b) recruited trained males $\dot{V}O_{2max}$ (55.9 ± 1.8 mL/kg/min) and found no difference in appetite between a control trial and brisk walking. The present study supports these observations as healthy un-trained males did not show differences in appetite when a standardised semi-solid meal was consumed instead of an *ad libitum* meal post exercise.

It must be noted that there were some differences observed for appetite AUC, as fullness was significantly lower after the HIGH trial compared to the LOW and nausea increased during the HIGH trial compared to control but not the LOW exercise trial. An increase in nausea within the present study and a decrease in fullness may suggest participants' perception of appetite might have been reduced post exercise as

the feelings of nausea may override the physical desire to eat. It is important to add, regardless of this result, appetite was unchanged in the short 2 h monitoring period after consuming a standardised semi-solid meal and should not be misinterpreted.

It is unknown whether exercise intensity within the present study would have affected appetite responses in the days following. Food intake and therefore EI was not measured post-trial or over the following 24-72 h, so potential compensation in EI cannot be estimated. However, the majority of the available research suggests that exercise does not stimulate any changes in EI >20-h after exercise (King et al., 1997, Hanlon et al., 2012). Although, in contrast, Rocha et al, (2013) demonstrated compensation in EI immediately post-trial and three-days after an acute bout of aerobic exercise. Further investigations are required to clarify subsequent EI and whether this response differs if the energy expenditure for exercise is matched.

GER in humans has been shown to respond to ingested volume, nutrient content and may be influenced by previous dietary intake (Noakes et al., 1991, Costill and Saltin, 1974, Rehrer et al., 1989). This is the first study, to have investigated gastric emptying responses to a semi-solid meal after continuous aerobic exercise at different intensities. Evans et al, (2016) observed that exercise intensity appeared to have little effect on GER of a glucose solution after the completion of an exercise stimulus. The results of this study support these observations. GER is regulated by splanchnic blood flow and central nervous system activity during exercise while some circulating gut-derived hormones have been shown to influence this variable. It would seem as though the changes in splanchnic blood flow and CNS activity that occur during exercise are likely to return to pre-exercise levels relatively quickly after completion of exercise thus having less of an influence on GI function in recovery. In this study, little difference was observed in circulating gut-derived hormone response after exercise which could also explain the lack of difference in GER of the test meal.

Ad libitum water consumption during the trial was significantly different with the largest fluid consumption within the HIGH and the lowest in the CONTROL, as would be expected. This difference in fluid intake may have affected GER; however, whether these fluid results in the present study indicate a potential mechanism for the results observed is questionable as the influence of hydration level during exercise on

GER is unclear. This suggests exercise intensity, at least between 40-70% $\dot{V}O_{2peak}$, has no effect on GER of a semi-solid meal.

No significant differences for blood glucose, total cholesterol and triglyceride concentrations were observed in the present study. These findings are consistent with previous research that also reports significantly higher glucose 30-60 min after food ingestion (King et al., 2010b, Clayton et al., 2016). Interestingly, Clayton et al (2016), investigated 24 h severe energy restriction (providing 25% of energy requirements) and revealed that following 24 h energy restriction, similar to the CONTROL trial in the present study, plasma glucose concentration 60-minutes after consuming a standardised breakfast was increased compared to an energy balanced control trial. Within the current study, NEFA increased from pre to post exercise in both exercise conditions. Aligned with previous literature (Martins et al., 2007a, Clayton et al., 2016, Yau et al., 2017a), NEFA concentrations fall after the ingestion of food containing carbohydrate due to its stimulatory effect on insulin release. NEFA AUC was significantly increased after the HIGH trial compared to the control, but not the LOW. The current study might suggest NEFA increases after exercise in a fasted state regardless of intensity. These findings are consistent with McIver et al (2018), as they reported participants who walked at a relatively low intensity (50% $\dot{V}O_{2peak}$) in a fasted state for 45min had significantly more circulating NEFA than participants who had eaten breakfast.

Triglyceride concentration was unchanged, with no differences between trials regardless of exercise intensity. Triglycerides usually increase within the blood in response to changes in energy demands (Aldiss et al., 2017). The lack of difference between the trials is to be expected as GER was unaffected by exercise intensity and thus nutrients would have been available for absorption at a similar rate.

No significant differences were observed for gut peptide concentrations (GLP-1, PYY and PP) or insulin). Nevertheless, ghrelin AUC was significantly lower during the HIGH trial compared to the LOW trial. As ghrelin acts as an appetite stimulant, it would be expected that appetite response would have been different in response to this change in circulating ghrelin. However, within the present study acylated ghrelin only responded to exercise intensity differently immediately after exercise as serum concentrations decreased by ~27% in the HIGH and increased by ~12% in the LOW.

This decrease in ghrelin has consistently been observed within the literature for exercise intensity >70% $\dot{V}O_{2max}$ (Broom et al., 2007, King et al., 2011a). These changes were not significantly different post exercise in this study and consideration must be taken when interpreting the ghrelin AUC data. Despite these changes showing no significance previous research has also failed to observe an immediate difference in ghrelin following an acute bout of exercise (King et al., 2010b). Investigations have shown an inverse relationship between ghrelin and insulin (Solomon et al., 2008, Saad et al., 2002, Flanagan et al., 2003, Blom et al., 2005). This was not observed within the current study, as no changes in ghrelin were seen regardless of the rise in insulin concentrations post-meal. The findings for GLP-1 and PP are consistent with previous literature regarding elevated levels following food consumption (Ueda et al., 2013, Koska et al., 2004, Orskov et al., 1994). However, within the current study the increase 30-min post meal was not significant. There was also a lack of variation for PYY within the current study. However, De Silva and Bloom (2012), suggest PYY may be increased dependent on the energy content of the meal consumed. Therefore, the PYY findings within the current study may be due to the relatively small standardised semi-solid lunch provided. These findings suggest that increasing the exercise intensity potentially represents a viable strategy to increase the likely negative energy balance augmented by exercise, without increasing short-term appetite later in the day. If subsequent metabolic responses are sustained in the long-term this could have important implications for weight management.

4.4.1. Limitations

This study has strengths and limitations. The main strength of this study is the crossover design, with the comparison to a control trial. This allowed direct comparisons between the two-exercise intensity. Similarly, this study is one of the few that has examined how a standardised semi-solid meal responds after different exercise intensities with the primary outcome to examine GER. Although, limitations must be acknowledged as sample numbers within the blood measurements were relatively low (n=8) due to complications with blood sampling. For this reason, caution must be considered when interpreting this data as it may be seen as being underpowered. However, this situation is a common issue within research of this nature (Broom et al., 2007), and future research within this area must consider larger

sample size to continue expanding research in relation to appetite regulation. Secondly, another limitation of the present study is the short 2-h monitoring period after exercise, as a longer monitoring period after exercise may offer more information on longer-term appetite regulation. Further studies should examine how post-exercise energy demands may cause different appetite response in the hours or days after exercise, which might lead to increased EI and therefore a negative effect on weight loss. Finally, some further limitations must be acknowledged. The meal challenge provided was in accordance with a single serving suggested by the food manufacturers and, as such, the energy content provided was relatively low. If this were to be increased, some subtle difference in GER may be observed. This is in agreement with the results of Moore et al, (1984) who reported the volume and energy density of the meal is crucial to the speed at which gastric emptying occurs. The rate is exponentially related to the volume in the stomach, therefore the fuller the stomach the faster the rate of emptying. This suggests using a standardised meal size is an important consideration when designing further research within the area.

4.4.2. Conclusion

In conclusion, post-exercise GER was similar regardless of exercise intensity. There was no effect of exercise intensity on subjective appetite after consuming a standardised semi-solid meal post-exercise. While some minor deviations in gut hormone response were noted, these were not statistically different between trials. These findings suggest exercise intensity does not affect GI measurements within a 2 h recovery period. Furthermore, the additional significant physiological demands of the high intensity trial did not result in compensatory alterations in the hormonal regulation of appetite or subjective appetite responses in the time period measured.

Future research is required to investigate the mechanisms responsible for changes in appetite, GER and circulating hormones as a consequence of different modes of exercise to explore if appetite regulation is affected when exercise intensity is matched but the modality changes (comparing continuous to intermittent exercise). Additionally, extending the observation period to include EI post-trial would also provide a greater indication on whether compensatory effects occur 24 h after participants leave the laboratory.

Chapter 5.

A Comparison Between Moderate and Low Intensity Intermittent and Continuous Exercise on Gastric Emptying Rate, Appetite and 24-h Energy Intake in Males[3, 4]

[3] Some of the data from this study contained within this chapter was presented as a poster communication and the abstract published in "Mattin LM, VJ. Yau, A. James, LJ. Evans, GH. (2018). A comparison of the effect of high and low intensity intermittent and continuous exercise on gastric emptying rate, appetite and substrate utilisation in healthy males. Europhysiology. The QEII Centre, London, UK: *The physiology Society*, 2018:376-7"

[4] The data from this study contained within this chapter has been accepted for publication in "Mattin LR, McIver VJ, Yau AMW, James LJ, Evans GH. (2020). A Comparison of Intermittent and Continuous exercise Bouts at Different Intensities on Appetite and Postprandial metabolic Responses in Healthy Men. Nutrients. 12(8):2370"

5.1 Introduction

As outlined in Chapter 4, GER was unaffected by continuous aerobic exercise performed at a low or high intensity and whether this was the direct effect of exercise regardless of intensity or the capability of the GI system to respond to the relatively small semi-solid meal provided (~242 Kcal) warrants further investigation. In contrast, considerable research has focused on the effectiveness of intermittent exercise on improving physical fitness and reducing cardiovascular disease risk (Laursen et al., 2005, Heydari et al., 2013). Although, some evidence, but not all, suggests intermittent exercise might facilitate greater weight loss (Jakicic et al., 1999) than continuous aerobic exercise, possibly related to a greater suppression of appetite post-exercise (Trapp et al., 2008, Boutcher, 2011, Heydari et al., 2012). Therefore, comprehending how physical activity, such as intermittent cycling affects the 'appetite response' post-exercise is critical to improving long-term modifications in EI following acute exercise.

Evidence has suggested GER of a liquid solution is delayed during exercise, when performed continuously (Rehrer et al., 1989, Maughan et al., 1990) and intermittently (Leiper et al., 2001a, Leiper et al., 2005) at a high intensity. However, post-exercise Evans et al, (2016) established that mode of exercise appears to have little effect on GER of a glucose solution ingested after completing moderate intense continuous exercise (MICE) for 30-min at 33% peak power output (PPO) and a HIIE bout (10 × 1 min at PPO). Only a small number of studies have examined GER in the postprandial periods after exercise (McIver et al., 2019, Evans et al., 2018a, McIver et al., 2018, Evans et al., 2016, Clayton et al., 2014) and studies that have investigated the impact of moderate to high exercise intensity on appetite and EI have not included GER as a primary measurement (King et al., 1997, King et al., 2010a, Martins et al., 2015). However, despite these findings, research from Poon et al, (2018) demonstrated the interval nature of HIIE had no effect on EI nor appetite regulation when compared to a continuous exercise bout and once again no measurement of GER was documented. Several reviews focusing on acute exercise have indicated further work is needed to better comprehend how intermittent or interval exercise bouts might affect EI in the hours after exercise (Schubert et al., 2013, Deighton and Stensel, 2014, Hazell et al.,

2016). It is currently unclear whether intermittent exercise at different intensities differentially affects GER or stimulates a compensatory increase in appetite response. We do, however, expect the same response in GER post-exercise as has been found previously during intermittent exercise.

MICE is the preferred exercise type for recreational activities (King et al., 2001) and favoured during acute energy deficit research (Wasse et al., 2013a, Martins et al., 2015, Ueda et al., 2009a, Deighton et al., 2013b). Whilst the effects of continuous exercise on post-exercise appetite/metabolism have been relatively well documented, less is known about the effects of intermittent exercise in this regard. Nevertheless, Sim et al, (2015) found performing three high intensity intermittent exercise sessions per week for 12 weeks caused variations in appetite regulation, leading to reduced EI compared to an equivalent period of MICE and control. This suggests long-term benefits of training in an intermittent manner that might increase weight loss. Additional work from this group found when a single exercise session was matched for total workload, EI remained lower after HIIE compared with MICE as EI was attenuated in the post-exercise meal (Sim et al., 2014). Despite showing minor changes in EI, intermittent exercise results in additional physiological adaptation, such as improved muscle oxidative capacity (Hood et al., 2011, Gillen and Gibala, 2014), that may lead to greater health benefits than continuous exercise.

To date, no investigation has compared the postprandial responses to a single bout of intermittent and continuous exercise at a moderate and low intensity to assess if the modality of exercise is as important as intensity. Therefore, it was hypothesised that intermittent exercise, regardless of intensity, would delay GER and lead to a greater suppression of appetite post-exercise than continuous exercise. The null hypothesis therefore is there would be no differences between exercise conditions. The purpose of this study was to compare how intermittent and continuous exercise at a low and moderate intensity would influence postprandial response to a standardized semi-solid meal on 1) GER and feelings of appetite, 2) Leading to a modified metabolite response, and 3) Altering 24 h post-trial EI.

5.2 Methods

5.2.1. Participants

Fourteen healthy men were recruited from central Manchester, UK (Table 10). Verbal and written explanations of the experimental procedures were provided before the start of the trials and written informed consent to participate was obtained. Two participants withdrew prior to completing the study, meaning twelve participants completed the study. An a priori calculation was conducted using data from chapter 4 (Mattin et al., 2018b). An effect size $\eta^2p = 0.135$ from a repeated measures ANOVA model, attributing GER as the primary outcome measure, and using an α of 0.05 and a statistical power of 0.8 determined that ≥ 12 participants would be required to reject the null hypothesis (G*Power 3.0.10, Heinrich Hein Universitat, Dusseldorf, Düsseldorf, Germany).

Table 10: S-2, Baseline Participant's Characteristics[1]

	Males (*n*=12)
Age, (y)	30 ± 6
Height, (m)	1.79 ± 0.08
Weight, (Kg)	79.1 ± 9.0
BMI, (kg/m²)	24.6 ± 2.0
Body fat, (%)	17.8 ± 3.9
Systolic BP, (mmHg)	132 ± 13
Diastolic BP, (mmHg)	79 ± 11
$\dot{V}O_{2max}$, (ml/kg/min)	38 ± 6
LOW-CON, (W)	76 ± 26
LOW-INT, (W)	221 ± 76
MOD-CON, (W)	114± 31
MOD-INT, (W)	417 ± 90

[1] Values are means ± SDs

5.2.2. Experimental Trials

Participants reported to the laboratory on four occasions, each separated by a minimum of 7 days. In addition to the pre-trial familiarisation outlined in general methods. Each participant was also required to standardise their diet and physical activity before each trial (see section 3.7. Pre-trial standardisation).

Upon arrival at the laboratory, participants' post-void body mass was obtained before they completed seated rest for 10 min, during this period baseline measurements for appetite (VAS questionnaire) were completed, before a capillary blood sample was collected. Expired gas samples were collected for 15 min in a semi-supine position on a bed, and this data was used to calculate substrate utilisation. The procedures for these baseline measurements are outlined in general methods.

Participants then completed a 60 min exercise protocol either at 60% $\dot{V}O_{2peak}$ (MOD) or 40% $\dot{V}O_{2peak}$ (LOW) in a continuous (CON) or intermittent (INT) manner. CON consisted of 60-min continuous cycling and INT consisted of 20 × 1 min exercise bouts interspersed by 2 min rest period. INT and CON at each intensity were matched for total work output, which is documented below (5.2.3 exercise intensity). HR and RPE (Borg, 1973) were recorded every 5 min in CON (5, 10, 15, 20, 25, 30, 45, 50, 55 and 60 min) and at the end of each 1 min exercise bout (1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, 52, 55 and 58 min) and at the end of each 2 min rest period in INT. The heart rate data is reported as individual points during exercise (Figure 25) and average heart rate across the 60 min exercise period (Table ***11***).

During exercise, each participant was provided with a standardized amount of water (125 mL) at 15, 30, 45 and 60 min resulting in a total of 500 mL during the exercise period. Furthermore, a capillary blood sample was collected mid-exercise (30 min) and at the end of the exercise bout 60 min (post-exercise). After completion of the exercise bout a further VAS was completed, before participants were given 30 min to shower and change their clothes.

Following collection at 90 min of a pre-meal blood sample and VAS, participants were fed a standardised meal consisting of two cans (~800 g) of vegetable soup (see section; 3.6. Meal Details), in which they were given a maximum of fifteen minutes to consume the standardised meal and instructed to consume it as quickly as

they were able. Time taken to eat this meal was recorded. After the meal, no more food or drink was consumed until participants left the laboratory at 225 min. Participants remained in a semi-supine position throughout the remaining 2h sampling procedure. Additional blood and expired gas samples were obtained every 30min (135, 165, 195 and 225 min), gastric emptying rate was obtained pre-meal ingestion and at (120, 135, 150, 165, 180, 195, 210 and 225 min); with appetite response being documented at (105, 120, 135, 150, 165, 180, 195, 210 and 225 min). The measurements within this trial are described in general methods. Following the collection of the final samples, participants were free to leave the laboratory. A schematic diagram of the experimental protocol is presented in (Figure 23).

5.2.3. Exercise Intensity

Exercise intensity was calculated in a similar manner as described within Chapter 4. Workload was then calculated using the liner trend line equation to evoke a work rate equivalent to 40% $\dot{V}O_{2Peak}$ (Low intensity) and 60% $\dot{V}O_{2Peak}$ (Moderate intensity) duration continuous exercise trial. Intermittent exercise was matched for power output compared to the continuous counterpart. The established W intensity for the continuous low/moderate trials were used to determined energy expenditure kilojoules (KJ) using *Equation6*. Using the notion (1W = 1 joule per second). The total energy expenditure (KJ) for the 60 min continuous trials were used to calculate 1 min W intensity for the intermittent trials. This ensured equal power output between continuous and intermittent trials at the required low and moderate exercise intensity.

Equation 6:

$$Joule's[J] = (W \times Time\ [s])$$

$$KJ = (J \div 1000)$$

5.2.4. Statistical Analysis

All data are presented as mean ± standard deviation (SD) unless otherwise stated. Data was tested for normality of distribution using the Shapiro-Wilk test. Pre-trial body mass, exercise and environmental measurements, time to eat soup, AUC calculations, gastric emptying T_{lag} and $T_{1/2,}$ and EI were analysed using a two-way repeated measures analysis of variance (ANOVA) for intensity and modality of exercise. Substrate utilisation, DOB, blood glucose, HR and appetite were analysed using a three-way repeated measures ANOVA for time, intensity and modality of exercise. Sphericity for repeated measures was assessed and Greenhouse-Geisser epsilon < 0.75 and the Huynh-Feldt correction adopted for less severe differences to correct for violations. Significant main effects were followed by paired student's t-Test or one-way repeated ANOVA with Bonferroni adjusted pairwise comparisons as appropriate. Effect size was documented as partial eta squared (η^2_p) or Cohen's (d) and 95% confidence intervals (CI) (see section 3.19.2. Post-Statistics Effect Size).

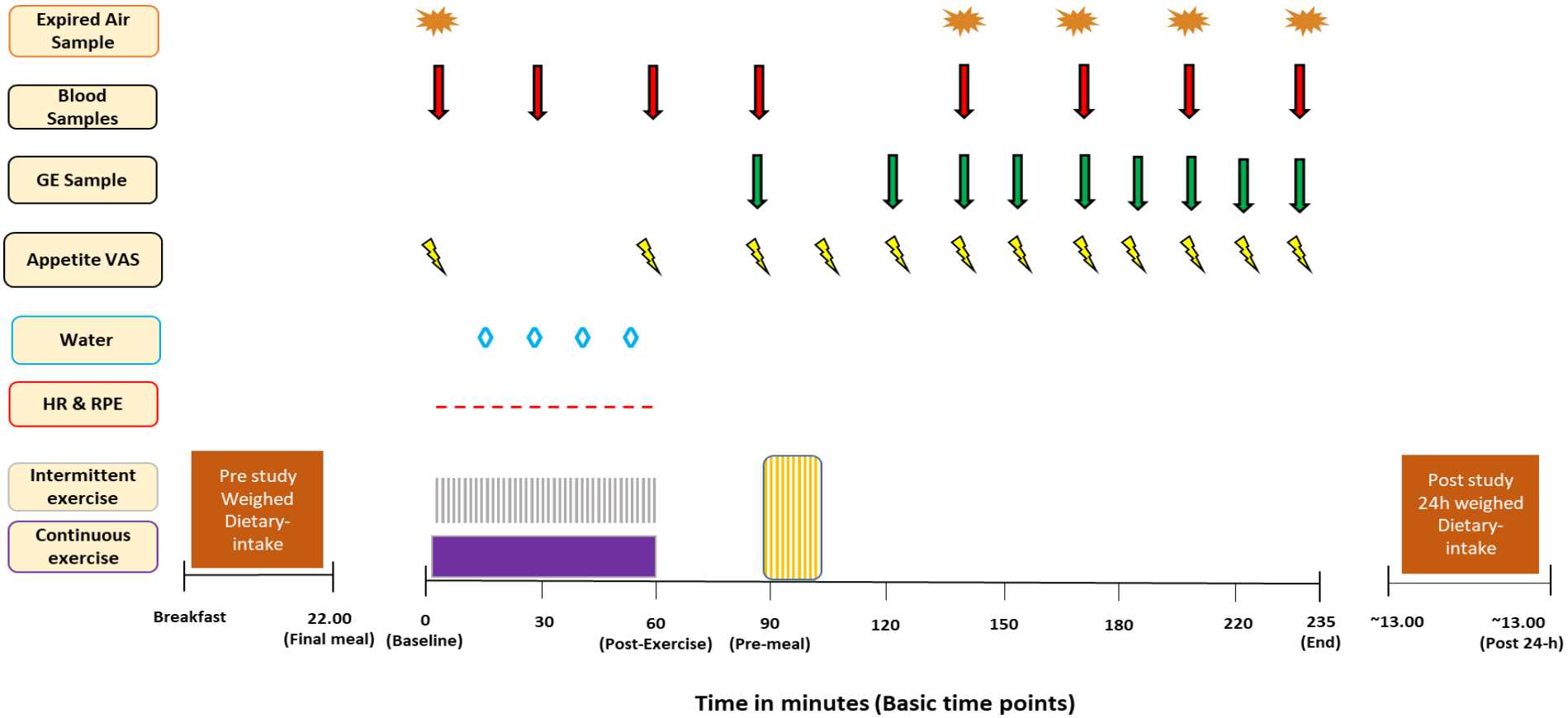

Figure 23: Schematic diagram of the experimental trial protocol S-2. Yellow lined rectangle represents 15 min standardised semi-solid meal period. HR, heart rate. RPE, rating of perceived exertion. GE, gastric emptying. VAS, visual analogy scale. Expired Air, (Substrate Utilisation). Intermittent, (LOW-INT and MOD-INT). Continuous, (LOW-CON and MOD-CON)

5.3 Results

5.3.1. Standardisation Measurements

Pre-trial body mass was not significantly different between trials with statistical analysis showing no effect of intensity ($p = 0.256$, $\eta^2_p = 0.116$), modality ($p = 0.388$, $\eta^2_p = 0.068$) and intensity × modality interaction effect ($p = 0.726$, $\eta^2_p = 0.012$). There was no difference in environment temperature between trials with statistical analysis showing no effect of intensity ($p = 0.262$, $\eta^2_p = 0.113$; $p = 0.061$, $\eta^2_p = 0.283$), modality ($p = 0.592$, $\eta^2_p = 0.027$; $p = 0.793$, $\eta^2_p = 0.007$) and intensity × modality interaction effect ($p = 0.297$, $\eta^2_p = 0.097$; $p = 0.752$, $\eta^2_p = 0.009$).

No main effect of modality ($p = 387$, $\eta^2_p = 0.019$) was shown for average heart rate (HR) during the 60 min exercise period, however a main effect of intensity ($p < 0.001$, $\eta^2_p = 0.969$) and an intensity × modality interaction ($p < 0.001$, $\eta^2_p = 0.582$) was observed. Post-hoc tests revealed HR was significantly higher between MOD-CON compared to LOW-CON (139 ± 18 vs. 104 ± 16 bpm: $p < 0.001$, d = 2.15, 95% CI = −8.04 –11.20 bpm) and MOD-INT compared to LOW-INT was reported (130 ± 17 vs. 106 ± 16 bpm: $p < 0.001$, d = 1.52, 95% CI = −8.10 –10.57 bpm)

No main effect of modality ($p = 0.118$, $\eta^2_p = 0.207$) nor intensity × modality interaction ($p = 0.119$, $\eta^2_p = 0.150$) was shown for total estimated work completed during exercise (KJ), however a main effect of intensity ($p < 0.001$, $\eta^2_p = 0.975$) was observed. Post-hoc tests revealed significantly more work was completed between LOW-CON compared to MOD-CON (277 ± 94 vs. 524 ± 104 KJ: $p < 0.001$, d = 2.60, 95% CI = −56.24 –55.79 W) and LOW-INT compared to MOD-INT was reported (271 ± 73 vs. 518 ± 103 KJ: $p < 0.001$, d = 2.89, 95% CI = −55.39 – 44.19 KJ)

Exercise intensity (W) was significantly different between all trials, with statistical analysis demonstrating an effect of intensity ($p < 0.001$, $\eta^2_p = 0.944$), modality ($p < 0.001$, $\eta^2_p = 0.963$) and an intensity × modality interaction ($p < 0.001$, $\eta^2_p = 0.0957$). Post-hoc tests revealed significantly lower exercise intensity for LOW-CON compared to LOW-INT (77 ± 26 vs. 222 ± 73 W: $p < 0.001$, d = 2.76, 95% CI = −38.54 – 17.47 W) and MOD-CON compared to MOD-INT was reported (145 ± 29 vs. 423 ± 86 W: $p < 0.001$, d = 4.52, 95% CI = −44.13 – 20.93 W).

Finally, differences in time to eat the soup were found between trial modality (p = 0.005, η^2_p = 0.519) but not for intensity (p = 0.253, η^2_p = 0.117) nor an intensity x modality interaction (p = 0.999, η^2_p = 0.010). Post-hoc tests revealed time to eat soup was significantly longer during MON-INT compared to MOD-CON (434 ± 91 vs. 405 ± 101 s: p = 0.011, d = 0.32, 95% CI = −51.95 – 57.32 s) but not between LOW-CON and LOW-INT (313 ± 166 vs. 343 ± 91 s: p = 0.456, d = 0.23, 95% CI = −51.25 – 94.16 s, Table 11).

Table 11: S2, Standardisation Measurements During Laboratory Visit

Measurements	LOW-CON	LOW- INT	MOD-CON	MOD-INT	P = Value
Pre-Trial Measurements					
Body Mass (Kg)	79.3 ± 8.8	79.3 ± 8.9	79.4 ± 8.7	79.6 ± 8.9	0.726
Exercise Measurements					
Average HR (60-min)	104 ± 16	106 ± 16	139 ± 18	130 ± 17	< **0.001**
Work Completed (KJ)	277 ± 94	271 ± 73	524 ± 104	518 ± 103	0.119
Exercise Intensity (W)	77 ± 26†	222 ± 73†	145 ± 29†	423 ± 86†	< **0.001**
Environment Temperature					
During Exercise (˚C)	20.8 ± 1.1	20.6 ± 0.9	20.7 ± 0.9	21.2 ± 1.1	0.297
During Recovery (˚C)	21.2 ± 1.1	21 ± 0.7	21.4 ± 0.8	21.4 ± 0.8	0.752
Semi-Solid Meal					
Weight of Soup Consumed (g)	773 ± 15	768 ± 10	771 ± 14	775 ± 10	0.058
Time to Eat Soup (s)	313 ± 166	343 ± 91	405 ± 101	434 ± 91*	0.999

Data are means ± SD. Values are significant $P < 0.05$; * MOD-INT is significantly different from MOD-CON; † Value is significantly different from all other trials. P = Values are documented as main interaction effect.

5.3.2. Energy and Macronutrient Intake

5.3.2.1. Pre-Trial

Pre-trial energy intake amounted to 2483 ± 721 kCal, 2474 ± 854 kCal, 2486 ± 660 kCal and 2396 ± 803 kCal during the LOW-INT, LOW-CON, MOD-INT and MOD-CON trials, respectively, and there was no significant effect for intensity (p =0.745, η^2_p = 0.010), modality (p = 0.621, η^2_p = 0.023) nor intensity x modality (p = 0.665, η^2_p = 0.018, Figure 24A).

This led to a similar proportion of energy from carbohydrates (P = 0.770, η^2_p = *0.188*); fats (P = 0.239, η^2_p = *0.193*); protein (P = 0.594, η^2_p = *0.090*) and fibre (P = 0.775, η^2_p = *0.009*). In addition, fluid consumption before was not significantly different between trials (P = 0.587, η^2_p = *0.056*, Table 12).

5.3.2.2. Post-Trial

No main effect of the intensity × modality (p = 0.093, η^2_p = 0.256) interaction was detected for 24 h post trial energy intake (kCal), however a main effect of intensity (p = 0.005, η^2_p = 0.556) and modality (p = 0.001, η^2_p = 0.667) was observed. Post-hoc tests revealed MOD-INT was significantly higher compared to MOD-CON (3500 ± 1419 vs. 2777 ± 1042 kCal: p < 0.001, d = 0.61, 95% CI = −802.25 – 590.16 kCal) but no difference was found between LOW-INT and LOW-CON (2556 ± 989 v 2320 ± 985 kCal: p = 0.258, d = 0.25, 95% CI = −559.32 –557.56 kCal, Figure 24B).

This led to a different proportion of energy from carbohydrates. There was a main effect of intensity × modality (p = 0.022, η^2_p = 0.425) interaction, intensity (p = 0.003, η^2_p = 0.611) and modality (p = 0.002, η^2_p = 0.635) observed. Post-hoc tests revealed MOD-INT was significantly higher compared to MOD-CON (1639 ± 318 KCal and 1198 ± 325 KCal: p = 0.002, d = 1.44, 95% CI = −186.48 – 193.50 kCal) but no difference was found between LOW-INT and LOW-CON (1223 ± 296 KCal, 1094 ± 235 kCal p = 0.088, d = 0.51, 95% CI = −174.42 – 139.38 kCal, Table 12)

There was no main effect of intensity × modality (p = 0.240, η^2_p = 0.135) interaction nor modality (p = 0.074, η^2_p = 0.284) observed. However, there was a main effect of intensity (p < 0.001, η^2_p = 0.819) found for fat. Post-hoc tests revealed LOW-CON was significantly lower compared to MOD-CON (801 ± 247 KCal and 1024 ±

235 KCal: $p < 0.001$, d = 0.97, 95% CI = −137.90 – 146.94 kCal) and LOW-INT was significantly lower compared to MOD-INT (906 ± 279 KCal, 1241 ± 382 kCal p = 0.020, d = 1.05, 95% CI = −224.69 –165.93 kCal, Table 12)

There was no main effect of intensity × modality (p = 0.151, η^2_p = 0.015) interaction nor modality (p = 0.445, η^2_p = 0.045) observed. However, there was a main effect of intensity (p = 0.007, η^2_p = 0.534) found for protein. Post-hoc tests revealed LOW-CON was significantly lower compared to MOD-CON (388 ± 76 KCal and 515 ± 233 KCal: $p < 0.001$, d = 0.77, 95% CI = −136.92 – 45.68 kCal) but no difference was found between LOW-INT and MOD-INT (404 ± 114 KCal, 554 ± 130 kCal p = 0.098, d = 1.29, 95% CI = −75.54 – 68.66 kCal, Table 12).

There was no main effect of intensity × modality (p = 0.106, η^2_p = 0.240) interaction, modality (p = 0.221, η^2_p = 0.145) nor intensity (p = 0.415, η^2_p = 0.067) observed for fibre (23 ± 13 KCal, 39 ± 27 KCal, 37 ± 27 KCal and 40 ± 16 kCal, Table 12) for LOW-INT, LOW-CON, MOD-INT and MOD-CON trials, respectively.

In addition, fluid consumption post (2054 ± 857 g, 2013 ± 1347 g, 2395 ± 1089 g and 2187 ± 1160 g: P = 0.087, η^2_p=*0.99*, Table 12) LOW-INT, LOW-CON, MOD-INT and MOD-CON trials was not significantly different between trials

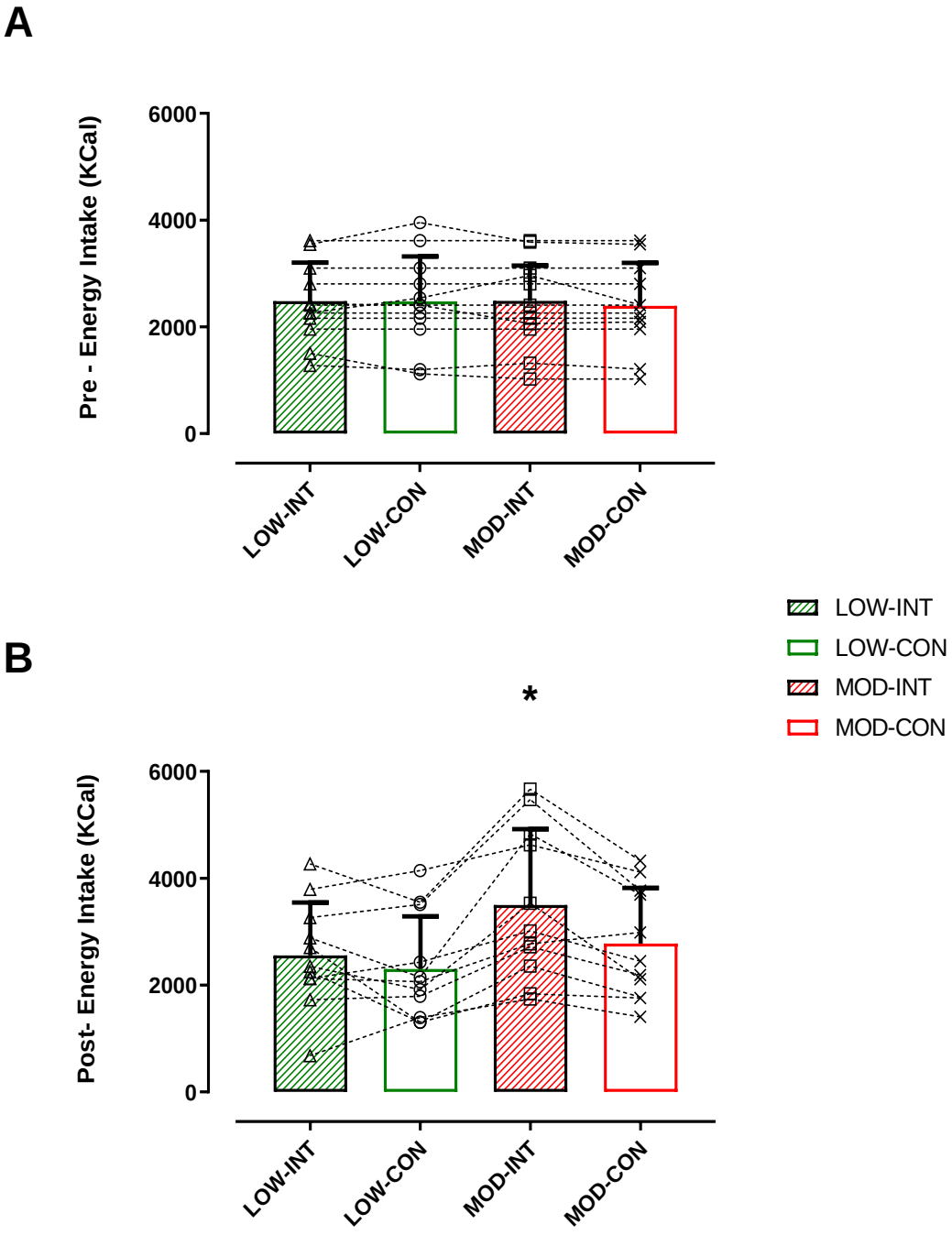

Figure 24: Representative of mean values for pre-energy intake (KCal) (A, n= 12) and Post-energy intake (KCal) (B, n = 11) with vertical error bars display SD's. Individual data is represented as (Δ) LOW-INT, (○) LOW-CON, (□) MOD-INT and (×) represents MOD-CON. There were no intensity, modality nor interaction main effects for pre-trial energy intake ($P > 0.05$), examined by a one-way repeated-measured ANOVA. An intensity and modality effect was observed ($P < 0.05$). However, no interaction main effects for post-trial energy intake ($P > 0.05$), examined by a two-way repeated-measures ANOVA. * Indicates MOD-INT were significantly different than MOD-CON, determined by Bonferroni adjusted paired *t*-test ($p<0.05$).

Table 12: Pre- and Post-Trial Macronutrient Content (KCal) from Total Energy Intake for LOW-CON, LOW-INT. MOD-CON and MOD-INT

Macronutrient	LOW-CON	LOW-INT	MOD-CON	MOD-INT	P= Value
		Pre-Trial Macronutrients (KCal)			
Carbohydrate	1131 ± 394	1120 ± 347	1109 ± 349	1201 ± 337	0.770
Protein	449 ± 211	449 ± 184	413 ± 218	425 ± 179	0.594
Fat	856 ± 442	874 ± 335	834 ± 402	821 ± 322	0.239
Fibre	39 ± 17	40 ± 17	40 ± 17	40 ± 18	0.775
Water (g)	2635 ± 1744	2777 ± 1704	2728 ± 1740	2678 ± 1838	0.587
		Post-Trial Macronutrients (KCal)			
Carbohydrate	1094 ± 235	1223 ± 296	1198 ± 325	*****1639 ± 318	**0.022**
Protein	388 ± 76	404 ± 114	515 ± 233	554 ± 130	0.151
Fat	801 ± 247	906 ± 279	1024 ± 235	1241 ± 382	0.240
Fibre	38 ± 27	23 ± 13	40 ± 40	37 ± 27	0.106
Water (g)	2031 ± 1347	2054 ± 857	2187 ± 1160	2395 ± 1489	0.087

Data are means ± SD. Values are significant $P < 0.005$ * Indicates MOD-INT were significantly larger than MOD-CON. P = Values are documented as main interaction effect

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5.3.3. Heart rate (HR) and RPE

No main effect for intensity x modality x time interaction ($p = 0.942$, $\eta^2_p = 0.025$) was observed, however a main effect of intensity ($p < 0.001$, $\eta^2_p = 0.827$), modality ($p < 0.001$, $\eta^2_p = 0.945$) and time ($p < 0.001$, $\eta^2_p = 0.930$) was observed for RPE during exercise.

Post-hoc test revealed there was an effect for modality as MOD-INT was significantly higher than MOD-CON and LOW-INT was significantly higher than LOW-CON; main interaction effect ($p < 0.001$, $\eta^2_p = 0.449$) and an intensity effect as LOW-INT was significantly lower than MOD-INT and LOW-CON was significantly lower than MOD-CON; main interaction effect ($p < 0.001$, $\eta^2_p = 0.436$) , all time points were highly significant ($p < 0.001$) apart from interval one (Baseline) and two (10 min) ($p > 0.05$) (Figure 25A)

No main effect of time ($p = 0.089$, $\eta^2_p = 0.999$) was observed, however a main effect of intensity ($p < 0.001$, $\eta^2_p = 0.833$), modality ($p < 0.001$, $\eta^2_p = 0.945$) and intensity x modality x time interaction ($p = 0.045$, $\eta^2_p = 0.318$) was observed for heart rate during exercise. Post-hoc test revealed there were no further differences for modality as MOD-INT was not significantly different than MOD-CON, main interaction effect ($p = 0.337$, $\eta^2_p = 0.094$) and LOW-INT was also not significantly different than LOW-CON ($p = 0.070$, $\eta^2_p = 0.138$). Although, there was an intensity effect as LOW-INT was significantly lower than MOD-INT ($p < 0.001$, $\eta^2_p = 0.801$), all time points were highly significantly ($p < 0.001$) apart from interval one (124 ± 24 v 120 ± 12 bpm: $P = 0.580$, d = 0.22, 95% CI = -13.36 – 7.40 bpm), and LOW-CON was significantly lower than MOD-CON ($p < 0.001$, $\eta^2_p = 0.395$), all time points were highly significantly ($p < 0.001$; Figure 25B)

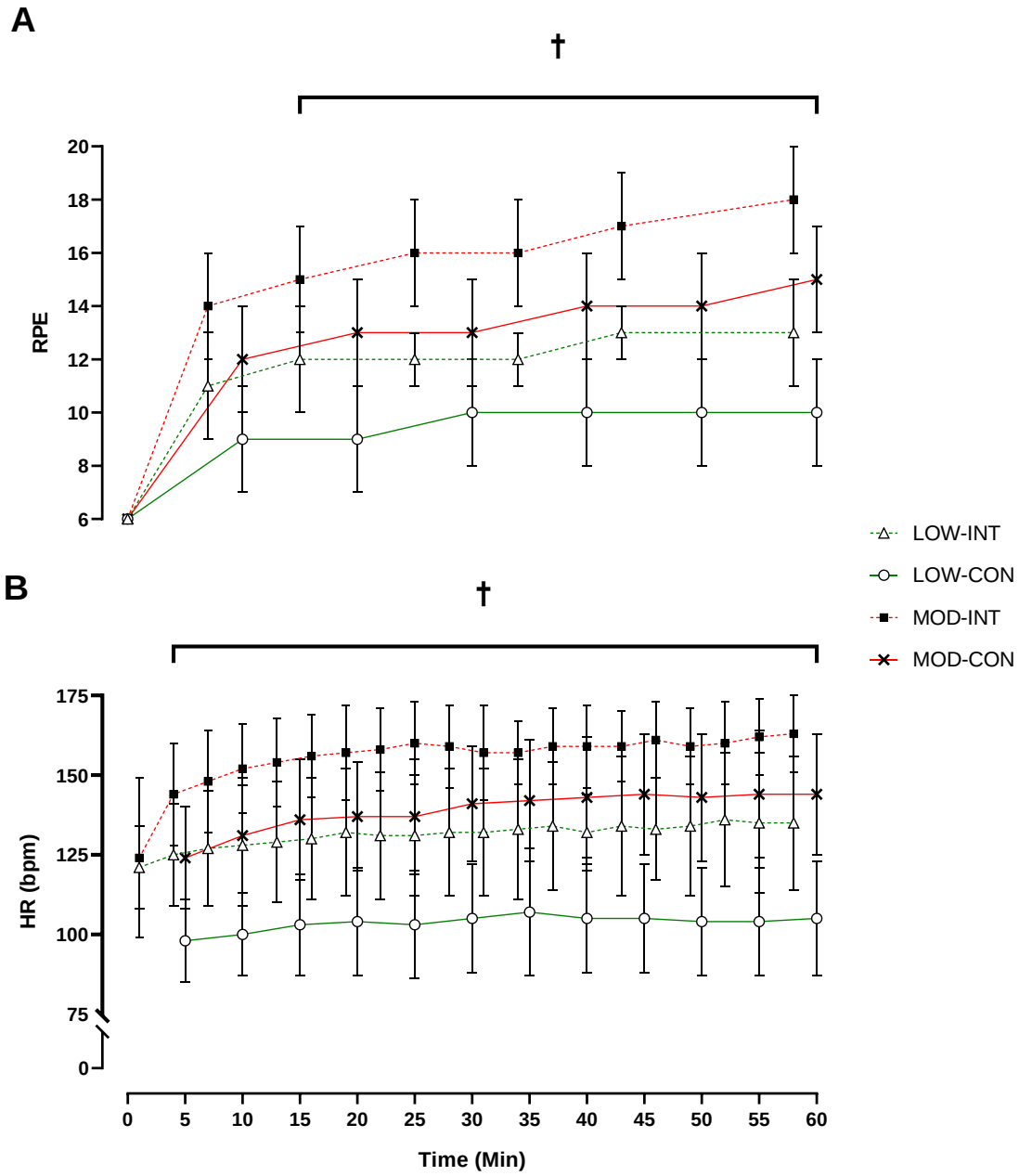

Figure 25: Representative of mean RPE (A) and HR (B), with vertical error bars display SDs (n = 12). There was no main effect of time ($p > 0.05$). However, there were a main effects for modality, intensity and a main interaction effect ($p < 0.05$) for HR and RPE during exercise, examined by a three-was repeated-measured ANOVA. † Indicates LOW-CON vs MOD-CON and LOW-INT vs MOD-INT were significantly different at each time point determined by Bonferroni adjusted paired t-test ($p < 0.05$). RPE; rate of perceived exertion. HR, heart rate; bpm, beats per minute.

5.3.4. Gastric Emptying

The time taken to empty half of the soup from the stomach (T_{half}) amounted to 111 ± 31 min, 106 ± 29 min, 95 ± 20 min and 107 ± 13 min on LOW-INT, LOW-CON, MOD-INT and MOD-CON, respectively. There was also no main effect of intensity (p = 0.591, η^2_p = 0.027), modality (p = 0.262, η^2_p = 0.113) or intensity x modality interaction effect (p = 0.055, η^2_p = 0.259; Figure 26B) observed for $T_{1/2}$. The time of maximal emptying rate (T_{lag}) amounted to 58 ± 15 min, 62 ± 15 min, 60 ± 17 min and 59 ± 15 min on LOW-INT, LOW-CON, MOD-INT and MOD-CON, respectively. No main effect of intensity (p =0.581, η^2_p = 0.029), modality (p = 0.990, η^2_p = 0.011) or intensity × modality interaction (p = 0.595, η^2_p = 0.027; Figure 26A) was observed for T_{lag}.

No main effect of intensity (p = 0.745, η^2_p = 0.010) was shown for delta over baseline AUC, however a main effect of modality (p = 0.003, η^2_p = 0.560) and intensity × modality interaction (p = 0.041, η^2_p = 0.328) were observed. Post-hoc test revealed significantly lower AUC for MOD-INT compared to MOD-CON (2246 ± 467 vs. 2670 ± 412 $^{13}CO_2$:$^{12}CO_2^{-1}$ 120 min^{-1}: p = 0.002, d = 1.01, 95% CI = −232.10 – 265.23 120 min^{-1}; Figure 27A).

No main effect of modality (p = 0.760, η^2_p = 0.009) or intensity × modality x time interaction (p = 0.302, η^2_p = 0.711) was observed for delta over baseline. A main effect of time (p < 0.001, η^2_p = 0.987) and intensity (p = 0.003, η^2_p = 0.568) was observed. Post-hoc test revealed MOD-INT was significantly lower than MOD-CON at **30-min** (20.33 ± 5.3 v 23.34 ± 4.1 $^{13}C0_2$:$^{12}C0_2$: P = 0.028, d = 0.66, 95% CI = −1.66 – 3.66 $^{13}C0_2$:$^{12}C0_2$); **45-min** (21.87 ± 4.8 v 25.39 ± 4.3 $^{13}C0_2$:$^{12}C0_2$: P = 0.019, d = 0.80, 95% CI = −1.64 – 3.51 $^{13}C0_2$:$^{12}C0_2$); **60-min** (22.76 ± 4.6 v 26.53 ± 4.5 $^{13}C0_2$:$^{12}C0_2$: P = 0.004, d = 0.87, 95% CI = −1.68 – 3.47 $^{13}C0_2$:$^{12}C0_2$); **75-min** (21.74 ± 4.2 v 26.15 ± 4.3 $^{13}C0_2$:$^{12}C0_2$: P = 0.001, d = 1.08, 95% CI = −1.35 – 3.46 $^{13}C0_2$:$^{12}C0_2$); **90-min** (20.59 ± 4.1 v 24.76 ± 4.2 $^{13}C0_2$:$^{12}C0_2$: P = 0.003, d = 1.04, 95% CI = −1.30 – 3.47 $^{13}C0_2$:$^{12}C0_2$), **105-min** (18.11 ± 4.2 v 22.84 ± 3.9 $^{13}C0_2$:$^{12}C0_2$: P = 0.001, d = 1.05, 95% CI = −1.33 – 3.37 $^{13}C0_2$:$^{12}C0_2$); and **120-min** (16.48 ± 4 v 20.47 ± 3.8 $^{13}C0_2$:$^{12}C0_2$: P = 0.001, d = 1.07, 95% CI = −1.08 – 3.33 $^{13}C0_2$:$^{12}C0_2$) post-meal

ingestion. These differences were not seen between the LOW-INT and LOW-CON trials ($P > 0.05$). All trials and time points were significantly increased from baseline to 120min ($p < 0.001$; Figure 27B).

Cumulative dose of percent $^{13}CO_2$, final values at 120 min were similar (LOW-INT, 22 ± 4 $^{13}CO_2$ %; LOW-CON, 23 ± 3 $^{13}CO_2$ %; MOD-INT, 21 ± 3 $^{13}CO_2$ % and MOD-CON, 24 ± 3 $^{13}CO_2$ %; $P = 0.104$, $\eta^2_p = 0.122$) (Figure 28)

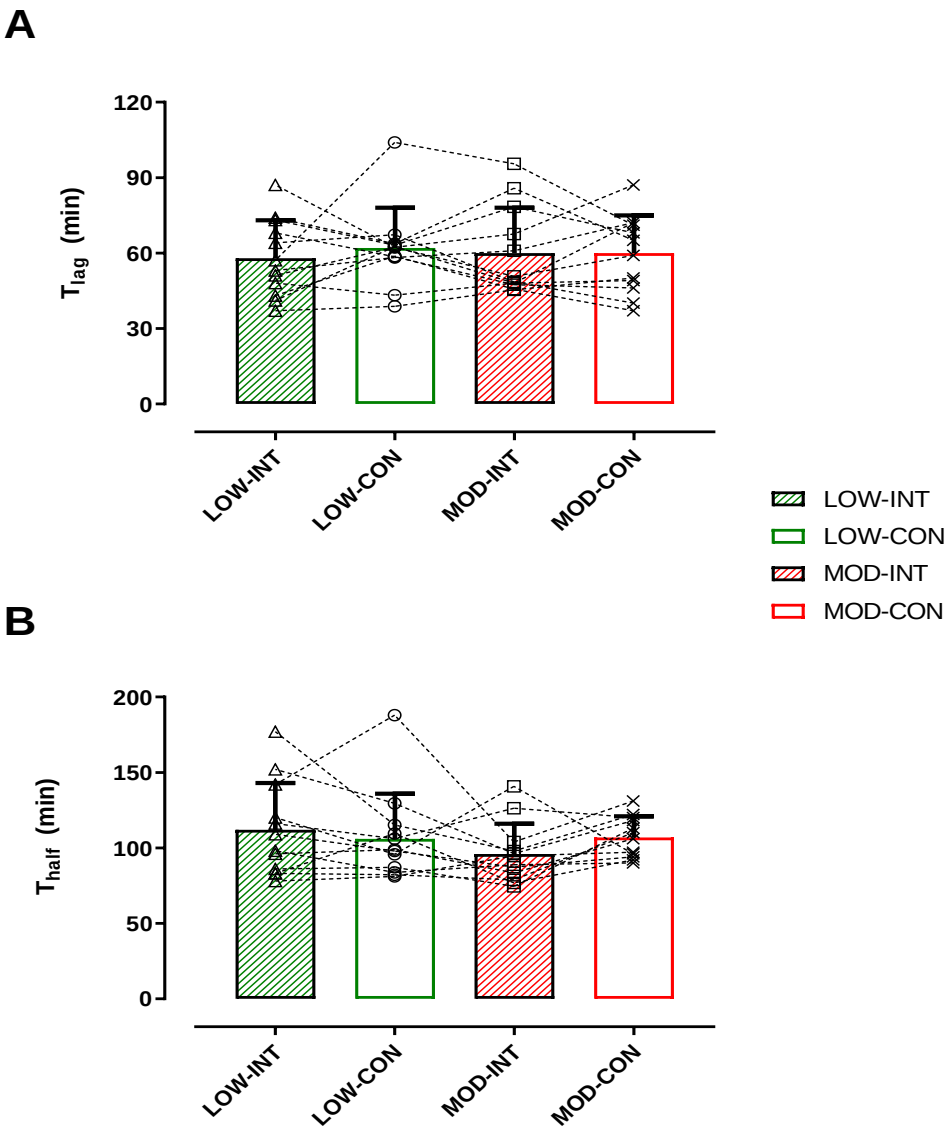

Figure 26: Representative of mean T_{lag} (A) and $T_{1/2}$ (B), with vertical error bars display SDs (n = 12). Individual data is represented as (Δ) LOW-INT, (○) LOW-CON, (□) MOD-INT and (×) represents MOD-CON. There were no main intensity, modality nor interaction effects observed for T_{lag} and $T_{1/2}$ ($P > 0.05$), examined by a two-way repeated-measures ANOVAs. T_{lag}, Time of maximal emptying rate; $T_{1/2}$, Half emptying time.

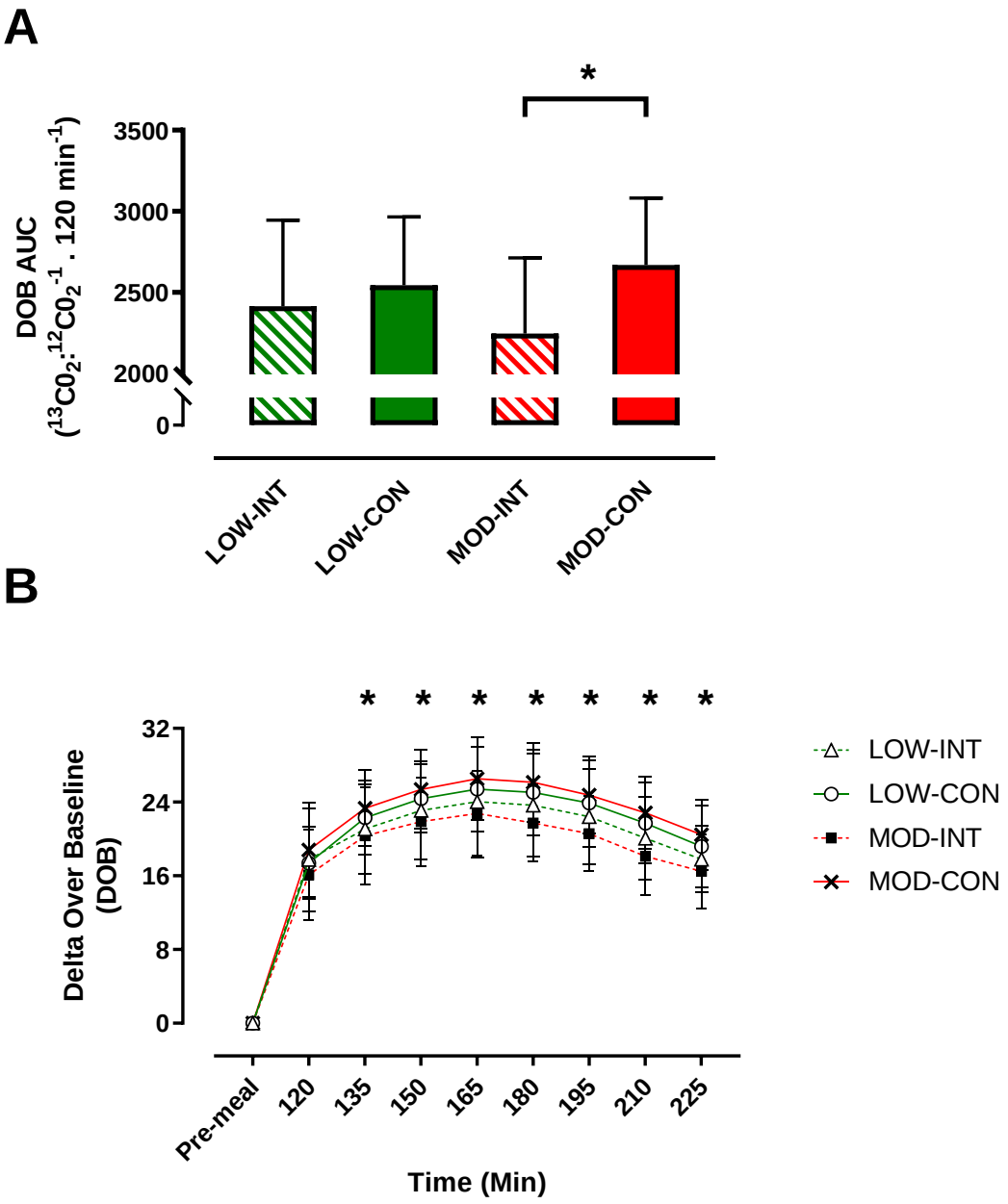

Figure 27: Representative of mean DOB AUC (0-120 min^{-1}) (A) and DOB respose (B), with vertical error bars display SDs (n = 12). There was no main effect of intensity ($p > 0.05$), but there was for modality and a main interaction effect for DOB-AUC ($p < 0.05$), examined by a two-way repeated-measures ANOVA. There were no main effects of modality nor interaction ($p > 0.05$). However, there was an effect for intensity and time ($p < 0.05$) for DOB, examined by a three-way repeated-measured ANOVA. * Indicates MOD-INT values are significantly different than MOD-CON, determined by Bonferroni adjusted paired t-test ($p < 0.05$). DOB, delta over baseline; AUC, area under curve.

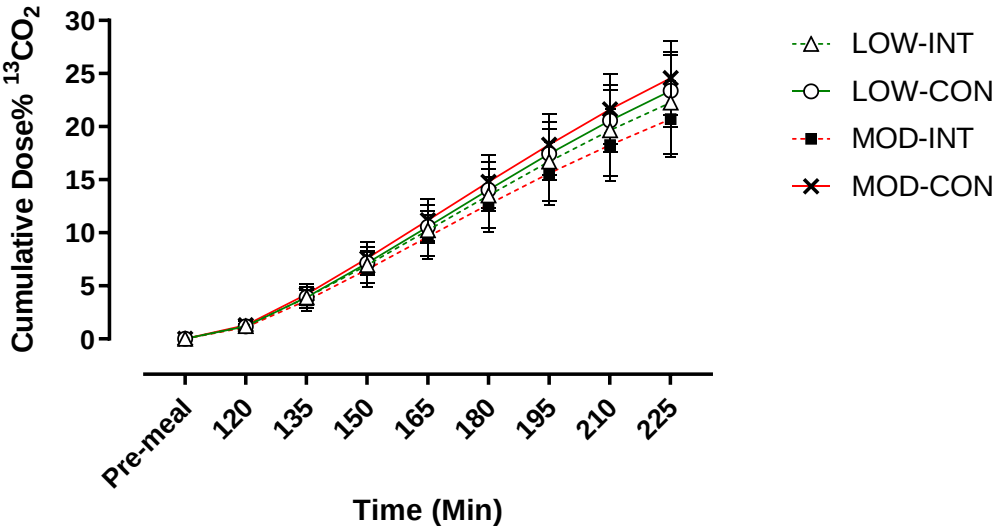

Figure 28: Representative of mean for cumulative dose of % $^{13}CO_2$, with vertical error bars display SDs (n = 12). There was a main effect of time (P < 0.001). However, no modality, intensity nor interaction effect were found for dose % $^{13}CO^2$ (P > 0.05), examined by three-way repeated-measures ANOVA.

5.3.5. Appetite

5.3.5.1. Hunger

Hunger decreased in all trials, apart from LOW-CON immediately post-exercise. Moreover, hunger also decreased after food ingestion before gradually rising throughout recovery in all trials. A main effect of time was observed for hunger (p < 0.001, η^2_p = 0.510). However, there were no intensity or modality effects for hunger (p = 0.222; η^2_p = 0.132: p = 0.895, η^2_p = 0.002) and therefore no intensity x modality x time interaction effect observed (p = 0.387, η^2_p = 0.976) (Figure 29B)

No main effect of intensity (p = 0.667, η^2_p = 0.017) nor a main effect of intensity × modality (p = 0.073, η^2_p = 0.264) was detected, however a main effect of modality (p = 0.034, η^2_p = 0.346) was observed for hunger AUC. A significantly lower AUC for MOD-INT compared to MOD-CON was reported (10011 ± 96 vs. 3103 ± 93 mm. 225 min: p = 0.002, d = 0.60, 95% CI = −2583.37 – 1756.26 mm. 225 min; Figure 29*A*), but not between LOW-INT compared to LOW-CON was reported (11363 ± 4567 vs. 11548 ± 2730 mm. 225 min: p = 0.835, d = 0.05, 95% CI = −1544.56 – 2584.03 mm. 225 min).

5.3.5.2. Fullness

Fullness increased in all trials, apart from LOW-CON immediately post-exercise. Moreover, fullness also increased after food ingestion before gradually decreasing throughout recovery in all trails. A main effect of time was observed for fullness ($p < 0.001$, $\eta^2_p = 0.617$). Although, there were no intensity or modality effects for fullness ($p = 0.437$, $\eta^2_p = 0.056$; $p = 0.237$, $\eta^2_p = 0.125$), but there was an intensity x modality x time interaction effect observed ($p = 0.041$, $\eta^2_p = 0.201$), However, post-hoc tests revealed no further differences between trials for fullness (Figure 30B)

No main effect of intensity ($p = 0.177$, $\eta^2_p = 0.159$), modality ($p = 0.254$, $\eta^2_p = 0.116$) nor a main effect of intensity × modality ($p = 0.985$, $\eta^2_p = 0.001$) was detected, for fullness AUC (Figure 30A).

5.3.5.3. PFC

PFC decreased in all trials, apart from LOW-CON immediately post-exercise. Moreover, PFC also decreased after food ingestion before gradually rising throughout recovery in all trials. A main effect of time was observed for PFC ($p < 0.001$, $\eta^2_p = 0.534$). However, there were no intensity or modality effects for PFC ($p = 0.300$, $\eta^2_p = 0.097$; $p = 0.485$, $\eta^2_p = 0.045$), but there was an intensity x modality x time interaction effect observed ($p = 0.04$, $\eta^2_p = 0.147$). Post-hoc tests revealed MOD-INT was significantly lower post-exercise compared to the other three trials ($p = 0.011$, d = 0.54, 95% CI = −8.18 – 15.84 mm) (Figure 31B)

No main effect of intensity ($p = 0.314$, $\eta^2_p = 0.092$), modality ($p = 0.052$, $\eta^2_p = 0.302$) nor a main effect of intensity × modality ($p = 0.220$, $\eta^2_p = 0.133$) was detected, for PFC AUC (Figure 31A).

5.3.5.4. Satisfaction

Satisfaction remained relatively stable in all trials immediately post exercise. Moreover, satisfaction increased after food ingestion before gradually decreasing throughout recovery in all trails. A main effect of time was observed for satisfaction ($p < 0.001$, $\eta^2_p = 0.640$). Although, there were no intensity or modality ($p = 0.353$, η^2_p

= 0.079; p = 0.530, η^2_p = 0.037) nor intensity x modality x time interaction effects observed for satisfaction (p = 0.430, η^2_p = 0.082) (Figure 32B)

No main effect of intensity (p = 0.581, η^2_p = 0.029), modality (p = 0.209, η^2_p = 0.139) nor a main effect of intensity × modality (p = 0.939, η^2_p = 0.001) was detected, for satisfaction AUC (Figure 32A).

5.3.5.5. Bloat

Bloat remained relatively stable in all trials apart from a small increase within MOD-INT immediately post exercise, Moreover, bloat increased after food ingestion before gradually decreasing throughout recovery in all trails. A main effect of time was observed for bloat (p < 0.001, η^2_p = 0.373). Although, there were no intensity or modality effects (p = 0.222, η^2_p = 0.076; p = 0.895, η^2_p = 0.075) nor intensity x modality x time interaction effect observed for bloat (p = 0.47, η^2_p = 0.100) (Figure 33B)

No main effect of intensity (p = 0.557, η^2_p = 0.032), modality (p = 0.263, η^2_p = 0.112) nor a main effect of intensity × modality (p = 0.341, η^2_p = 0.083) was detected, for bloat AUC (Figure 33A).

5.3.5.6. Nausea

Nausea remained relatively stable in all trials across the whole trial, apart from an increase within MOD-INT immediately post exercise. A main effect of time (p < 0.001, η^2_p = 0.107), intensity (p < 0.018, η^2_p = 0.413) and an intensity x modality x time interaction effect observed for nausea (p < 0.001, η^2_p = 0.377). However, there was no eftect for modality (p = 0.069, η^2_p = 0.270). Post-hoc tests revealed MOD-INT was higher post-exercise (p = 0.011, d = 1.24, 95% CI = −13.47 – 4.18 mm; Figure 34B)

There was a main effect of intensity (p = 0.032, η^2_p = 0.353), modality (p = 0.012, η^2_p = 0.448) and a main effect of intensity × modality (p = 0.014, η^2_p = 0.439) was observed for nausea AUC. A significantly higher AUC for MOD-INT compared to MOD-CON was reported (1925 ± 512 vs. 588 ± 186 mm. 225 min: p = 0.009, d =

3.63, 95% CI = −286.06 – 108.86 mm. 225 min; Figure 29A), but not between LOW-INT compared to LOW-CON was reported (599 ± 188 vs. 604 ± 237 mm. 225 min: p = 0.974, d = 0.02, 95% CI = −134.07 – 106.39 mm. 225 min).

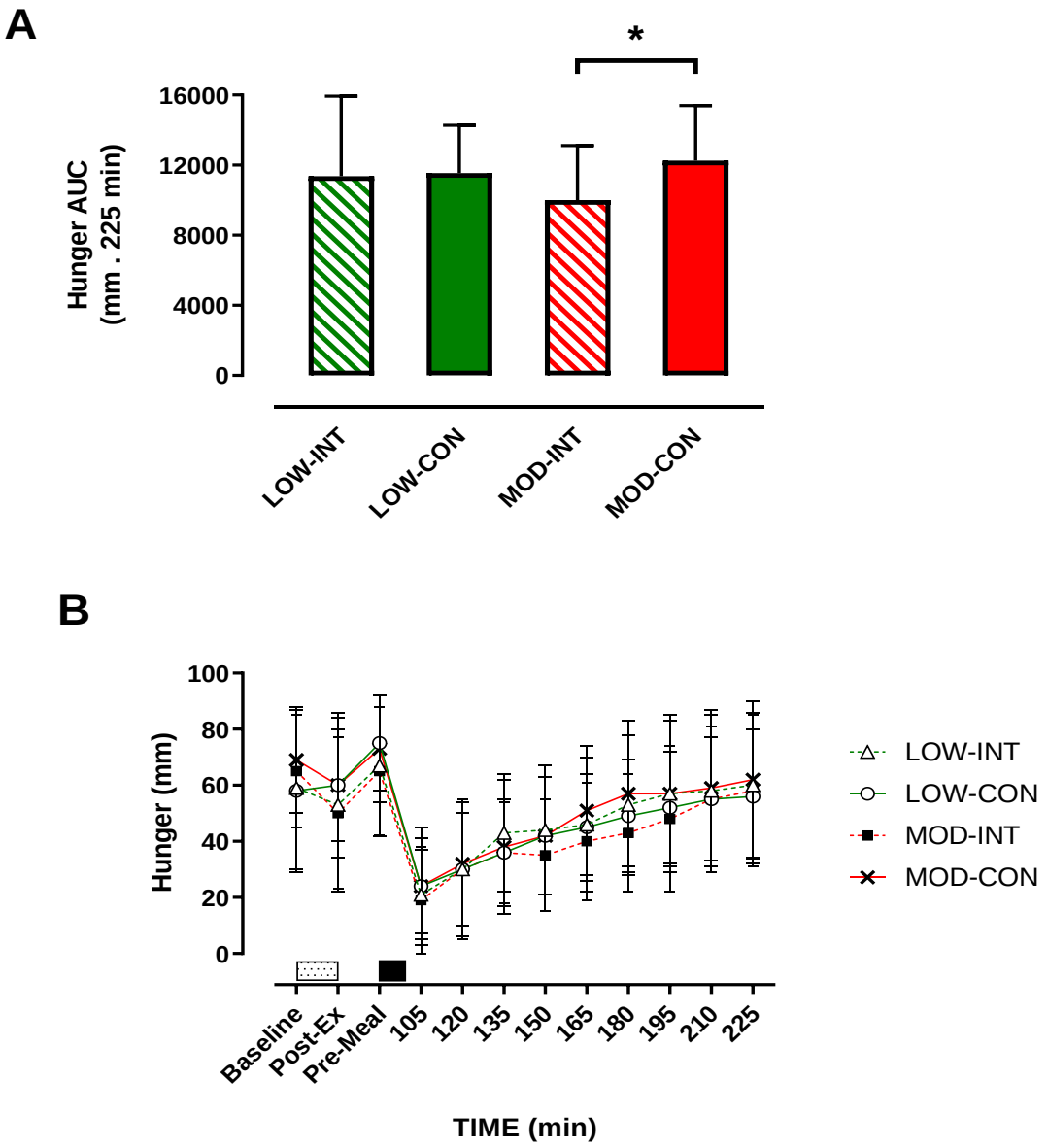

Figure 29: Representive of mean hunger AUC (0-225 min) (A) and hunger VAS response (B), with vertical error bars display SDs (n = 12). *Unfilled rectangle with black spots* indicates 60-min exercise period; *filled black rectangle* indicates a semi-solid meal. There was no main effect of intensity ($p > 0.05$), but there was for modality and a main interaction effect for hunger AUC ($p < 0.05$), examined by a two-way repeated-measured ANOVA. There were no main effect of modality, intensity nor interaction ($p > 0.05$). However, there was an effect for time ($p < 0.05$) for hunger, examined by a three-was repeated-measured ANOVA. * Indicates MOD-INT values were significantly different than MOD-CON, determined by Bonferroni adjusted paired t-test ($p < 0.05$). AUC, area under curve.

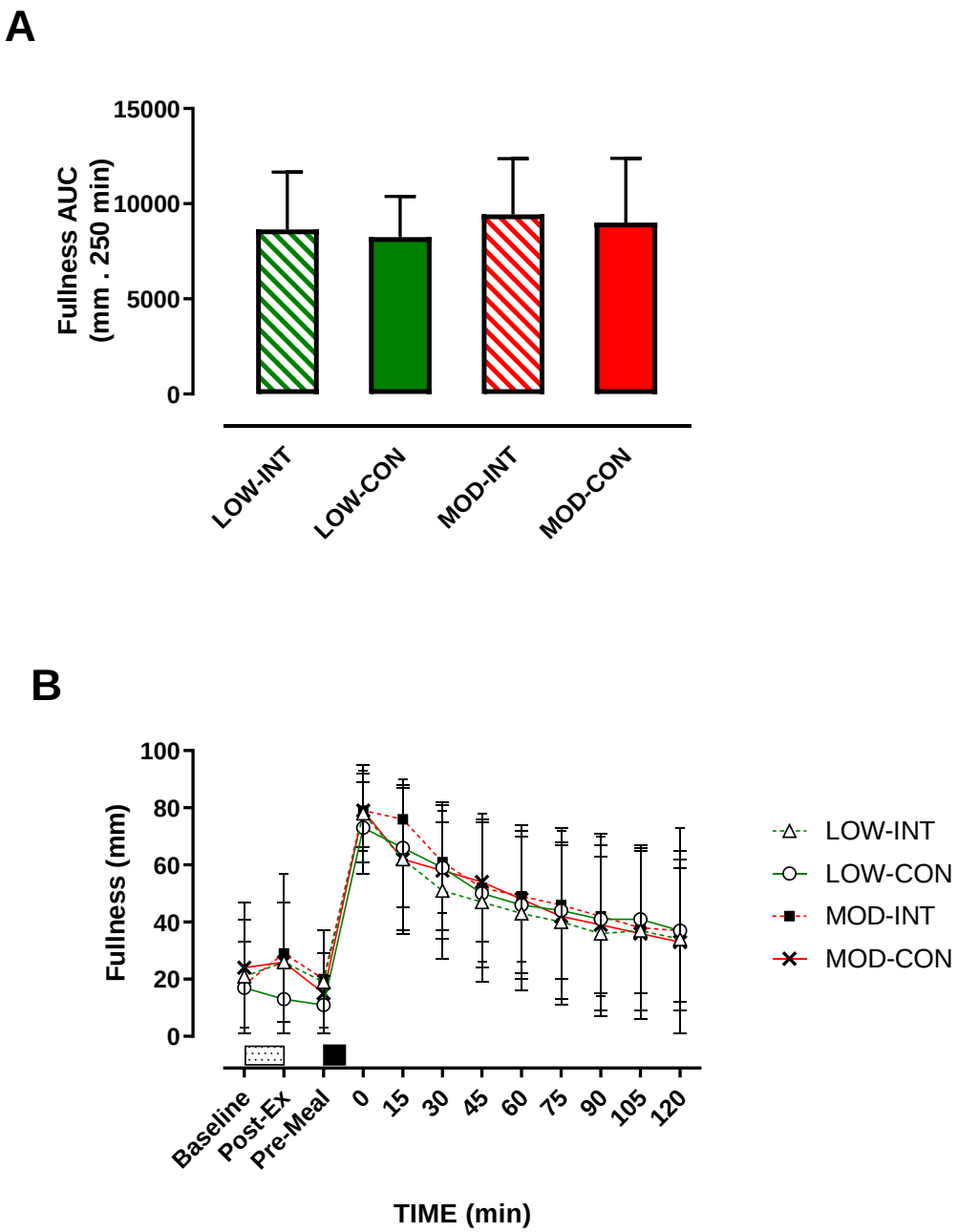

Figure 30: Representative of mean fullness-AUC (0-225 min) (A) and fullness VAS response (B), with vertical error bars display SDs (n = 12). *Unfilled rectangle with black spots* indicates 60-min exercise period; *filled black rectangle* indicates a semi-solid meal. There was no main effect of intensity, modality nor main interaction effect for fullness-AUC ($p > 0.05$), examined by a two-way repeated-measured ANOVA. There were no main effect of modality nor intensity ($p > 0.05$). However, there were main effects for interaction and time observed for fullness ($p < 0.05$), examined by a three-way repeated-measured ANOVA. AUC, area under curve.

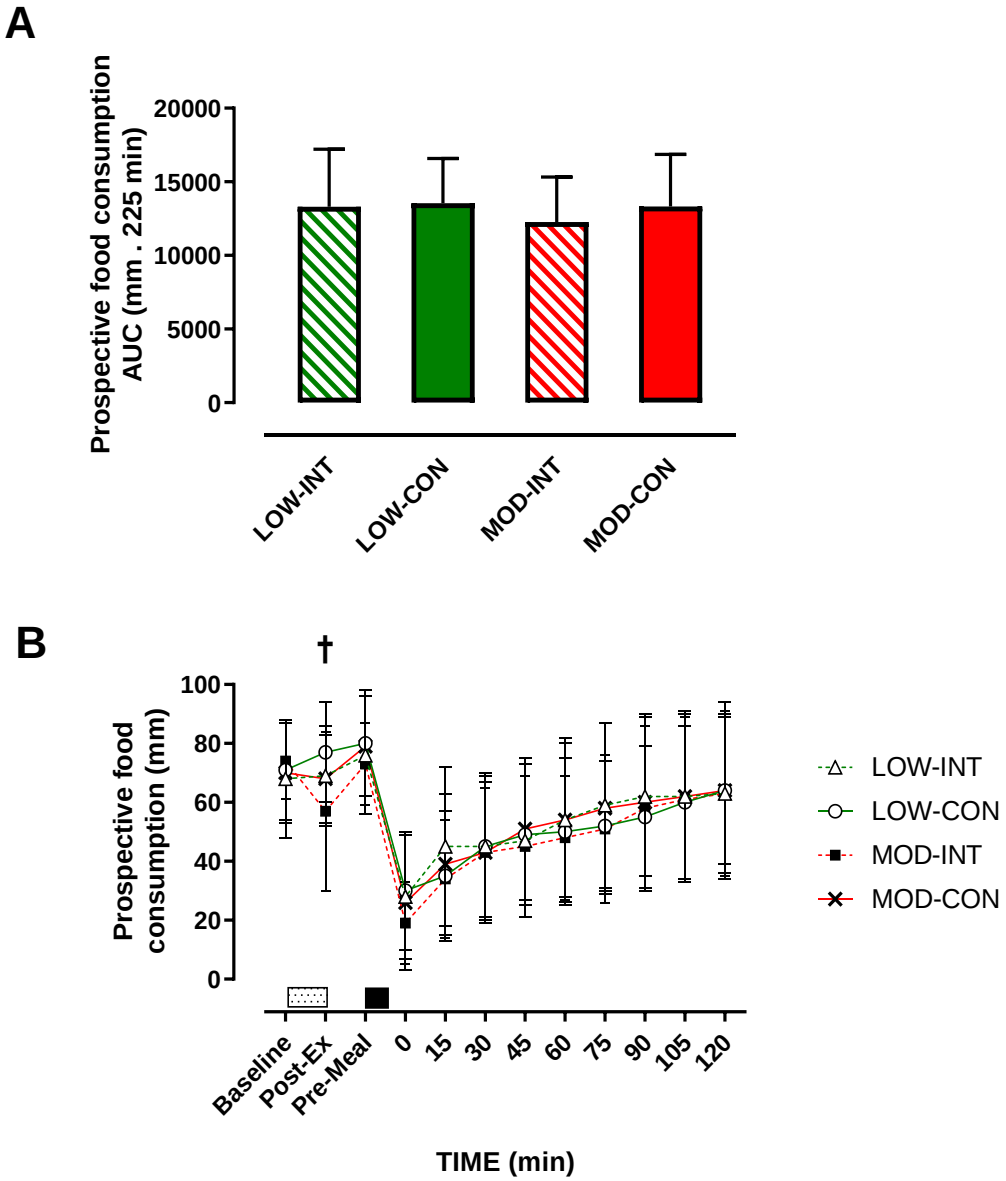

Figure 31: Representative of mean PFC AUC (0-225 min) (A) and PFC VAS response (B), with vertical error bars display SDs (n = 12). *Unfilled rectangle with black spots* indicates 60-min exercise period; *filled black rectangle* indicates a semi-solid meal. There were no main effects of intensity, modality nor main interaction effect for PFC-AUC ($p > 0.05$), examined by a two-way repeated-measured ANOVA. There were no main effect of modality nor intensity ($p > 0.05$). However, there was for time, a main interaction effect ($p < 0.05$), examined by a three-way repeated-measured ANOVA. † Indicates MOD-INT were significantly different from all other trials determined by Bonferroni adjusted paired t-test ($p < 0.05$). PFC, prospective food consumption; AUC, area under curve.

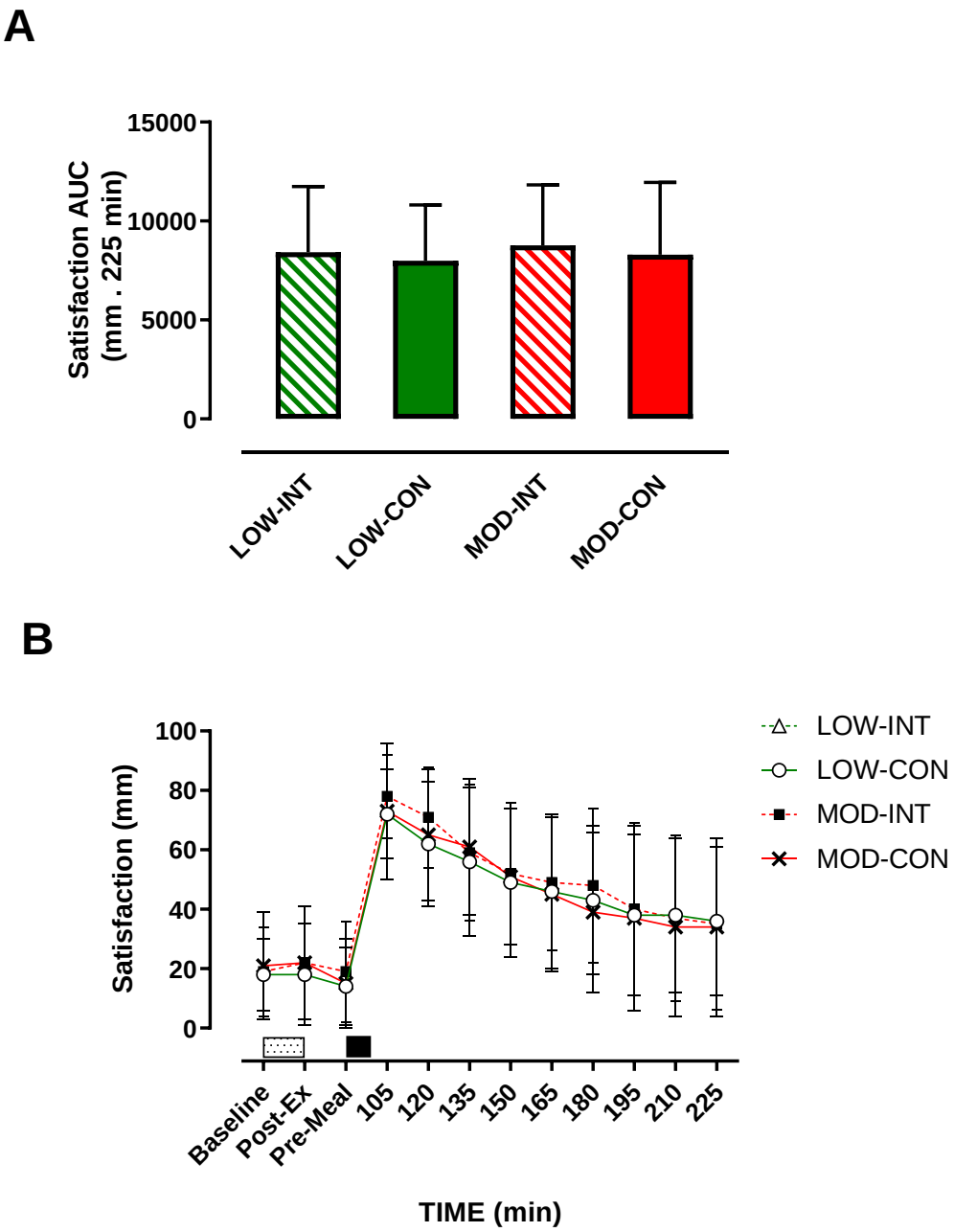

Figure 32: Representative of mean satisfaction AUC (0-225 min) (A) and satisfaction VAS response (B), with vertical error bars display SDs (n = 12). *Unfilled rectangle with black spots* indicates 60-min exercise period; *filled black rectangle* indicates a semi-solid meal. There was no main effect of intensity, modality nor main interaction effect for satisfaction AUC ($p > 0.05$), examined by a two-way repeated-measured ANOVA. There were no main effects of modality, intensity nor interaction effect ($p > 0.05$). However, there was a main effect for time observed for satisfaction ($p < 0.05$), examined by a three-way repeated-measured ANOVA. AUC, area under curve.

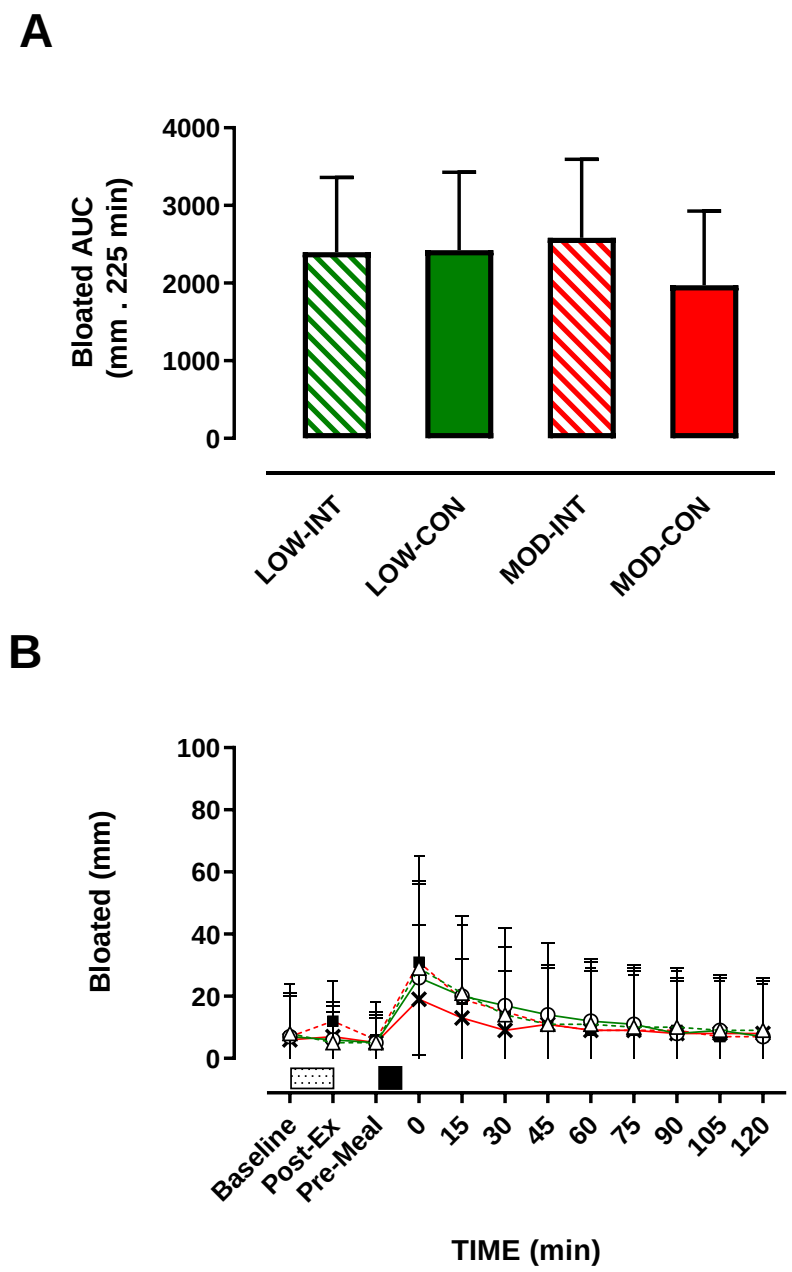

Figure 33 Representative of mean bloat AUC (0-225 min) (A) and bloat VAS response (B), with vertical error bars display SDs (n = 12). *Unfilled rectangle with black spots* indicates 60-min exercise period; *filled black rectangle* indicates a semi-solid meal. There weas no main effect of intensity, modality nor main interaction effect for bloat AUC ($p > 0.05$), examined by a two-way repeated-measured ANOVA. There were no main effects of modality, intensity nor interaction effect ($p > 0.05$). However, there was a main effect for time observed ($p < 0.05$), examined by a three-way repeated-measured ANOVA. AUC, area under curve

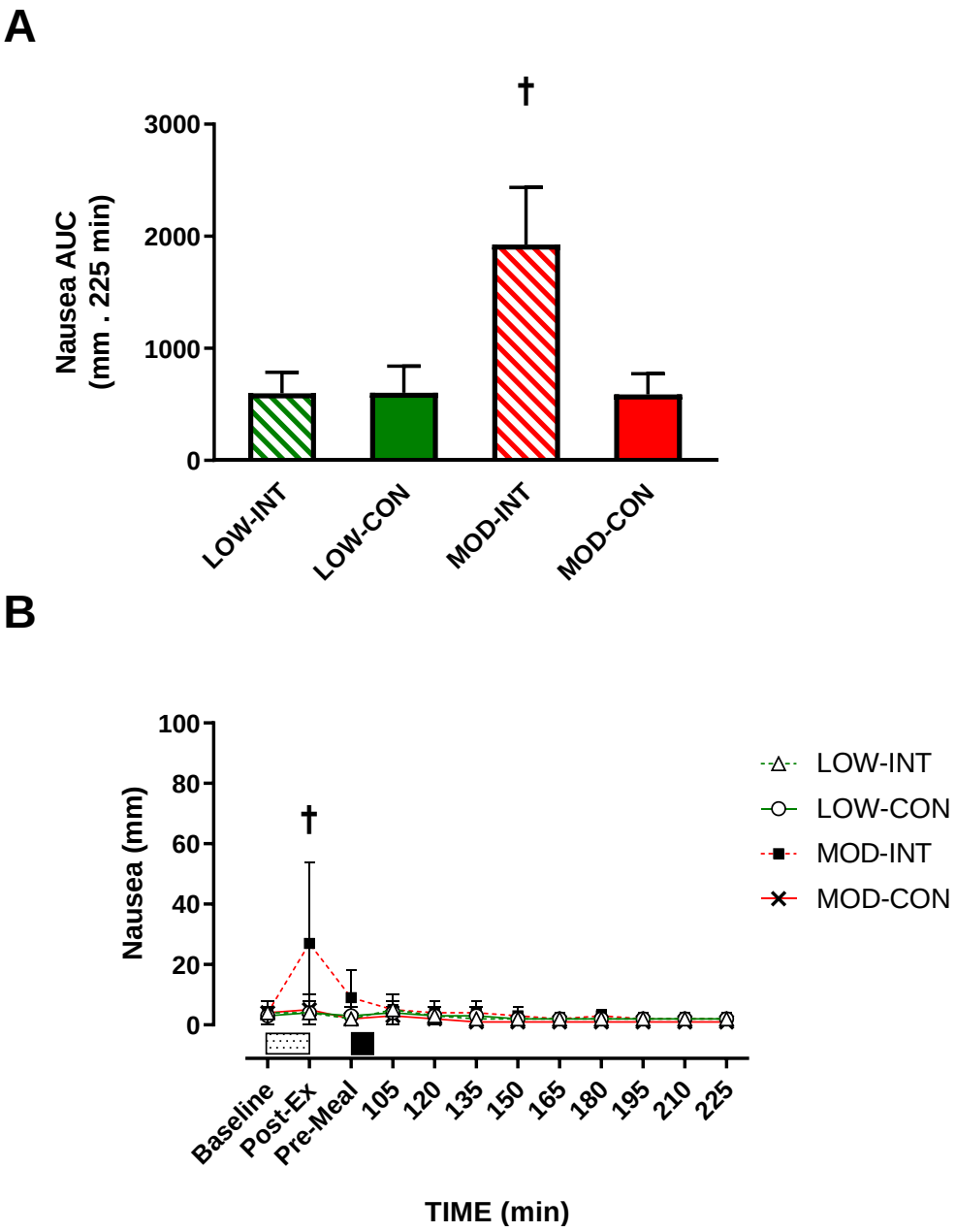

Figure 34: Representative of mean nausea AUC (0-225 min) (A) and nausea VAS response (B), with vertical error bars display SDs (n = 12). *Unfilled rectangle with black spots* indicates 60-min exercise period; *filled black rectangle* indicates a semi-solid meal. A main intensity, modality and main interaction effect for nausea AUC was observed ($p > 0.05$), examined by a two-way repeated-measured ANOVA. There was no main effect for modality ($p > 0.05$). However, there was for intensity, time and main interaction effect ($p < 0.05$), examined by a three-way repeated-measures ANOVA. † Indicates MOD-INT were significantly different from all other trials determined by Bonferroni adjusted paired t-test ($p < 0.05$). AUC, area under curve.

5.3.6. Blood Glucose Concentration

No main effect of modality ($p = 0.638$, $\eta^2_p = 0.021$) was observed, however a main effect of intensity ($p = 0.036$, $\eta^2_p = 0.342$), time ($p < 0.001$, $\eta^2_p = 0.882$) and intensity x modality x time interaction ($p = 0.009$, $\eta^2_p = 0.210$) was observed for blood glucose concentration. Post-hoc tests revealed that MOD-INT was significantly higher mid-exercise (4.9 ± 0.4 mmol.L: $p = 0.016$, $\eta^2_p = 0.266$) compared to the other three trials. LOW-CON was significantly higher than LOW-INT post-exercise (4.8 ± 0.5 vs. 4.4 ± 0.5 mmol.L: $p = 0.004$, d = 0.84, 95% CI = 0.55 – 1.12 mmol.L). Subsequently, blood glucose was higher 30-min post-meal ingestion for LOW-CON compared to LOW-INT (7.6 ± 1.0 vs. 7.0 ± 1.0 mmol.L: $p < 0.001$, d = 0.64, 95% CI = 0.07 – 1.20 mmol.L). This was also replicated during the MOD trials as blood glucose was higher during MOD-CON compared to MOD-INT (7.5 ± 0.9 vs. 6.5 ± 0.9 mmol.L: $p < 0.001$, d = 1.07, 95% CI = 0.55 – 1.57 mmol.L; Figure 35B). There were no further differences for blood glucose during the remaining recovery period post-meal (135-225 min).

No main effect of intensity ($p = 0.634$, $\eta^2_p = 0.021$) nor modality ($p = 0.107$, $\eta^2_p = 0.164$) was detected, however a main effect of intensity × modality ($p = 0.022$, $\eta^2_p = 0.394$) was observed for blood glucose AUC. A significantly lower AUC for LOW-INT compared to LOW-CON was reported (1027 ± 96 vs. 1085 ± 93 mmol.L^{-1} 225 min^{-1}: $p = 0.003$, d = 0.64, 95% CI = −51.98 – 54.96 225 min^{-1}; Figure 35A), but not between MOD-INT compared to MOD-CON was reported (1042 ± 61 vs. 1057 ± 77 mmol.L^{-1} 225 min^{-1}: $p = 0.533$, d = 0.20, 95% CI = −43.36 – 43.77 min^{-1}).

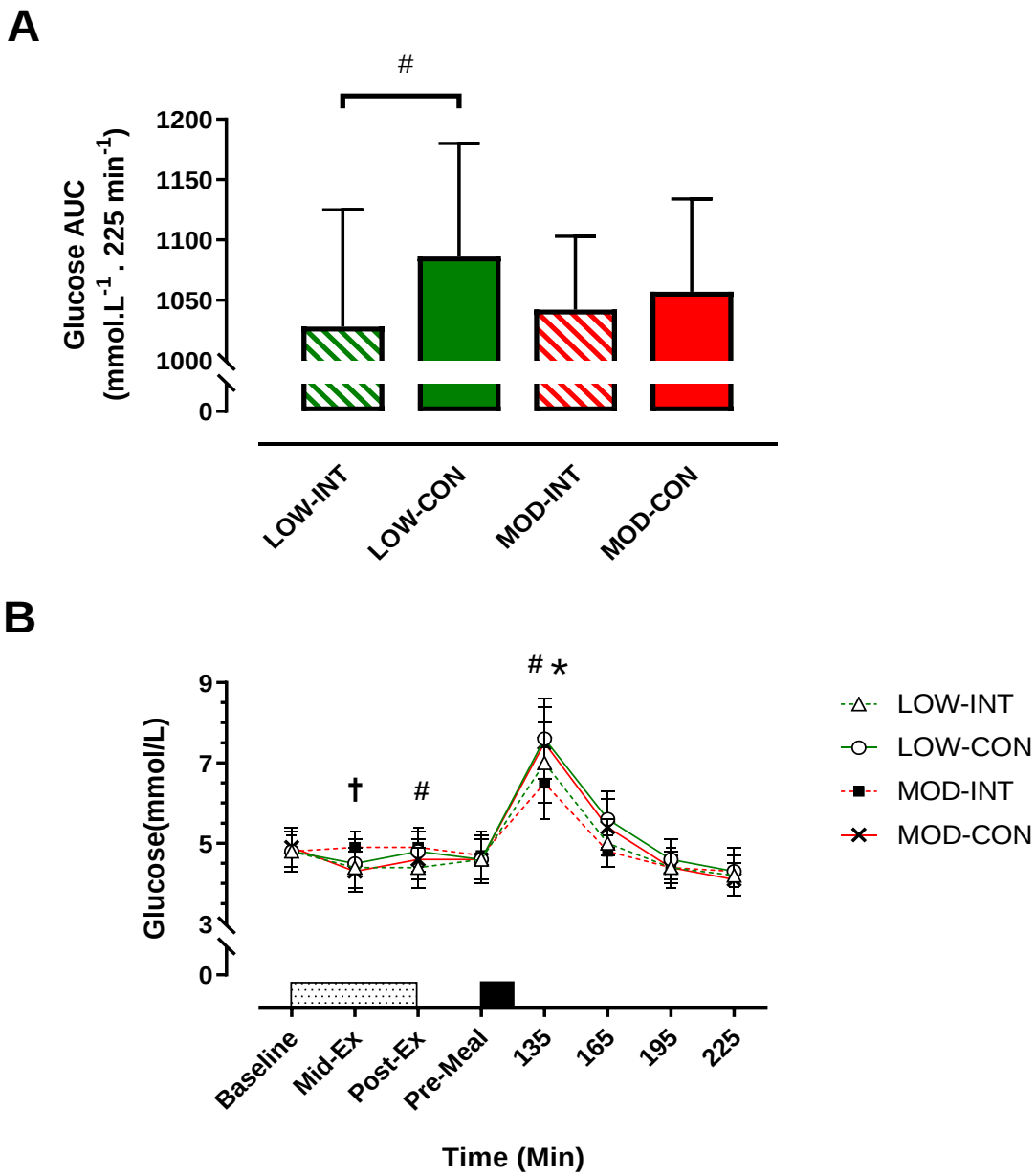

Figure 35: Representative of mean blood glucose AUC (0-225 min) (A) and blood glucose response (B), with vertical error bars display SDs (n = 12). *Unfilled rectangle with black spots* indicates 60-min exercise period; *filled black rectangle* indicates a semi-solid meal. There were no main effects of intensity nor modality ($p > 0.05$), but there weas a main interaction effect for blood glucose AUC ($p < 0.05$), examined by a two-way repeated-measured ANOVA. There was no main effect of modality ($p > 0.05$). However, there was a main intensity, time, and an interaction effect ($p < 0.05$) for blood glucose response, examined by a three-way repeated-measured ANOVA. † Indicates MOD-INT were significantly higher than all other trials. # Indicates LOW-INT were significantly different from LOW-CON. *Indicates MOD-INT were significantly different from MOD-CON, determined by Bonferroni adjusted paired t-test ($p < 0.05$). AUC, area under curve.

5.3.7. Substrate Utilisation

5.3.7.1. Carbohydrate Oxidation

No main effect of intensity ($p = 0.677$, $\eta^2_p = 0.016$), modality ($p = 0.346$, $\eta^2_p = 0.081$), or intensity × modality x time interaction ($p = 0.766$, $\eta^2_p = 0.023$) was observed for carbohydrate oxidation response. Although, a main effect of time ($p < 0.001$, $\eta^2_p = 0.409$) was observed. Post-hoc tests revealed carbohydrate oxidation significantly increased from baseline at 60-min post-meal ingestion ($P = 0.047$, $\eta^2_p = 0.123$) in all four trials. However, LOW-CON was the only trial to remain elevated 90-min post meal ingestion compared to baseline (0.31 ± 0.07 vs. 0.23 ± 0.08 g/min: $p = 0.001$, d = 1.11, 95% CI = 1.76 –1.16 g/min; Figure 36B)

There was no main effect of intensity ($p = 0.543$, $\eta^2_p = 0.035$), modality ($p = 0.445$, $\eta^2_p = 0.054$) nor intensity x modality interaction ($p = 0.158$, $\eta^2_p = 0.172$) for carbohydrate oxidation AUC (Figure 36A).

5.3.7.2. Fat Oxidation

No main effect for intensity ($p = 0.639$, $\eta^2_p = 0.021$), modality ($p = 0.170$, $\eta^2_p = 0.164$) or intensity × modality × time interaction ($p = 0.939$, $\eta^2_p = 0.018$) was observed for fat oxidation response. Although, a main effect of time ($p < 0.001$, $\eta^2_p = 0.512$) was observed as fat oxidation significantly increased from baseline at 30-min post-meal ingestion for MOD-INT (0.064 ± 0.04 v 0.113 ± 0.04 g/min: $P = 0.003$, d = 1.45, 95% CI = 1.42 – 1.46 g/min) and MOD-CON (0.063 ± 0.03 v 0.105 ± 0.03 g/min: $P = 0.001$, d = 1.46, 95% CI = 1.45 – 1.48 g/min). Although, LOW trials also increased this small increase was not significant; LOW-INT (0.057 ± 0.05 v 0.096 ± 0.04 g/min: $P = 0.051$, d = 0.90, 95% CI = 0.88 – 0.93 g/min) and LOW-CON (0.073 ± 0.04 v 0.100 ± 0.04 g/min: $P = 0.050$, d = 0.71, 95% CI = 0.68 – 0.73 g/min; Figure 37B)

There was no main effect of intensity ($p = 0.262$, $\eta^2_p = 0.113$) or modality ($p = 0.921$, $\eta^2_p = 0.001$). An intensity × modality interaction was observed for fat oxidation AUC ($p = 0.041$, $\eta^2_p = 0.327$; Figure 37A), however, post-hoc tests revealed no further differences between trials for fat oxidation AUC.

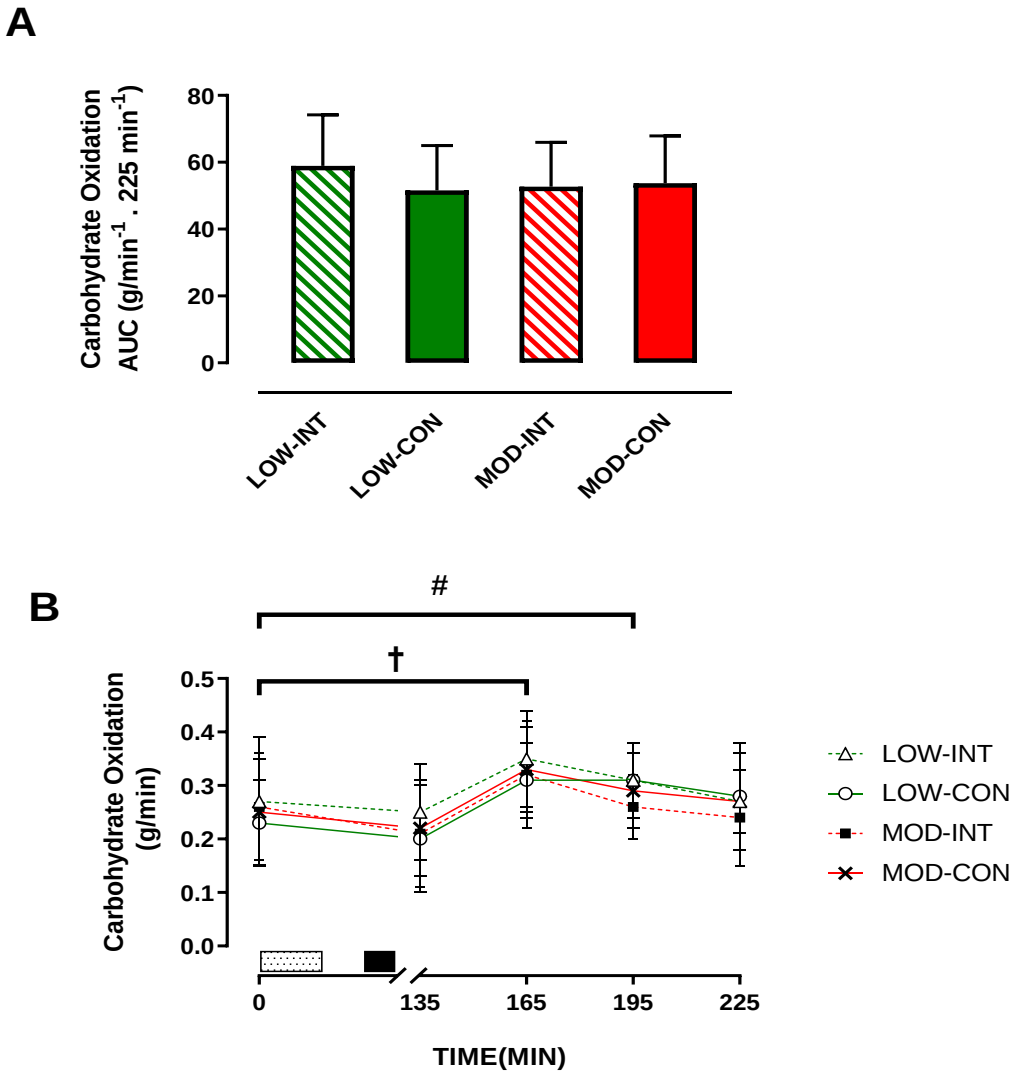

Figure 36: Representative of mean carbohydrate oxidation AUC (0-225 min) (A) and carbohydrate oxidation response (B), with vertical error bars display SDs (n = 12). *Unfilled rectangle with black spots* indicates 60-min exercise period; *filled black rectangle* indicates a semi-solid meal. There were no main effects of intensity, modality nor main interaction effect for carbohydrate oxidation AUC ($p > 0.05$), examined by a two-way repeated-measured ANOVA. There were no main effects of modality, intensity nor main interaction effect ($p > 0.05$). However, there was a main effect for time ($p < 0.05$) for carbohydrate oxidation response, examined by a three-way repeated-measured ANOVA. † Indicates all trials significantly increased from baseline to 60-min post meal. # Indicates LOW-CON trial significantly increased from baseline to 90-min post-meal determined by Bonferroni adjusted paired t-test ($p < 0.05$). AUC, area under curve.

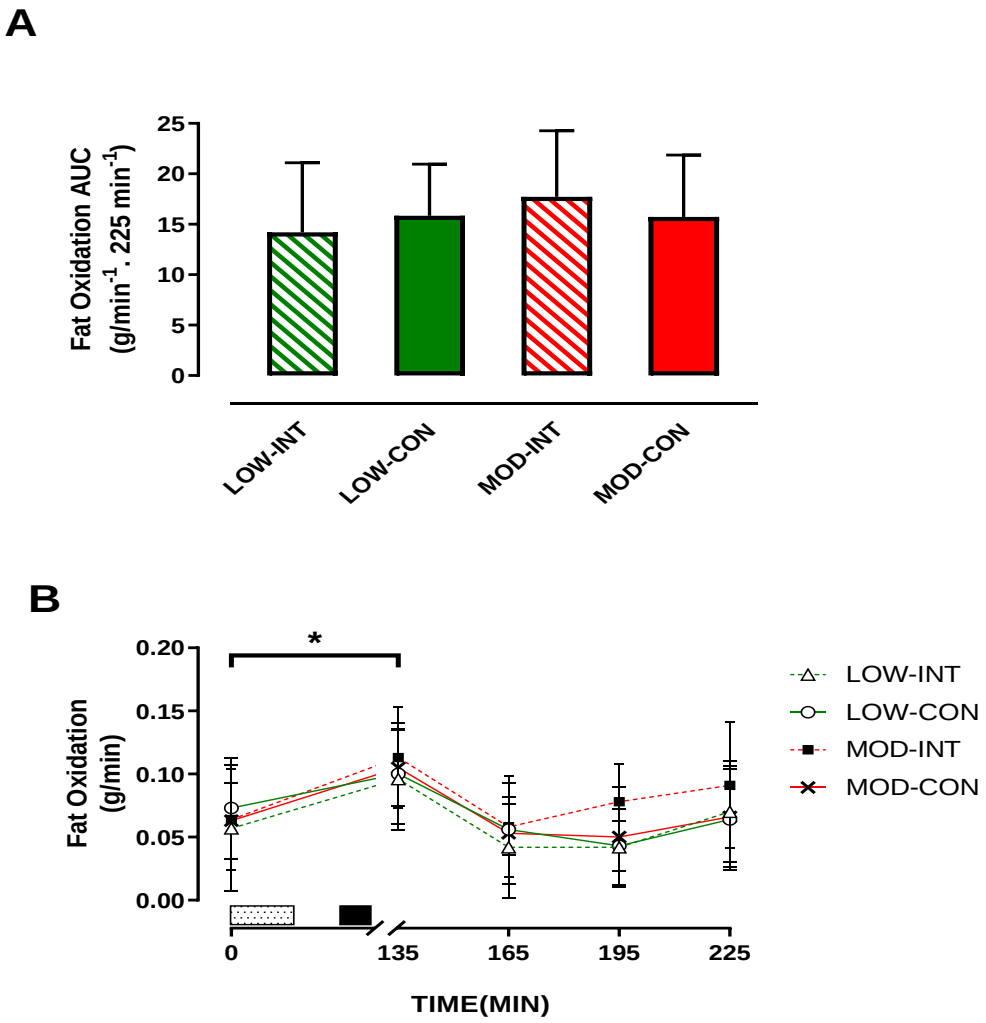

Figure 37: Representative of mean fat oxidation AUC (0-225 min) (A) and fat oxidation response (B), with vertical error bars display SDs (n = 12). *Unfilled rectangle with black spots* indicates 60-min exercise period; *filled black rectangle* indicates a semi-solid meal. There were no main effects of intensity nor modality ($p > 0.05$). However, there was a main interaction effect for fat oxidation AUC ($p < 0.05$), examined by a two-way repeated-measured ANOVA. There were no main effects of modality, intensity nor main interaction effect ($p > 0.05$). However, there was a main effect for time ($p < 0.05$) for fat oxidation response, examined by a three-way repeated-measured ANOVA. * Indicates MOD-INT and CON were significantly increased from baseline to 30-min post-meal determined by Bonferroni adjusted paired t-test ($p < 0.05$). AUC, area under curve.

5.4 Discussion

The primary aim of this investigation was to examine the effect of INT/CON exercise at different exercise intensities on GI responses and subsequent appetite response following ingestion of a standardized semi-solid meal. The main findings were that modality of exercise appears to have little impact on the markers measured during this study when performed at a low and moderate intensity. However, 24-h post-exercise EI increased by approximately ~21% in the MOD-INT compared to MOD-CON despite the activities being matched for power output. Further studies are required to determine whether GER, might have influenced the increase in 24-h EI post-exercise. It is also important to add that the participants in the present study were healthy, physically active men, so whether these findings extend to different populations (females or overweight/obese) is currently unknown.

To date, this is the first study to examine GER of a semi-solid meal after moderate intensity intermittent exercise compared to an energy matched continuous exercise bout. There are a number of physiological factors that regulate GER, including GI hormones such as ghrelin, peptide YY (PYY) and glucagon-like peptide-1 (GLP-1). GLP-1 and PYY have been shown to increase post-exercise which may increase parasympathetic activation, providing a potential mechanism why GER was unaffected in the present study (Schubert et al., 2014).

In contrast, ghrelin levels are usually high pre-exercise and decline immediately after exercise before gradually increasing prior to food intake as seen within chapter 4 (Mattin et al., 2018b). Although appetite regulating hormones were not measured within the present study, appetite was assessed using a VAS. Previous studies that have used VAS have reported exercise intensity > 60% $\dot{V}O_{2max}$ results in suppression of appetite in untrained individuals (Martins et al., 2007a, Becker et al., 2012, King et al., 2013a). In the present study, PFC was significantly lower post-exercise and nausea was higher only during the MOD-INT trial. An increase in nausea within the present study may suggest why subjective measures of hunger were subdued in all trials apart from LOW-CON which increased immediately post-exercise, however, there was no significant difference observed. It is important to add; regardless of this result, hunger in the short 2-h monitoring period after consuming a standardized semi-solid meal

responded similarly in recovery, irrespective of modality of exercise or intensity. These findings are consistent with Holliday et al (2017c), who reported no significant reduction in subjective appetite when participants completed a bout of high intensity aerobic exercise. Although subjective appetite was unchanged, the time taken to eat the semi-solid meal was marginally longer during the MOD-INT trial compared to MOD-CON. It should be considered that when participants were challenged to consume the whole semi-solid meal 30 min after exercise, it took 29 s longer to consume the meal on the MOD-INT trial compared to its counterpart. This result may suggest a possible suppression in appetite post-exercise may also be indicated by the prospective food consumption data as food volume, energy density and macronutrient composition all influence postprandial fullness (Rolls et al., 1998, Marciani et al., 2015), and so in the present study, all of these components of the meal were standardized. Proposing the delay in meal consumption did not compromise appetite regulation during the 2-h recovery period after exercise helping to maintain an energy deficit after both exercise modality and intensity.

Despite appetite responding similarly within the postprandial period in the current study, EI was ~21% (723 kCal) higher 24-h post-trial for MOD-INT compared to MOD-CON. In contrast, the majority of the available research suggests that exercise does not stimulate any changes in EI > 20-h after exercise (King et al., 1997, Hanlon et al., 2012) when using a self-reported measurement of food intake. In addition, King et al, (2010a) provided each participant with an overnight food bag and also found EI remained unchanged. Intermittent exercise has been suggested to evoke greater weight loss than traditional endurance exercise due to greater reductions in appetite during the post-exercise period (Trapp et al., 2008, Boutcher, 2011, Deighton and Stensel, 2014). These findings indicate that the moderate intensity intermittent trial stimulated an increase in EI 24-h post-trial. The mechanism for this increase in EI after the MOD-INT trial is not directly clear. Hengist et al, (2020) assessed the metabolic responses to maximal eating and discovered that participants who consumed on average nearly double the EI when compared to *ad libitum* eating, had marginal differences in physiological responses and glycaemic control within the post-prandial period, suggesting the increase in EI documented in the current study undoubtedly had very small physiological effects after a one-off single bout of intermittent exercise.

However, it must be considered that consuming excess energy will eventually lead to weight gain and therefore increase the risk of developing obesity. When comparing the present findings to previous data, it has been demonstrated among the majority of the scientific literature that land-based exercise does not stimulate increases in EI in the hours after exercise (King et al., 2010a, Deighton and Stensel, 2014). It must be noted, examining EI via a weighed dietary assessment may cause recall bias, as the nature of any documentation data collection method has potentially high participant variation. For this reason, caution must be used when interpreting this data.

Recent studies have provided a semi-solid meal after exercise and reported that low intensity (brisk walking) or cycling (Mattin et al., 2018b, McIver et al., 2018) did not affect GER after exercise. GER in humans has been shown to be affected by ingested volume and nutrient content (Noakes et al., 1991). For this reason, during the current study the meal provided was standardized for energy content and volume. It is well known that ingesting protein-rich food immediately after exercise stimulates muscle protein synthesis. Although strenuous continuous exercise delays GI function and delivery of nutrients to the circulation, Kashima et al, (2017) found that intermittent supramaximal cycling delayed GER of a 300 mL carbohydrate-protein drink when participants consumed the drink 5 and 30-min after exercise compared to a control. This discovery of delayed GER after intense exercise was suggested to be a result of small intestine mucosal damage as a significant increase in I-FABP was observed in both exercise trials. These findings suggest mucosal damage increases in response to strenuous exercise. Within the current study, a lower DOB for MOD-INT may indicate a potential mechanism, as delayed GER may also result in reduced intestinal absorption, affecting nutrient uptake post-exercise. Further research is needed to understand if small intestine permeability results in a delay in GER after strenuous exercise. However, this theory warrants further investigation as I-FABP was not measured in this study, hence whether increases in I-FABP may have an effect on GER is unclear.

Increased fat oxidation has been suggested to be beneficial for reducing fat mass (Achten and Jeukendrup, 2004). The current study found fat oxidation peaked 30-min post ingestion in all conditions compared to baseline values, but a significant increase was only seen during MOD-INT/CON trials. This increase in fat oxidation suggests

exercising at 60% $\dot{V}O_{2peak,}$ regardless of the modality of exercise results in an increase in fat metabolism up to 30-min post food consumption. Nevertheless, beyond 60 min fat oxidation reduced, resulting in no differences between exercise conditions in the postprandial period. This corresponds with existing literature, as fasted exercise increases fat metabolism and feeding carbohydrate induces a greater increase in carbohydrate metabolism (Achten and Jeukendrup, 2004, McIver et al., 2018).

Furthermore, carbohydrate oxidation significantly peaked during LOW-INT/CON at 60–90 min and during the 2-h recovery, fat oxidation increased at 90- and 120-min only during the MOD-INT which might suggest carbohydrate oxidation was more heavily relied on in the later stages of MOD-INT compared to other trials. Glucose levels increased mid-exercise during MOD-INT and, regardless of intensity, were lower 30 min after food ingestion when intermittent trials were compared to continuous trials. This suggests that, within a non-endurance trained population, carbohydrate became the primary source for energy during intermittent exercise. This may have resulted in increased muscle and liver glycogen replacement during recovery to maximize muscle glycogen resynthesis, which might account for the change in fat oxidation in the postprandial period. This result should not be misinterpreted, as AUC data for both fat oxidation and carbohydrate oxidation did not show any significant differences. Therefore, the intermittent exercise trial corresponds to similar work output, as 60% $\dot{V}O_{2peak}$ continuous exercise did not significantly increase energy metabolism in the postprandial period.

5.4.1. Limitations

This study presents with both strengths and limitations. The main strength of this study is the crossover design, as each modality was matched for power output at a low and moderate intensity. This study is also one of the few that examines GER. A limitation was that we have not accounted for changes in gut-derived hormone data. Previous research has shown that ghrelin regulates GER (Levin et al., 2006, Falkén et al., 2013) and this may modulate feelings of hunger and EI. Further studies should further examine the differences in post-exercise energy demands after moderate intensity intermittent exercise to understand the reason why EI was higher, which could have a negative effect on energy balance and possibly relevant exercise

outcomes (i.e., weight loss). The manner in which EI were assessed might also be considered as a limitation. When using weighed diet recall it is difficult to minimize mistakes made by the participants and, in addition, the post-exercise diet analysis was undertaken by an experienced member of the research team rather than a qualified dietician, which could have introduced error or bias. Blinding participants from the modality of exercise was impossible and therefore the elevation in EI after the MOD-INT trial was possibly established for the reason that participants thought they should consume more food after the 'hard' intermittent exercise. Further work is needed to establish how EI is assessed in the 24–48 h after exercise.

Another limitation was the nature of the moderate intensity intermittent exercise when matched for power output to moderate intensity continuous exercise, as the healthy, untrained participants within the current study found this session extremely difficult, which resulted in two participants having to withdraw as they were unable to complete MOD-INT trial. This is not unusual as Martins et al, (2015) found inactive overweight individuals also struggled when exercise induced an energy expenditure of 250 kCal. This would suggest modality of exercise is an important consideration when designing physical activity sessions.

5.4.2. Conclusions

There was no change in GER between trials and similar appetite and substrate utilisation response in the short 2-h monitoring period after all exercise trials. However, 24-h EI following MOD-INT was greater than MOD-CON. The mechanisms behind this are unclear as exercise was matched for power output. These findings may have important implications for current exercise prescription guidelines as the modality of exercise appears to have little impact on these markers when performed at a low intensity. Going forward, it may be important to consider if splitting exercise, by completing multiple exercise sessions over the same day would affect appetite regulation, as future studies should aim to develop whether the nature of exercising intermittently (stop start) leads to increased EI and therefore weight gain.

Chapter 6

The Effect of Exercising Once vs. Twice a Day on Gastrointestinal Function, Metabolic Response, and Appetite in Healthy Males: A Randomised Cross Over Trial.[1]

[5] Some of the data from the study contained within this chapter was presented as a poster communication and the abstract published in "Mattin LM, McIver VJ. Yau, A. James, LJ. Evans, GH.(2019). The effects of multiple exercise bouts on appetite and metabolic response to food ingestion. The Physiological Society annual conference. Aberdeen Exhibition and conference centre, Aberdeen, UK: *The physiology Society*."

6.1 Introduction

It has been previously shown, GER is unaffected after a low intense continuous bout of exercise (40% $\dot{V}O_{2Peak}$) and a high (70% $\dot{V}O_{2Peak}$), when a semi-sold meal is consumed 30 min after the exercise bout (Mattin et al, 2018). Moreover, continuous, and intermittent exercise performed at a low intensity (40% $\dot{V}O_{2Peak}$) and moderate intensity (60% $\dot{V}O_{2Peak}$), does not affect how fast a semi-solid meal empties from the stomach (Mattin et al, 2020). Therefore, performing an acute one-off exercise bout at various intensities and modes, does not appear to disrupt GER. No literature to date has examined whether GER is affected when exercising multiple times, also known-within the literature as 'exercise snacking' (Francois et al., 2014, Perkin et al., 2019). It would also be important to discover whether dividing exercise into two separate bouts across the same day compared to a one-off bout matched at a high intensity (70% $\dot{V}O_{2Peak}$) would provoke a similar appetite regulatory hormone and metabolic response to that of 'time of day feeding' research.

The majority of work to date that has focusing on time of day feeding after exercise within a, healthy population (Hagobian and Braun, 2006, Stephens et al., 2007, Hagobian et al., 2009), overweight (Hagobian et al., 2008, Kozey-Keadle et al., 2014b) and type 2 diabetics (Blankenship et al., 2019) and have identified metabolic health as their main outcome. To our knowledge, this is the first investigation to examine the acute effects of exercising 'Twice-a-day' on changes in GER and the association between EI.

It must be noted, modifying nutritional intake post exercise to optimise recovery and enhance performance has been well documented (Burke et al., 2004, Jeukendrup and Tipton, 2009, Rodriguez et al., 2009, Jeukendrup, 2017a). In particular, the use of feeding with carbohydrate rich nutrients to drive recovery was researched by Ivy et al, in (1988). They revealed that highly trained cyclists consuming a carbohydrate supplement at 2 g/Kg of body weight immediately after exercise re-synthesised muscle glycogen faster than if the same carbohydrate supplement was consumed 2 h post exercise (Ivy et al., 1988). For this reason, immediate feeding of carbohydrate after exercise is seen within the world of athletes as an optimal way to maximise performance (Thomas et al., 2016). Gut hormones such as GLP-1 and PYY

are thought to be co-localised and increase after consuming nutrient dense material (Neary et al., 2005). This change in hormones has been thought to increase parasympathetic activation, proving a potential mechanism as to why GER fluctuates post-exercise (Schubert et al., 2014). In contrast, circulating ghrelin levels are elevated when participants are in a fasted state or pre-nutrient intake (Broom et al., 2007, Broom et al., 2009, Wasse et al., 2013b) and decline immediately after nutrient intake (Mattin et al., 2018a), shown within Chapter 4. Alterations in the secretion of gut hormones may have important implications for GER and the delivery of nutrients to the small intestine which may be an understudied factor in present literature, focusing on time of day feeding, and appetite control (Geliebter, 1988).

Nevertheless, more emphasis has been placed on diabetic clinical patients as Devlin et al, (1987) discovered endogenous glucose production rates were 20% lower in the morning after a single bout of exercise compared to a no-exercised state within non-insulin dependent diabetic men. Furthermore, an investigation which examined time of day feeding by breaking up sedentary sitting periods with light physical activity after meals (breakfast, lunch and dinner), compared to a one-off continuous walk after breakfast, revealed a reduced glucose response in both conditions only after breakfast. One potential reason for the reduced glucose response after lunch and dinner were suggested to be the low intensity nature and frequency of the physical activity (Blankenship et al., 2019). Surprisingly, less emphasis has been given to the timing of nutrient intake after an exercise bout to improve health-related benefits within the general population (Stephens and Braun, 2008).

Literature focusing on the effects of appetite regulation post-exercise (Martins et al., 2007a, King et al., 2010a, Deighton et al., 2013b, Wasse et al., 2013b) have regularly used an exercise duration > 60 min when a single bout of exercise was prescribed. This follows a recommendation from the Institute of Medicine (IOM), suggesting that 60 min of physical activity is needed per day to prevent the decline in metabolic effects gained from embarking on exercise (Brooks et al., 2004).Therefore, without regular stimulus or sufficient quantity of exercise a natural decline in metabolic health will result in a gain in body fat over time. Despite this, the effects of time of day feeding after exercise within a healthy population for improving metabolic health, weight loss or enhancing insulin stimulated glucose metabolism are less-

established and warrant further investigation (Kozey-Keadle et al., 2014b, Kozey-Keadle et al., 2014a).

To our knowledge, no investigation has yet compared whether 'Twice-a-day' exercise influences appetite and GER response leading to a change in metabolic regulation within healthy males. We hypothesised that a single bout of exercise would exhibit compensatory changes in appetite, and acylated ghrelin response post-exercise leading to a slower GER post lunch. Hence, the null hypothesis was that there would be no difference between exercise conditions regardless of whether a single bout or multiple bouts of exercise were completed.

Therefore, the aim of this study was to explore the effect of exercising 'Twice-a-day' (two high intensity continuous exercise bouts for 30-min separated by 2 h recovery), compared to a one off 60-min single bout of high intensity continuous exercise on: 1) GER of a post-lunch semi-solid meal, 2) Subjective and hormonal appetite regulation, 3) Overall metabolic response, and 4) Whether an energy balanced feeding schedule would lead to differences in 24 h post trial EI.

6.2 Methods

6.2.1. Participants

Sixteen healthy men were recruited from central Manchester and undertook experimental trials between December 2018 and April 2019 in the Physiology Laboratories at Manchester Metropolitan University, UK (Table 13). Verbal and written explanations of the experimental procedures were provided before the start of the trials and written informed consent was obtained. Two participants withdrew prior to completing the study, meaning fourteen participants completed the study. An a priori calculation was conducted using data from Chapter 5 focusing on GER and appetite data (Mattin et al., 2020b). An effect size η^2p = 0.081 from a repeated measures ANOVA model, attributing GER as the primary outcome measure, and using an α of 0.05 and a statistical power of 0.8 determined that $\geq$ 14 participants would be required to reject the null hypothesis (G*Power 3.0.10, Heinrich Hein Universitat, Dusseldorf, Düsseldorf, Germany). This chapter was registered at

ISRCTN online, resource provided by BMC part of Springer Nature. Identifier: ISRCTN48264634; Is it more beneficial for appetite and weight management to exercise once or twice a day?

Table 13: S-3, Baseline Subject Characteristics[1]

	Males (*n*=14) [*]
Age, y	27 ± 6.0
Height, m	1.79 ± 0.09
Weight, Kg	79.3 ± 10.4
BMI, kg/m²	24.9 ± 2.0
Body fat, %	18.5 ± 4.1
Systolic BP, mmHg	133 ± 14
Diastolic BP, mmHg	74 ± 9.0
V02max, ml/kg/min	42 ± 9.0
Exercise Intensity, W	164 ± 37
ETEE, Kcal	556 ± 53

[1] Values are means ± SDs
[*] Two subjects withdrew from the study
ETEE; Estimated trial energy expenditure

6.2.2. Experimental Trials

Participants reported to the laboratory on two occasions to complete each ~7 h (0-420min) trial. Experimental trials commenced between 0730 and 0830 following an overnight fast from 22:00 with the exception of plain water consumption. In addition to the pre-trial familiarisation outlined in general methods, each participant was also required to standardise their diet and physical activity before each trial (see section 3.7. Pre-trial standardisation).

Upon arrival at the laboratory, participants were asked to completely empty their bladder before pre-trial body mass was obtained. Following this, participants rested for 15 min in a semi-supine position whilst a cannula was inserted into the antecubital vein to enable venous blood collection. Following collection of a baseline

blood sample (0 min), each participant was asked to strap a heart rate monitor (Polar H10, Kemple, Finland) around their chest so HR could be observed during the exercise periods. Following this VAS appetite questionnaires were completed, and expired gas samples were collected for 15 min from a semi-supine position on a bed. The procedures for these baseline measurements are outlined in general methods.

Participants then completed a 60-min continuous cycle (SINGLE) or a split 30-min morning (0-30 min) and 30-min afternoon cycle (210-240 min) (SPLIT) at the same intended intensity of ~70% $V02_{peak}$. SINGLE participants exercised continuously from 0-60-min and rested until 75-min. SPLIT participants exercised from 0-30-min and rested until 75-min. The rationale for using different amounts of rest after the first exercise period was to keep the timing of measurements identical in relation to the overall time scale of the study. This resulted in both trials starting breakfast at 75 min regardless of exercise condition. Therefore, the SINGLE exercise trial received 15 min rest and the SPLIT exercise trial 45 min rest before breakfast. This time difference has been illustrated towards the left of Figure 38. Furthermore, expired air was collected continuously during exercise periods using a breath-by-breath gas analyzer (Metalyzer 3b, Cortex, Leipzig, Germany), participants wore the expired air mask from 0-60 min during the first exercise period, and samples were analyzed at (15, 30, 45 and 60 min) and from 210-240 min during the second exercise period (210, 225, 240 min) regardless of whether the participants were exercising or resting. HR was also recorded every 5 min during the first exercise session in both SINGLE and SPLIT trials (5, 10, 15, 20, 25, 30, 45, 50, 55 and 60 min) and RPE (Borg, 1973) every 10 min (0, 10, 20, 30, 40, 50 and 60 min). HR was also collected during the second exercise session at (210, 215, 220, 225, 230, 235 and 240 min) and RPE (210, 220, 230 and 240 min). HR and RPE data were reported as; individual points during exercise period (Figure 40) and average HR across the total completed 60 min exercise period (Table 14).

At 75-min (pre-breakfast) a further blood sample and VAS were collected before participants were provided with a glass of semi-skimmed milk (see section 3.6. Meal Details), which amounted to 30% of ETEE (see section 3.6.1. Calculating Estimated Trial Energy Expenditure) and water so the total volume during this breakfast meal period amounted to a standardised 500ml (Table 14). Participants then

entered their first rest period, in which they rested in the laboratory for 2-h (90-210 minutes). During this period, participants were free to work, read or watch DVDs; expired air samples and VAS were collected every 30-min (120, 150, 180 and 210 min) and blood samples at 60-min intervals (150 and 210 min). During this recovery phase, each participant was provided with a standardized amount of water (125ml) every 15-min from (150-195 min) resulting in a total of 500 ml being consumed.

At 210-min (start of second exercise period) participants who were undertaking the SPLIT trial started their second 30 min afternoon exercise session and SINGLE participants continued to rest for the 30 min duration from (210-240 min). At this point all participants were given 30 min to shower and change their clothes before, a further blood sample and VAS was collected at 270 min (pre-lunch).

Participants were then provided with meal two (Lunch), a bowl of Heinz vegetable soup which amounted to the remaining 70% of ETEE. Water was added to the soup before heating in a microwave, in order for the final meal volume to be standardised to 1000g (Table 14). Each participant was given a maximum of 15 minutes to consume the standardised meal and instructed to consume it as quickly as they were able. Time taken to eat this meal was recorded.

Participants remained in a semi-supine position throughout the remaining 2h sampling procedure (second recovery period). Gastric emptying samples were obtained pre-meal ingestion (270 min) and every 15 min post-meal (300, 315, 330, 345, 360, 375, 390 and 405 min). Additional expired air samples and VAS were collected every 30-min (315, 345, 375 and 405 min) and blood samples were obtained every 60 min (345 and 405 min). Following the collection of the final samples (405 min), participants were provided with a 500 ml bottle of water and free to leave the laboratory at 420 min and start their post-trial 24 h weighed dietary intake. The measurements within this trial are described in general methods. A schematic diagram of the experimental protocol is presented in (Figure 38)

6.2.3. Exercise Intensity

Exercise intensity was calculated in a similar manner as described within Chapter 4. Workload was then calculated using the liner trend line equation to evoke a work rate equivalent to 70% $\dot{V}O_{2Peak}$ (High intensity). Each exercise bout was designed, so total estimated energy expenditure KJ during exercise was matched

between both trials, using *Equation 6* (see section 5.2.3). The work rate was consistent between the continuous one-off 60 min exercise bout and the split 2 x 30 min continuous exercise bouts.

6.2.4. Biochemical Analysis

Blood sample analysis is described in general methods. Gut hormone analysis was performed on 126 duplicate samples. Corresponding intra-assay CVs for active ghrelin, GLP-1, insulin, PP and PYY were 6.7%, 10.8%, 7.6%, 10% and 7.9% respectively. Inter-assay CVs were determined from concentrations across five different assays for active ghrelin, GLP-1, insulin, PP and PYY were 6.4%, 15.2%, 9.6%, 20.8 and 11.2% respectively.

Metabolite analysis was performed on 196 duplicate samples. Corresponding intra-assay CVs for glucose, triglycerides, cholesterol and NEFA were 1.2%, 1.2%, 1.2% and 7.4% respectively. Inter-assay CVs were determined from concentrations across five verification runs for glucose, triglycerides, cholesterol and NEFA and were 4.9%, 3.7%, 3.1% and 5.3% respectively.

I-FABP$_2$ analysis was performed on 84 duplicate samples. Corresponding intra-assay CVs for I-FABP$_2$ were 4%. Inter-assay CVs were determined from concentrations across two different assays for I-FABP$_2$ 20% respectively.

6.2.5. Statistical Analysis

All data are presented as mean ± standard deviation (SD) unless otherwise stated. Data was tested for normality of distribution using the Shapiro-Wilk test. Differences in standardisation measurements, pre-trial body mass, weight of soup consumed, volume of milk consumed, weight and volume of water, time to consume breakfast and lunch, Pre and post-EI, proportion of macronutrient, average HR, gastric emptying T_{lag}, $T_{1/2}$, and all AUC results were analysed using one-way repeated measures analysis of variance (ANOVA). Two-way repeated ANOVA were used to examine differences in; HR, RPE, I-FABP$_2$, carbohydrate and fat oxidation, gastric emptying DOB and cumulative dose %13C0$_2$ values, gut hormones concentrations, metabolites concentrations and appetite VAS scores. Sphericity for repeated measures was assessed and Greenhouse-Geisser epsilon < 0.75 and the Huynh-Feldt correction

adopted for less severe differences to correct for violations. Significant main effects were followed by paired student's t-Test or one-way repeated ANOVA with Bonferroni adjusted pairwise comparisons as appropriate. Effect size was documented as partial eta squared (η^2_p) or Cohen's (d) and 95% confidence intervals (CI) (see section 3.19.2. Post-Statistics Effect Size).

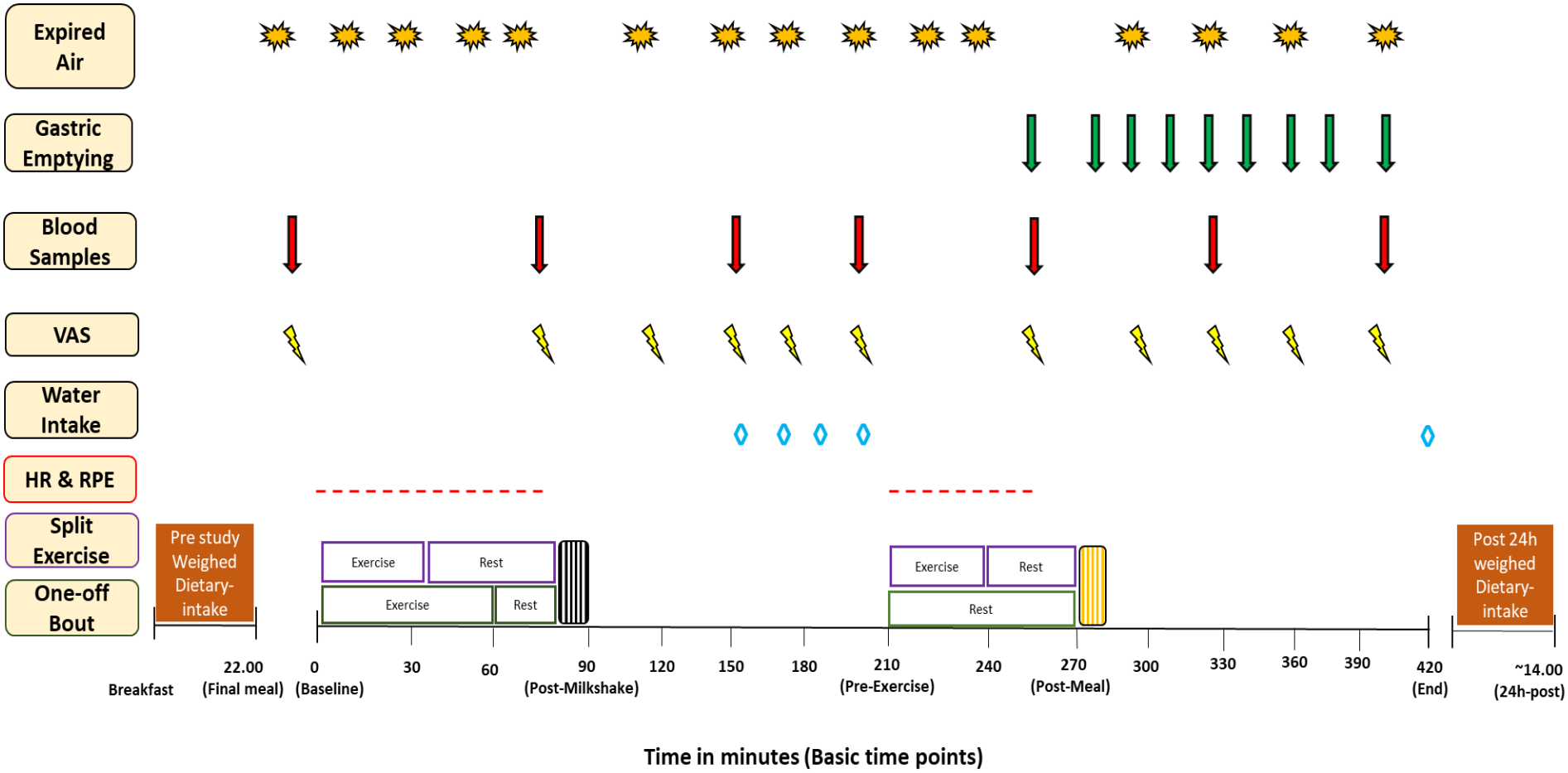

Figure 38: Schematic diagram of the experimental trial protocol S-3. Black lined rectangle represents breakfast drink period. Yellow lined rectangle represents 15 min standardised semi-solid meal period. HR, heart rate. RPE, rating of perceived exertion. GE, gastric emptying. VAS, visual analogy scale. Expired Air, (Substrate Utilisation). Split 2 x 30 min exercises bouts (SPLIT). One-off 60 min exercise bout (SINGLE)

6.3 Results

6.3.1. Standardisation Measurements

Pre-trial body mass was not significantly different between SINGLE compared to SPLIT (P = 0.432, η^2_p = 0.048). There were no differences between the weight of soup and the volume of milk consumed (P > 0.05), nor weight or volume of water (P > 0.05) during meal periods. Time to consume breakfast was not significantly different (P = 0.194, $\eta^2 p$ = 0.126) and this was the same for time to consume lunch (P = 0.554, $\eta^2 p$ = 0.028). Average heart rate over the total 60-min exercise period was not significantly different between SINGLE compared to SPLIT (P = 0.534, $\eta^2 p$ = 0.128, Table 14)

Table 14: S3, Standardisation Measurements During Laboratory Visit

	SINGLE	**SPLIT**	***P*=value**
	Pre-Trial Measurements		
Body Mass (Kg)	80.7 ± 11.2	80.6 ± 11.1	0.432
	Exercise Measurement		
Average HR (60-min)	156 ± 14	154 ± 14	0.534
	Breakfast Measurements		
Milk (mL)	334 ± 33	334 ± 33	1.00
Water (mL)	165 ± 32	165 ± 32	1.00
Total breakfast (S)	114 ± 80	82 ± 57	0.194
	Lunch Measurements		
Soup (g)	823 ± 82	823 ± 82	1.00
Water (g)	172 ± 79	172 ± 79	1.00
Total lunch (s)	420 ± 217	392 ± 198	0.554

Data are means ± SD. Values are significant P<0.05

6.3.2. Energy and Macronutrient Intake

Pre-trial energy intake was not significantly different between SINGLE compared to SPLIT respectively (2571 ± 1173 Kcal vs 2547 ± 1311 Kcal, P =0.779,

$\eta^2 p$ = 0.006, Figure 39A). This led to a similar proportion of energy from carbohydrates (P = 0.898, η^2_p=*0.001*), fats (P = 0.326, η^2_p=*0.074*), protein (P = 0.243, η^2_p=*0.103*) and fibre (P = 0.919, η^2_p=*0.001*). In addition, fluid consumption before was also not significantly different between trials (*P* = 0.483, η^2_p=*0.039*, Table 15).

24-hour post-trial energy intake revealed SPLIT was significantly higher compared to SINGLE (3240 ± 1171 vs 2556 ± 1049 Kcal: *P* =0.007, d = 0.64, 95% CI = -635.9–570.8 KCal, Figure 39B). Individual data indicated 10 out of 13 participants had a higher energy intake 24-h after the SPLIT trial compared to the SINGLE. This led to different proportion of macronutrients being consumed 24 hours after the trial had ended. There was no differences between; carbohydrates (P = 0.280, η^2_p=*0.096*), protein (P = 0.055, η^2_p=*0.274*), fibre (P = 0.525, η^2_p=*0.034*) and water (P = 0.488, η^2_p=*0.041*). However, participants did consume considerably more fat 24-h after completing the SPLIT trial compared to the SINGLE (*P* =0.037, d = 0.67, 95% CI = -364.8–266.4, Table 15)

Table 15: Pre and Post-Trial Macronutrient Content (KCal) from Total Energy Intake for SINGLE and SPLIT Trials.

	SINGLE	**SPLIT**	***P*=value**
	Pre-Trial Macronutrients (KCal)		
Carbohydrate	1141 ± 445	1132 ± 564	0.898
Protein	376 ± 152	403 ± 149	0.243
Fat	1021 ± 674	976 ± 703	0.326
Fibre	37 ± 18	38 ± 28	0.919
Water (g)	1929 ± 1118	1857 ± 968	0.483
	Post-Trial Macronutrients (KCal)		
Carbohydrate	1092 ± 615	1206 ± 597	0.280
Protein	393 ± 123	628 ± 412	0.055
Fat	994 ± 489	*****1373 ± 671	**0.037**
Fibre	34 ± 26	40 ± 30	0.525
Water (g)	1355 ± 741	1481 ± 1148	0.488

Data are means ± SD. Values are significant *P*<0.05
* Indicates SPLIT is significantly larger than SINGLE.

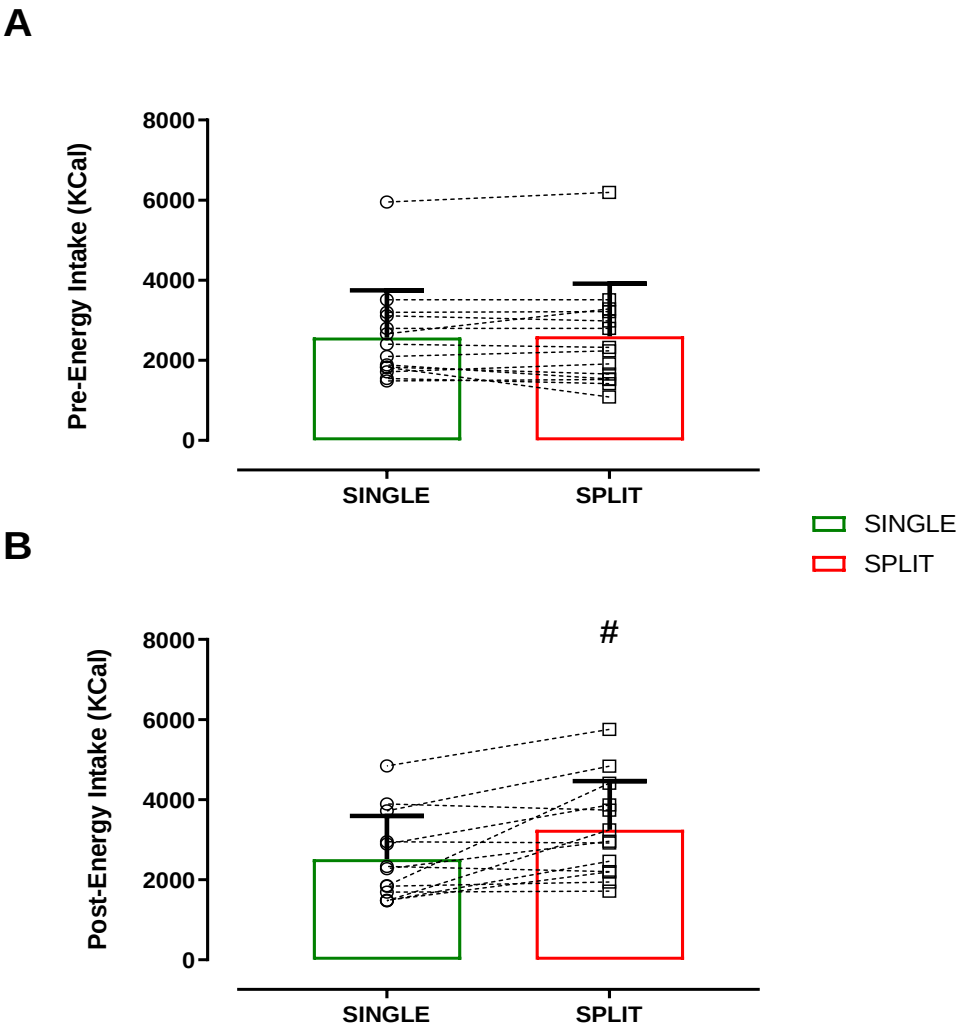

Figure 39: Representative of mean values for pre-energy intake (KCal) (A, n=14) and post-energy intake (KCal) (B, n=13) with vertical error bars display SD's. Individual data is represented as (○) for SINGLE and (□) represents SPLIT. There was no main effect observed for pre-energy intake (p >0.05) examined by a one-way repeated-measured ANOVA. However, there was a main effect observed for post-energy intake (p <0.05) examined by a one-way repeated-measured ANOVA. # Indicates, SPLIT was significantly different from SINGLE, determined by Bonferroni adjusted paired *t*-test (p <0.05).

6.3.3. Heart Rate and RPE

A main effect for trial (p <0.001, η^2p = 0.879), time (p <0.001, η^2p = 0.827) and trial x time interaction (p <0.001, η^2p = 0.918) was observed for RPE during exercise. Post-hoc test revealed there was an effect at bassline as SINGLE was marginally higher than SPLIT (9 ± 3 v 8 ± 2 RPE: P = 0.022, d = 0.41, 95% CI = -1.16–1.45 RPE). SINGLE was significantly higher from 40-60 min compared to SPLIT; all time point were highly significant (p <0.001). Unsurprisingly, SPLIT was

significantly higher from 210-240 min compared to SINGLE where all time points were highly significant (p <0.001; Figure 40A). A main effect for trial (p <0.001, η^2p = 0.939), time (p <0.001, η^2p = 0.885) and trial x time interaction (p <0.001, η^2p = 0.944) was observed for heart rate during exercise. Post-hoc test revealed SINGLE was significantly higher from 35-60 min compared to SPLIT, all time points were highly significant (p <0.001). Unsurprisingly, SPLIT was significantly higher from 215-240 min compared to SINGLE all time points were highly significant (p <0.001; Figure 40B).

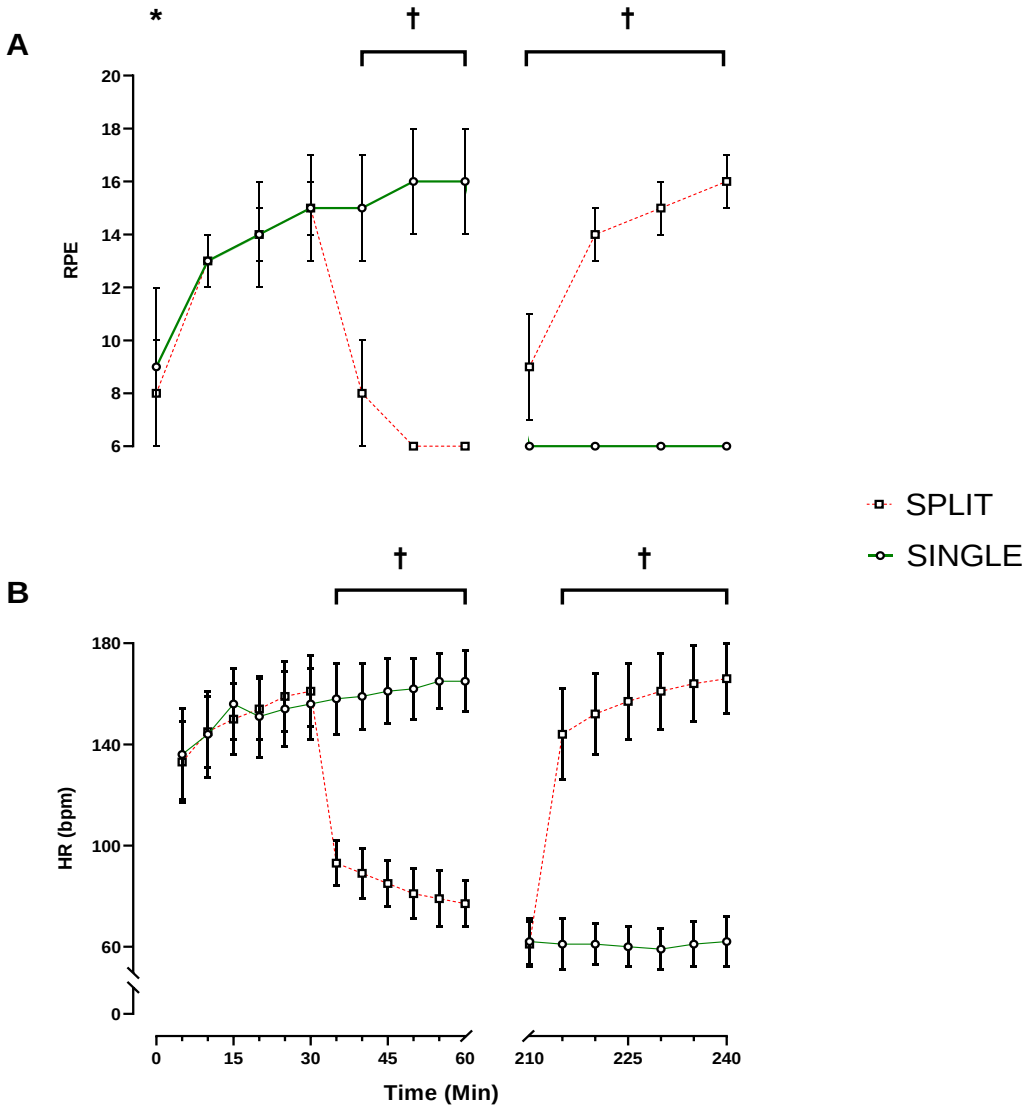

Figure 40: Representative of mean RPE (A) and HR (B), with vertical error bars display SDs. (n = 14). There was a main effect for trial, time and a main interaction effect (p <0.05) for HR and RPE during exercise, examined by a two-way repeated-measured ANOVA. † Indicates, SINGLE vs SPLIT was significantly different? at each time point (p <0.001). *Indicates, SINGLE vs SPLIT was significantly different (p <0.05). Post-hoc tests were determined by Bonferroni adjusted paired *t-test*. RPE; rate of perceived exertion, HR, heart rate; bpm, beats per minute.

6.3.4. Gastric Emptying

No main effect of trials (P = 0.086, η^2_p=*0.210*) was observed for DOB. However, a main effect of time (P <0.001, η^2_p=*0.936*) and interaction (P <0.01, η^2_p=*0.240*) was observed for DOB. Post-hoc test revealed for DOB, SPLIT was significantly higher at 330-min (23.9 ± 5 vs 21.5 ± 5.1 $^{13}C0_2$:$^{12}C0_2$: P =0.020, d = 0.49, 95% CI = -2.13–3.16 $^{13}C0_2$:$^{12}C0_2$) and 345-min (25.5 ± 5 vs 22.8 ± 4.9 $^{13}C0_2$:$^{12}C0_2$: P =0.013, d = 0.57, 95% CI = -2.05–3.13 $^{13}C0_2$:$^{12}C0_2$) Both trials increased at every time point from baseline (P<0.001, Figure 43B). There was no main effect observed for DOB AUC (p = 0.065, η^2p = 0.239, Figure 43A)

There was a main effect observed for T_{lag} as SPLIT was significantly faster compared to SINGLE (73 ± 15 vs 89 ± 24 min: P =0.007, d = 0.83, 95% CI = -11.74–8.69 min). There was also a main effect observed for $T_{1/2}$ as SPLIT was significantly faster compared to SINGLE (118 ± 29 vs 149 ± 49 min: P =0.027, d = 0.80, 95% CI = -24.87–15.99 min, Figure 41). There was a main effect observed for trials (P = 0.026, η^2_p=*0.326)*, time (P <0.001, η^2_p=*0.980*) and interaction (P =0.039, η^2_p=*0.276*) observed for percent $^{13}C0_2$. Post-hoc test revealed, SINGLE was significantly lower than SPLIT at **330-min** (5.8 ± 1.3 vs 6.5 ± 1.1 $^{13}CO_2$ %: P =0.024, d = 0.60, 95% CI = 0.03–1.28 $^{13}CO_2$ %), **345-min** (8.9 ± 1.8 vs 9.9 ± 1.7 $^{13}CO_2$ %: P =0.018, d = 0.59, 95% CI = -0.30–1.54 $^{13}CO_2$ %), **360-min** (12.1 ± 2.3 vs 13.4 ± 2.3 $^{13}CO_2$ %: P =0.019, d = 0.59, 95% CI = -0.62–1.79 $^{13}CO_2$ %) and **375-min** (15.3 ± 2.8 vs 16.9 ± 2.7 $^{13}CO_2$ %: P =0.025, d = 0.60, 95% CI = -0.81–2.07 $^{13}CO_2$ %). However, cumulative dose of percent $^{13}CO_2$, final values at 120 min were similar and not significantly different (SINGLE, 21.6 ± 3.5 $^{13}CO_2$ % vs SPLIT, 23.1 ± 3.3 $^{13}CO_2$ %;P = 0.053, η^2_p=*0.258,* Figure 42).

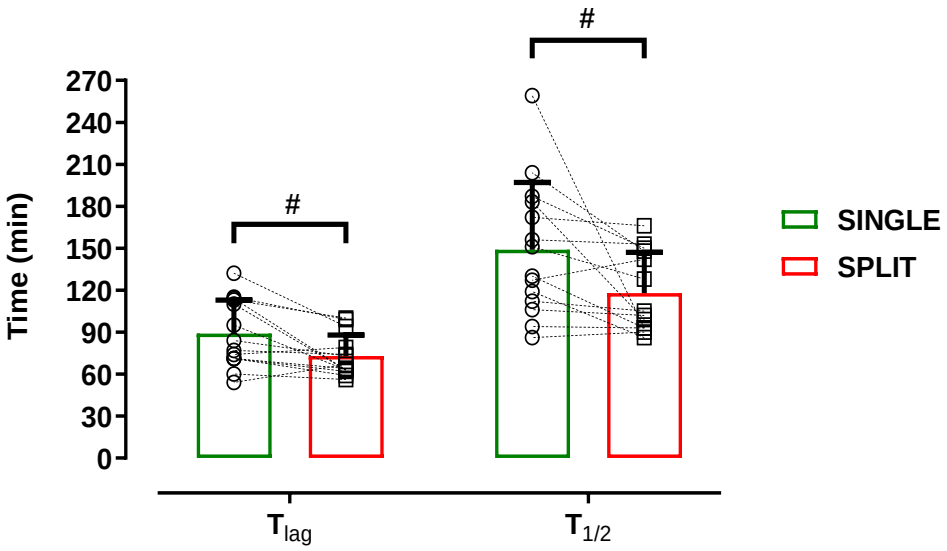

Figure 41: Representative of mean T_{lag} and $T_{1/2}$ with vertical error bars display SDs (n = 14). Individual data is represented as (○) for SINGLE and (□) represents SPLIT. There was a main effect observed for T_{lag} and $T_{1/2}$ ($p < 0.05$) examined by a one-way repeated-measured ANOVA. # Indicate, SPLIT was significantly different from SINGLE, determined by Bonferroni adjusted paired *t*-test ($p < 0.05$). T_{lag}, Time of maximal emptying rate; $T_{1/2}$, Half emptying time.

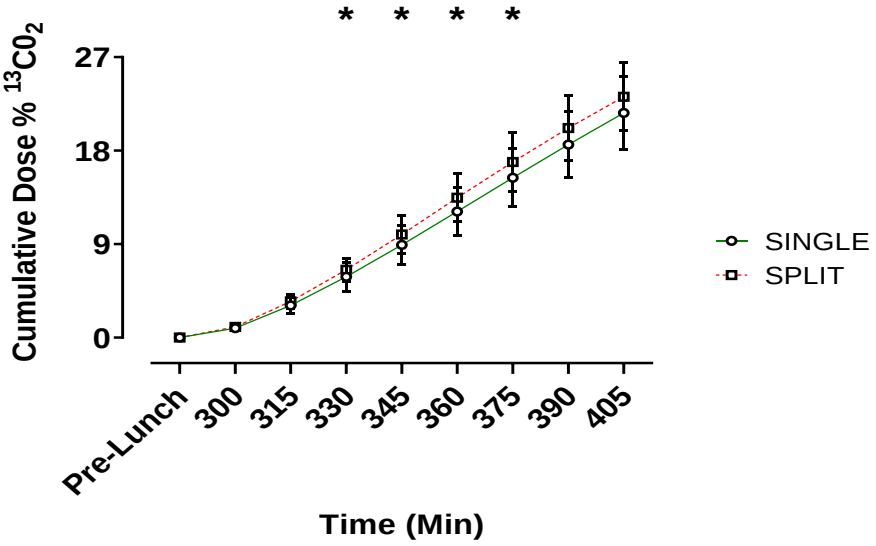

Figure 42: Representative of mean cumulative dose of % $^{13}CO_2$, with vertical error bars display SDs (n = 14). There was no main effect for trial and time ($p > 0.05$). Although, a main interaction effect observed ($p < 0.05$), examined by a one-way repeated-measured ANOVA * Indicate, SINGLE vs SPLIT was significantly different, determined by Bonferroni adjusted paired *t*-test ($p < 0.05$).

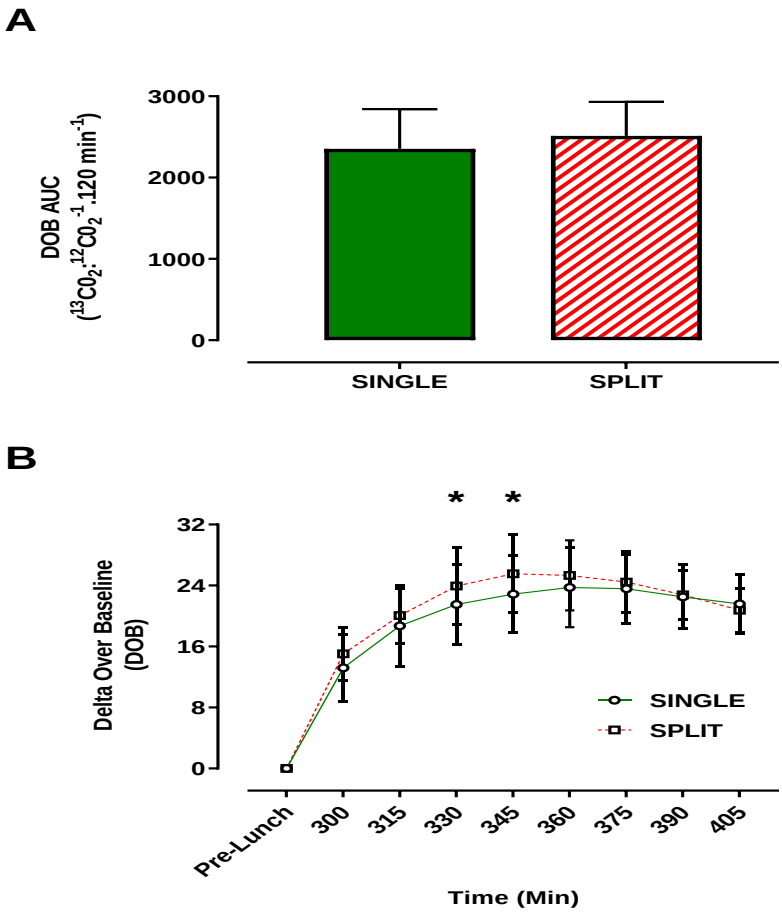

Figure 43: Representative of mean DOB AUC (0-120 min^{-1}) (A) and DOB response (B), with vertical error bars display SDs (n = 14). There was no main effect observed for DOB AUC (p >0.05), examined by a one-way repeated-measured ANOVA. There was no main effect for trial (p >0.05). Although, there was a main effect for time and interaction effect observed for DOB (p <0.05), examined by a two-way repeated-measured ANOVA. * Indicate, SINGLE vs SPLIT was significantly different, determined by Bonferroni adjusted paired *t*-test (p <0.05). DOB, delta over baseline; AUC, area under curve.

6.3.5. I-FABP$_2$

There was no main effect for I-FABP$_2$ AUC for SINGLE vs SPLIT respectively (P = 0.400, η^2p = 0.060, Figure 44A*)*. No main effect for trial (p =0.252, η^2p = 0.108) nor trial x time interaction (p =0.780, η^2p = 0.011) was observed. However, an effect for time (p <0.001, η^2p = 0.618) was identified for I-FABP$_2$.

Post-hoc test revealed SINGLE and SPLIT trials increased from baseline to pre-breakfast (0-75 min) and baseline to pre-lunch (0-270 min), both increases in both trials were highly significant ($p < 0.001$, Figure 44B).

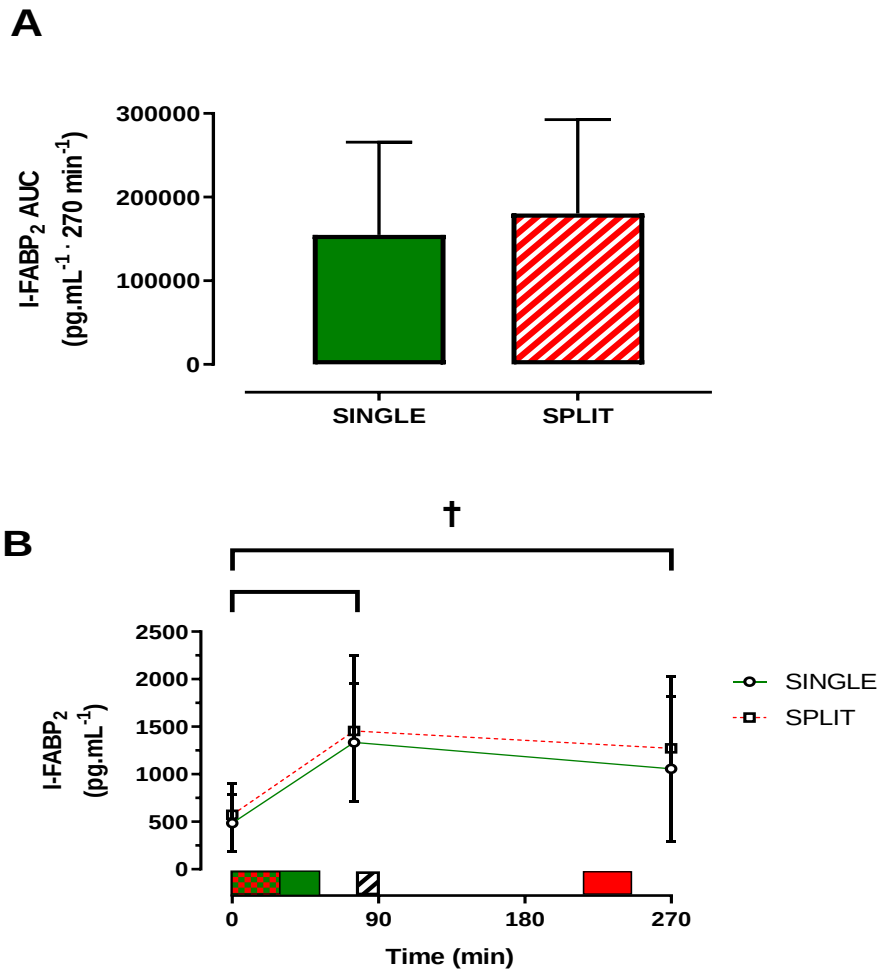

Figure 44: Representative of mean I-$FABP_2$–AUC (0-270 min^{-1}) (A) and I-$FABP_2$ response (B), with vertical error bars display SDs (n = 14). *Chequered red and green rectangle* indicates 30-min exercise period; *Filled green rectangle* indicates the remaining 30-min exercise period of the 60-min single bout; *Hashed black rectangle* indicates 30% ETEE milk-drink and *red rectangle* indicates 30-min exercise period for split. There was no main effect observed for I-$FABP_2$ AUC ($p > 0.05$), examined by a one-way repeated-measured ANOVA. There was no main effect for trial nor main interaction effect ($p < 0.05$). However, a main effect for time was observed ($p < 0.001$), examined by a two-way repeated-measured ANOVA. † Indicates a significant increase for time from baseline to 75 and 270 min within SINGLE vs SPLIT, determined by Bonferroni adjusted paired *t-test* ($p < 0.001$). I-$FABP_2$; Intestinal fatty acid binding protein 2.

6.3.6. Appetite

6.3.6.1. Hunger

Hunger increased during SPLIT and decreased during SINGLE post exercise compared to baseline. Moreover, hunger remained elevated during SPLIT until 150 min. Both trials decreased in hunger after food ingestion (breakfast) before gradually rising throughout recovery in all trials. A main effect of time ($p < 0.001$, $\eta^2p = 0.735$) and interaction effect ($p = 0.031$, $\eta^2p = 0.137$) was observed for hunger. However, there were no trial effects for hunger ($p = 0.108$; $\eta^2p = 0.187$; Figure 45B). Post-hoc tests revealed SPLIT was significantly increased at 75min (pre-breakfast) compared to SINGLE (70 ± 14 vs. 53 ± 22 mm: $p = 0.025$, d = 0.96, 95% CI = −6.38–12.48 mm) There was no main effect for hunger AUC ($p = 0.136$, $\eta^2p = 0.163$; Figure 45A).

6.3.6.2. Fullness

Fullness remained relatively stable in both trials until 120 min before fullness started to decrease in both trials until 270 min (pre-lunch). Both trials then increased after lunch before gradually decreasing throughout recovery in both trials. A main effect of time ($p < 0.001$, $\eta^2p = 0.740$) was observed for fullness. However, there were no trial ($p = 0.290$, $\eta^2p = 0.086$) nor main interaction effects observed ($p = 0.723$, $\eta^2p = 0.036$, Figure 46B). There was no main effect for fullness AUC ($p = 0.309$, $\eta^2p = 0.080$; Figure 46A).

6.3.6.3. PFC

PFC decreased from baseline during SINGLE and increased during SPLIT at 75 min (pre-breakfast), before remaining relatively stable in both trials until 270 min (pre-lunch). In both trials PFC decreased after lunch before gradually increasing throughout recovery. A main effect of time ($p < 0.001$, $\eta^2p = 0.721$) was observed for PFC. However, there were no trial ($p = 0.341$, $\eta^2p = 0.070$) nor main interaction effects observed ($p = 0.134$, $\eta^2p = 0.106$, Figure 47B). There was no main effect for PFC AUC ($p = 0.490$, $\eta^2p = 0.037$; Figure 47A).

6.3.6.4. Satisfaction

Satisfaction decreased from baseline during SPLIT and increased during SINGLE at 75 min (pre-breakfast), before SINGLE continued to increase until 120 min. Both

trials then remained relatively similar until 270 min (pre-lunch). Both trials then increased after lunch before gradually decreasing throughout recovery to 345 min were SPLIT decreased in satisfaction visible more than SINGLE at 375 and 405 min. A main effect of time ($p < 0.001$, $\eta^2p = 0.705$) was observed for satisfaction. However, there were no trial ($p = 0.203$, $\eta^2p = 0.121$) nor main interaction effects observed ($p = 0.361$, $\eta^2p = 0.079$, Figure 48B). There was no main effect for satisfaction AUC ($p = 0.207$, $\eta^2p = 0.119$; Figure 48A).

6.3.6.5. Bloat

Bloat remained relatively stable in both trials until 270 min (pre-lunch), before both trials then increased after lunch resulting in a gradual decrease throughout recovery in both trials. A main effect of time ($p =0.033$, $\eta^2p = 0.231$) was observed for bloat. However, there were no trial ($p = 0.427$, $\eta^2p = 0.049$) nor main interaction effects observed ($p = 0.377$, $\eta^2p = 0.075$, Figure 49B). There was no main effect for bloat AUC ($p = 0.428$, $\eta^2p = 0.049$; Figure 49A).

6.3.6.6. Nausea

Nausea increased during SINGLE and decreased during SPLIT at 75 min (pre-breakfast) compared to baseline. Both trials then remained relatively similar until 405 min. There was no main effect of time ($p =0.121$, $\eta^2p = 0.167$), trial ($p = 0.975$, $\eta^2p = 0.001$) nor main interaction effect observed ($p = 0.309$, $\eta^2p = 0.090$, Figure 50B). There was no main effect for nausea AUC ($p = 0.826$, $\eta^2p = 0.004$; Figure 50A).

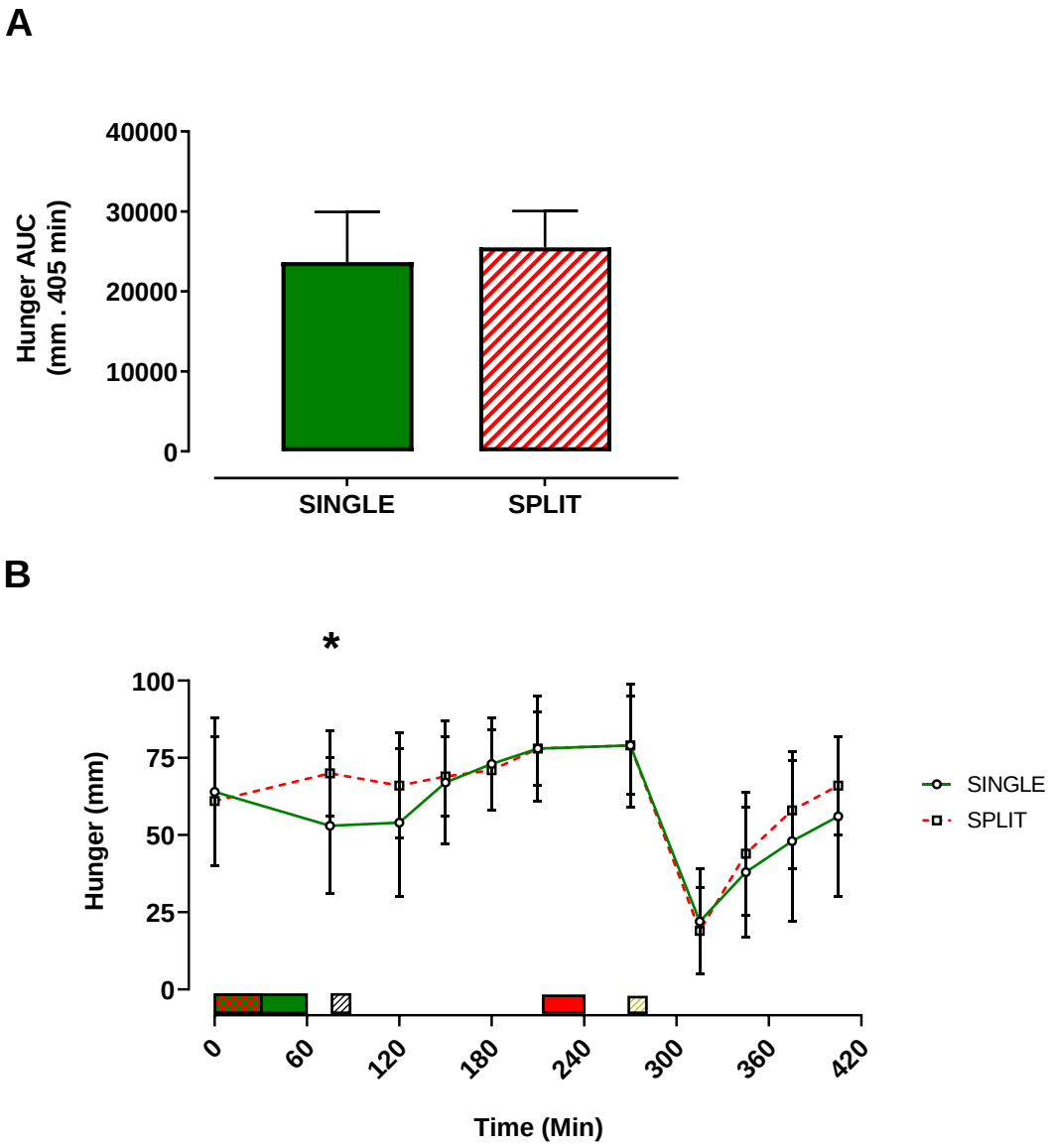

Figure 45: Representive of mean hunger AUC (0-405 min) (A) and hunger VAS response (B), with vertical error bars display SDs (n = 14). *Chequered red and green rectangle* indicates 30-min exercise period; *Filled green rectangle* indicates the remaining 30-min exercise period of the 60-min single bout; *Hashed black rectangle* indicates 30% ETEE milk-drink. *Red rectangle* indicates remaining 30-min exercise period for split exercise; *Hashed yellow rectangle* indicates ingestion of 70% ETEE semi-solid meal. There was no main effect observed for hunger AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial effect observed for hunger ($p > 0.05$). However, there was a main effect for time and a main interaction effect ($p < 0.05$) examined by a two-way repeated-measured ANOVA. * Indicates post-hoc tests were significantly different between SINGLE vs SPLIT, determined by Bonferroni adjusted paired t-test ($p < 0.05$). AUC, area under curve.

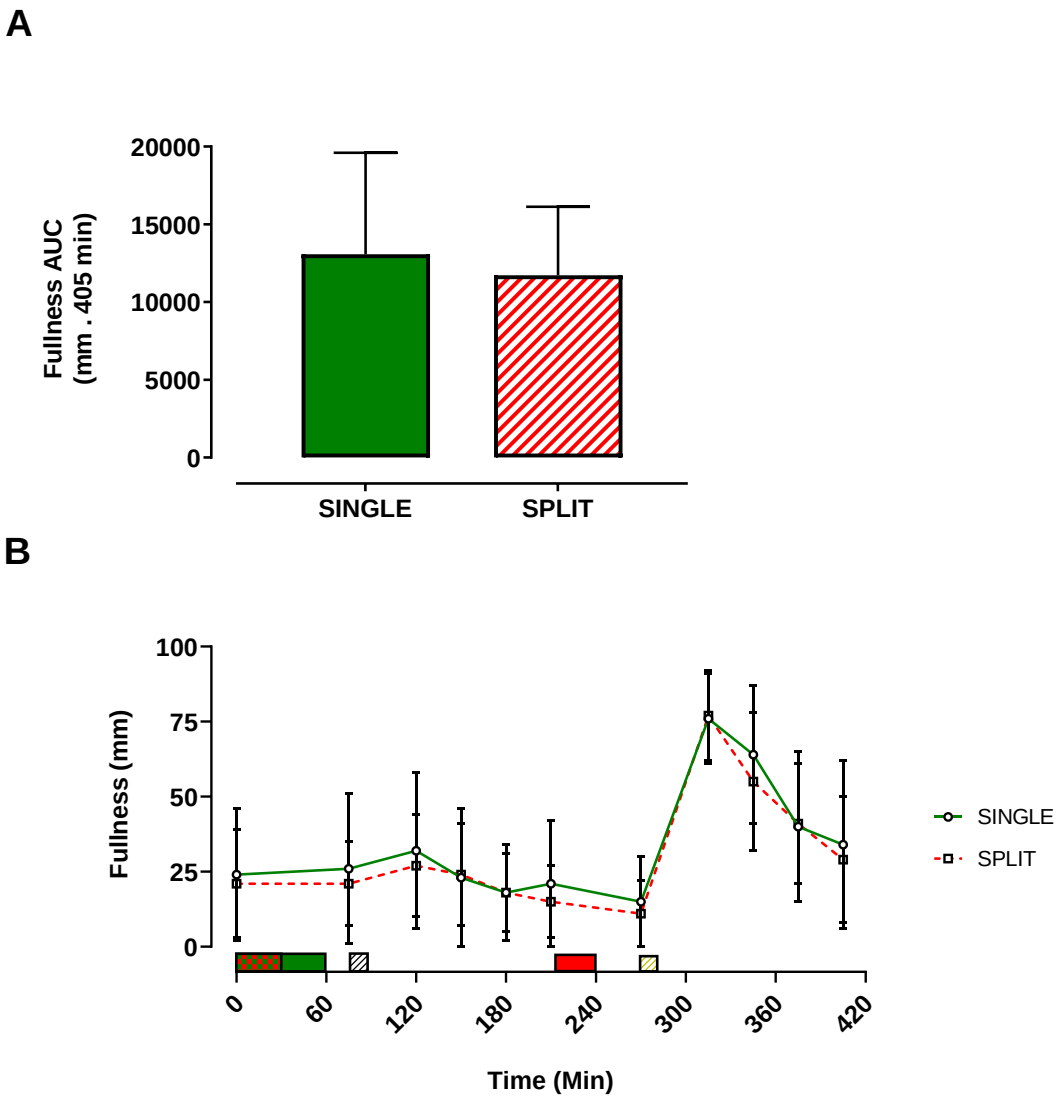

Figure 46: Representive of mean fullness AUC (0-405 min) (A) and fullness VAS response (B), with vertical error bars display SDs (n = 14). *Chequered red and green rectangle* indicates 30-min exercise period; *Filled green rectangle* indicates the remaining 30-min exercise period of the 60-min single bout; *Hashed black rectangle* indicates 30% ETEE milk-drink. *Red rectangle* indicates remaining 30-min exercise period for split exercise; *Hashed yellow rectangle* indicates ingestion of 70% ETEE semi-solid meal. There was no main effect observed for fullness AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial, time nor main interaction effect observed for fullness ($p > 0.05$) examined by a two-way repeated-measured ANOVA. AUC, area under curve.

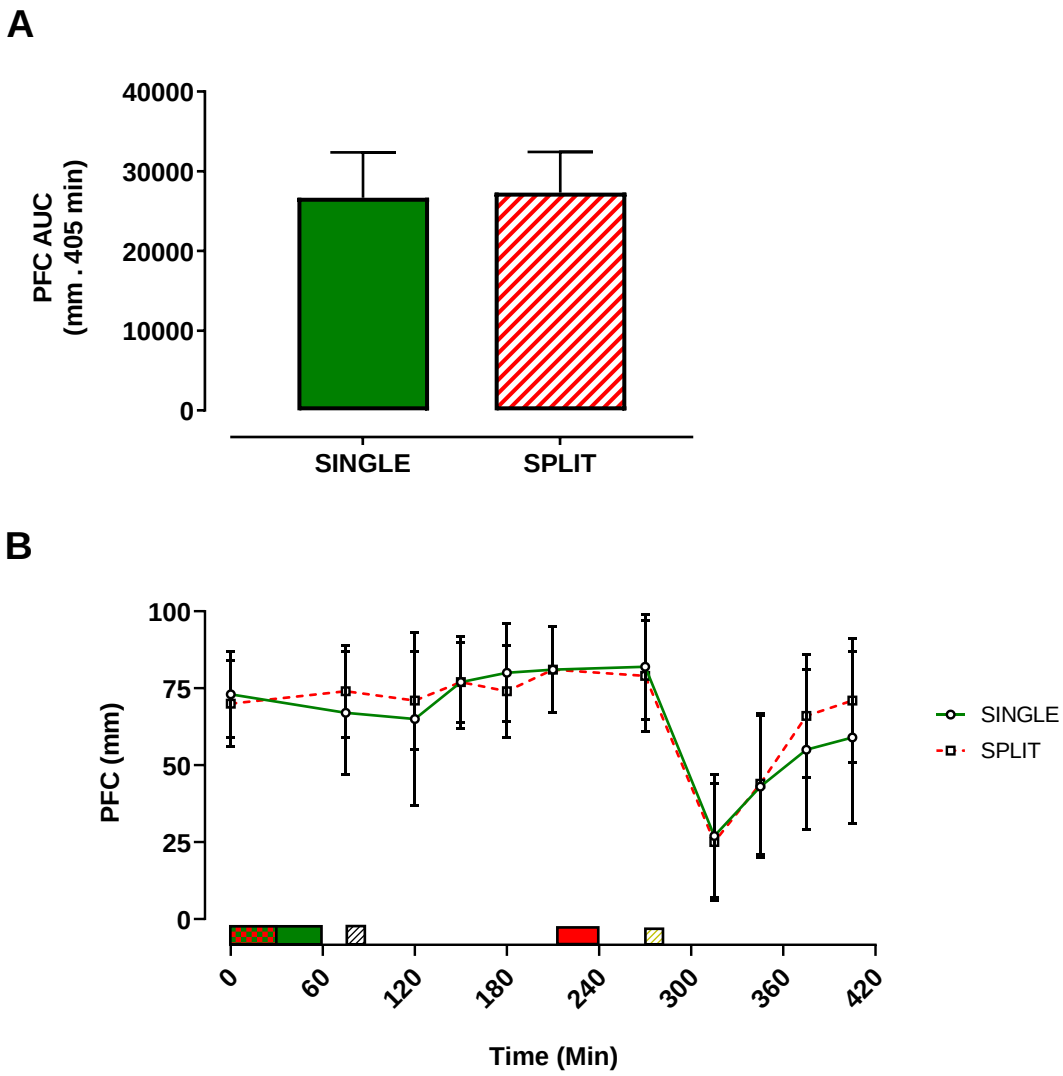

Figure 47: Representive of mean PFC AUC (0-405 min) (A) and PFC VAS response (B), with vertical error bars display SDs (n = 14). *Chequered red and green rectangle* indicates 30-min exercise period; *Filled green rectangle* indicates the remaining 30-min exercise period of the 60-min single bout; *Hashed black rectangle* indicates 30% ETEE milk-drink. *Red rectangle* indicates remaining 30-min exercise period for split exercise; *Hashed yellow rectangle* indicates ingestion of 70% ETEE semi-solid meal. There was no main effect observed for PFC AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial, time nor main interaction effect observed for PFC ($p > 0.05$) examined by a two-way repeated-measured ANOVA. AUC, area under curve; PFC, prospective food consumption

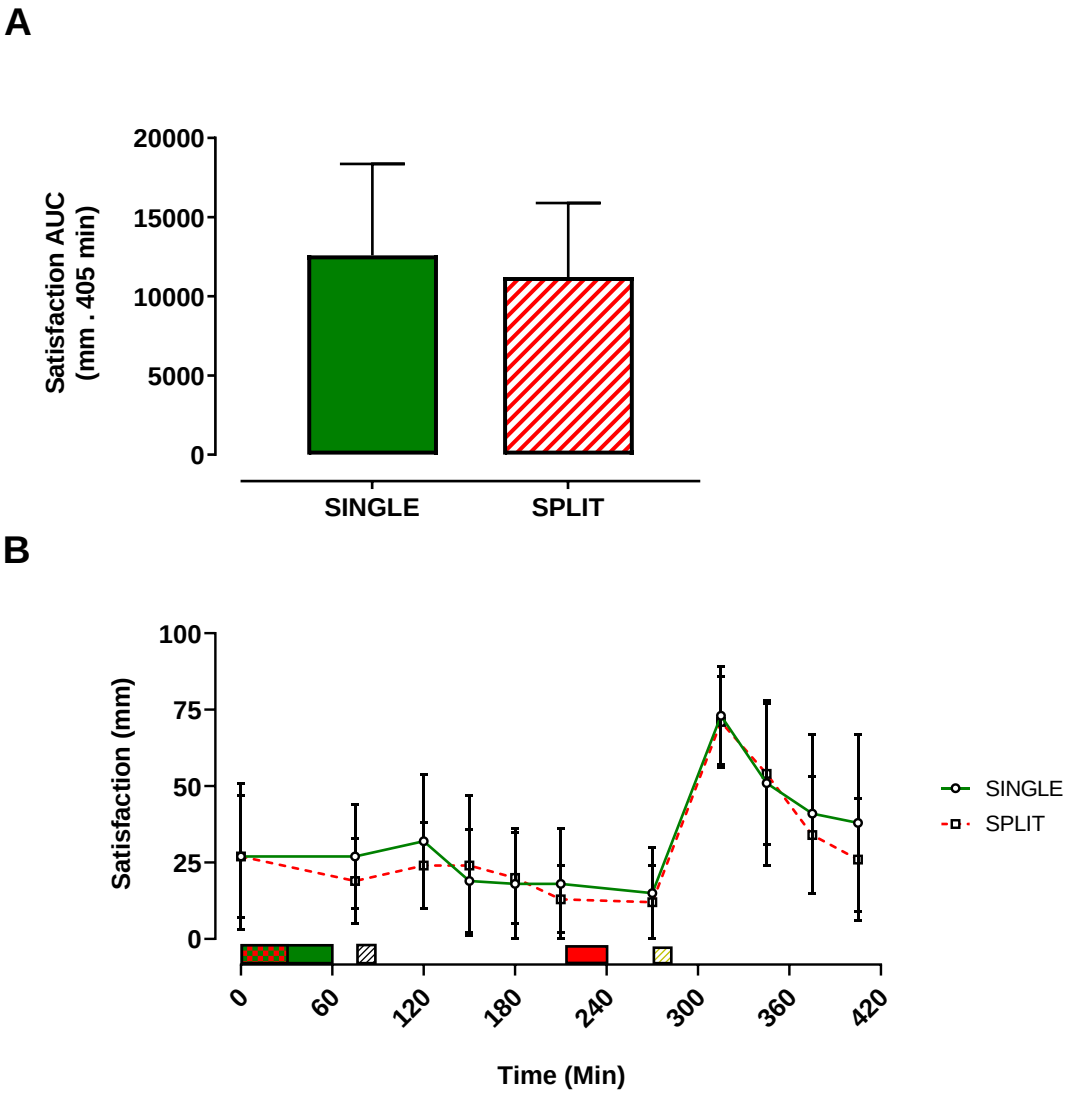

Figure 48: Representive of mean satisfaction AUC (0-405 min) (A) and satisfaction VAS response (B), with vertical error bars display SDs (n = 14). *Chequered red and green rectangle* indicates 30-min exercise period; *Filled green rectangle* indicates the remaining 30-min exercise period of the 60-min single bout; *Hashed black rectangle* indicates 30% ETEE milk-drink. *Red rectangle* indicates remaining 30-min exercise period for split exercise; *Hashed yellow rectangle* indicates ingestion of 70% ETEE semi-solid meal. There was no main effect observed for satisfaction AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There were no main trial, time nor main interaction effects observed for satisfaction ($p > 0.05$) examined by a two-way repeated-measured ANOVA. AUC, area under curve.

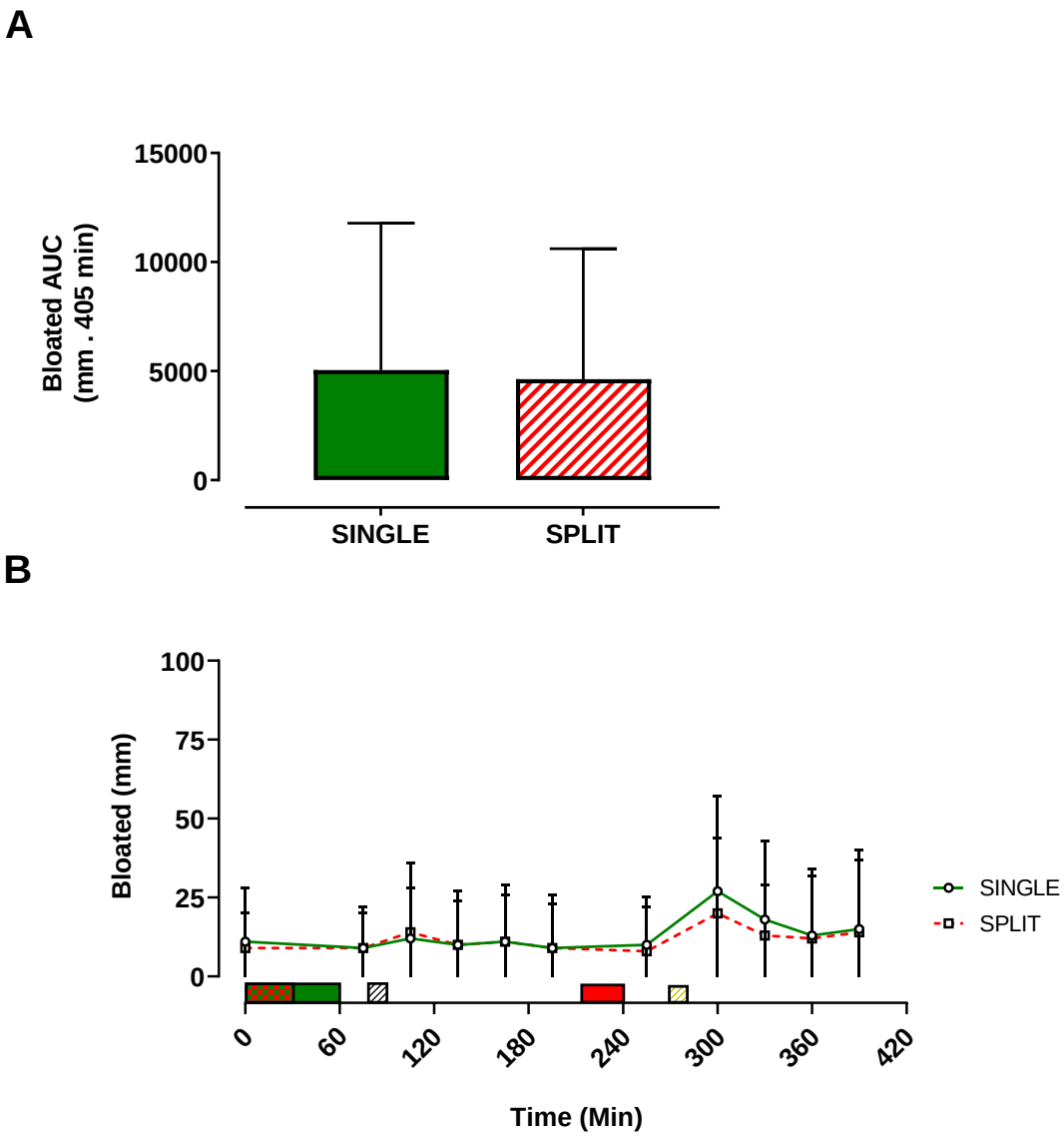

Figure 49: Representive of mean bloat AUC (0-405 min) (A) and bloat VAS response (B), with vertical error bars display SDs (n = 14). *Chequered red and green rectangle* indicates 30-min exercise period; *Filled green rectangle* indicates the remaining 30-min exercise period of the 60-min single bout; *Hashed black rectangle* indicates 30% ETEE milk-drink. *Red rectangle* indicates remaining 30-min exercise period for split exercise; *Hashed yellow rectangle* indicates ingestion of 70% ETEE semi-solid meal. There was no main effect observed for bloat AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial, time nor main interaction effect observed for bloat ($p > 0.05$) examined by a two-way repeated-measured ANOVA. AUC, area under curve.

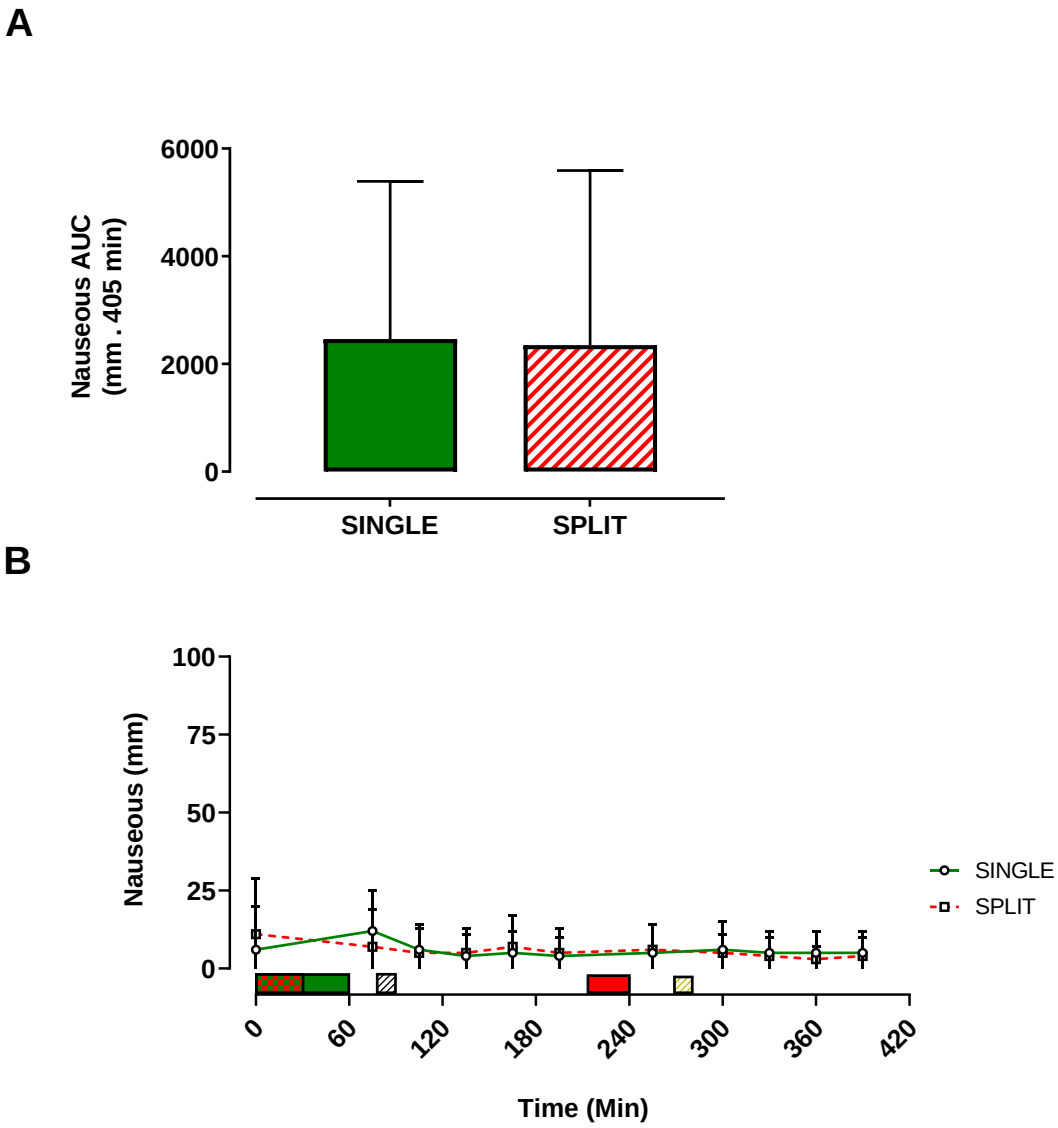

Figure 50: Representive of mean nausea AUC (0-405 min) (A) and nausea VAS response (B), with vertical error bars display SDs (n = 14). *Chequered red and green rectangle* indicates 30-min exercise period; *Filled green rectangle* indicates the reaming 30-min exercise period of the 60-min single bout; *Hashed black rectangle* indicates 30% ETEE milk-drink. *Red rectangle* indicates remaining 30-min exercise period for split exercise; *Hashed yellow rectangle* indicates ingestion of 70% ETEE semi-solid meal. There was no main effect observed for nausea AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial, time nor main interaction effect observed for nausea ($p > 0.05$) examined by a two-way repeated-measured ANOVA. AUC, area under curve.

6.3.7. Blood Metabolites

6.3.7.1. Glucose

Glucose responded differently post exercise at 75 min (pre-breakfast) as SINGLE increased and SPLIT decreased compared to baseline. Glucose remained similar until 270 min, before glucose increased during the SINGLE trial compared to the SPLIT. This increase remained at 345 and 405 min during the SINGLE trial compared to SPLIT. A main effect of time ($p < 0.001$, $\eta^2p = 0.693$), trial ($p = 0.006$, $\eta^2p = 0.474$) and interaction effect was observed for glucose ($p = 0.048$, $\eta^2p = 0.208$, Figure 51B). Post-hoc tests revealed SINGLE was significantly increased at 270 min (pre-lunch) compared to SPLIT (4.5 ± 0.3 vs. 4.3 ± 0.2 mmol.L: $p = 0.015$, d = 0.81, 95% CI = −0.66–0.92 mmol.L) and 405 min (5.1 ± 0.4 vs. 4.5 ± 0.3 mmol.L: $p < 0.001$, d = 1.76, 95% CI = 1.55–1.92 mmol.L). There was a main effect for glucose AUC as SINGLE was significantly increased compared to SPLIT (1725 ± 124 vs. 1654 ± 80 mmol.L^{-1}. 405 min^{-1}: $p = 0.031$, d = 0.70, 95% CI = −64.3–42.7 mmol.L^{-1}. 405 min^{-1}, Figure 51A).

6.3.7.2. Cholesterol

Cholesterol responded similarly across the duration of the whole study in both the SINGLE and the SPLIT trials. Although SINGLE tended to be elevated over and SPLIT across most time points. A main effect of time ($p < 0.001$, $\eta^2p = 0.509$), trial ($p = 0.050$, $\eta^2p = 0.283$) and interaction effect was observed for cholesterol ($p = 0.001$, $\eta^2p = 0.306$, Figure 52B). Post-hoc tests revealed SINGLE was significantly increased at 75 min (pre-breakfast) compared to SPLIT (4.6 ± 0.9 vs. 4.0 ± 0.6 mmol.L: $p = 0.009$, d = 0.81, 95% CI = −0.34–1.13 mmol.L), 345 min (4.3 ± 0.9 vs. 3.9 ± 0.6 mmol.L: $p = 0.037$, d = 0.59, 95% CI = −0.17–0.90 mmol.L) and 405 min (4.3 ± 0.8 vs. 3.9 ± 0.6 mmol.L: $p = 0.049$, d = 0.58, 95% CI = 0.12–0.91 mmol.L). There was no main effect for cholesterol AUC. However, SINGLE did approach significance compared to SPLIT ($p = 0.051$, $\eta^2p = 0.283$, Figure 52A).

6.3.7.3. Triglycerides

Triglycerides responded similarly across the duration of the whole study in both the SINGLE and the SPLIT trials until SPLIT stated to elevate more than SINGLE at 345 and 405 min. A main effect of time ($p =0.048$, $\eta^2p = 0.223$) and interaction effect

(p = 0.004, η^2p = 0.379) was observed for triglycerides. However, there were no trial effects for triglycerides (p = 0.275; η^2p = 0.098; Figure 53B). Post-hoc tests revealed SPLIT was significantly increased at 345 min compared to SINGLE (0.95 ± 0.45 vs. 0.80 ± 0.34 mmol.L: p = 0.031, d = 0.39, 95% CI = −0.15–0.57 mmol.L) and 405 min (0.83 ± 0.5 vs. 0.67 ± 0.34 mmol.L: p = 0.041, d = 0.39, 95% CI = 0.13–0.57 mmol.L). There was no main effect for triglycerides AUC (p = 0.381, η^2p = 0.064, Figure 53A).

6.3.7.4. NEFA

NEFA responded differently post exercise at 75 min (pre-breakfast) as SINGLE increased and SPLIT decreased compared to baseline. NEFA then decreased at 150 min (60 min post breakfast) in both trials, although remained elevated in the SINGLE trial compared to the SPLIT. NEFA then continued to rise in both trials until 270 min (post-second exercise), where NEFA peaked in the SPLIT trial compared to SINGLE, before decreasing in both trials 60 min post-lunch. NEFA began to elevate once more at 405 min in the SPLIT trial compared to SINGLE. A main effect of time (p <0.001, η^2p = 0.801) and interaction effect (p < 0.001, η^2p = 0.692) was observed for NEFA. However, there were no trial effects for NEFA (p = 0.084; η^2p = 0.228; Figure 54B). Post-hoc tests revealed SINGLE was significantly increased at 75 min (pre-breakfast) compared to SPLIT (1.23 ± 0.45 vs. 0.38 ± 0.12 mmol.L: p < 0.001, d = 2.68, 95% CI = 2.44–2.74 mmol.L) and 150 min (0.34 ± 0.23 vs. 0.19 ± 0.09 mmol.L: p =0.038, d = 0.89, 95% CI = 0.77–0.94 mmol.L). Post second exercise bout post-hoc tests revealed SPLIT was significantly increased at 270 min compared to SINGLE (1.21 ± 0.50 vs. 0.88 ± 0.38 mmol.L: p =0.027, d = 0.77, 95% CI = 0.51–0.97 mmol.L) and 405 min (0.88 ± 0.04 vs. 0.24 ± 0.16 mmol.L: p =0.007, d = 5.07 95% CI = 5.67-5.78 mmol.L).

There was a main effect for NEFA AUC as SINGLE was significantly increased compared to SPLIT (231 ± 84 vs. 180 ± 45 mmol.L^{-1}. 405 min^{-1}: p = 0.022, d = 0.79, 95% CI = −43.3–24.3 mmol.L^{-1}. 405 min^{-1}, Figure 54A).

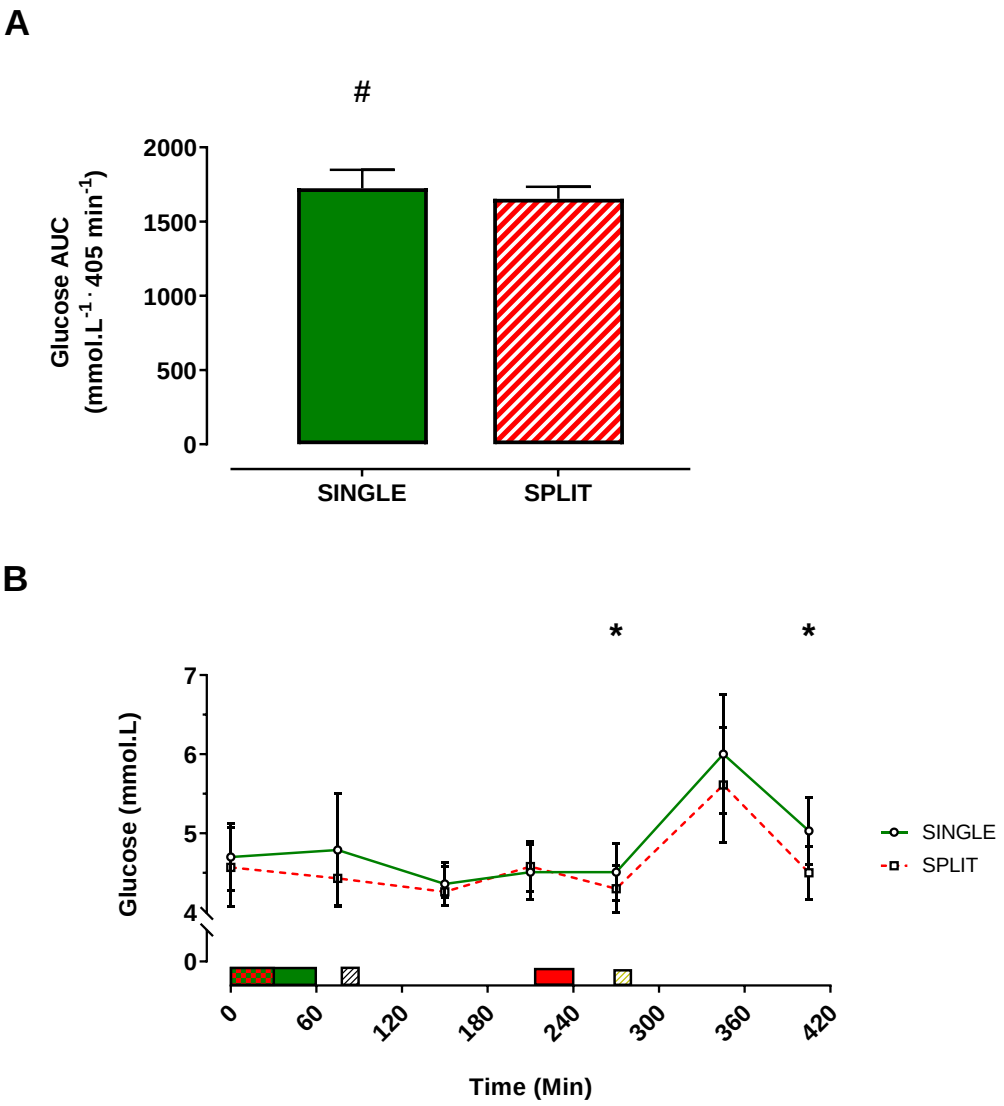

Figure 51: Representive of mean glucose AUC (0-405 min) (A) and glucose VAS response (B), with vertical error bars display SDs (n = 14). *Chequered red and green rectangle* indicates 30-min exercise period; *Filled green rectangle* indicates the remaining 30-min exercise period of the 60-min single bout; *Hashed black rectangle* indicates 30% ETEE milk-drink. *Red rectangle* indicates remaining 30-min exercise period for split exercise; *Hashed yellow rectangle* indicates ingestion of 70% ETEE semi-solid meal. There was a main effect observed for glucose AUC ($p < 0.05$) examined by a one-way repeated-measured ANOVA. There was a main trial, time and a main interaction effect observed for glucose ($p < 0.05$) examined by a two-way repeated-measured ANOVA. *, # Indicates post-hoc tests were significantly different between SINGLE vs SPLIT, determined by Bonferroni adjusted paired *t-test* ($p < 0.05$). AUC, area under curve.

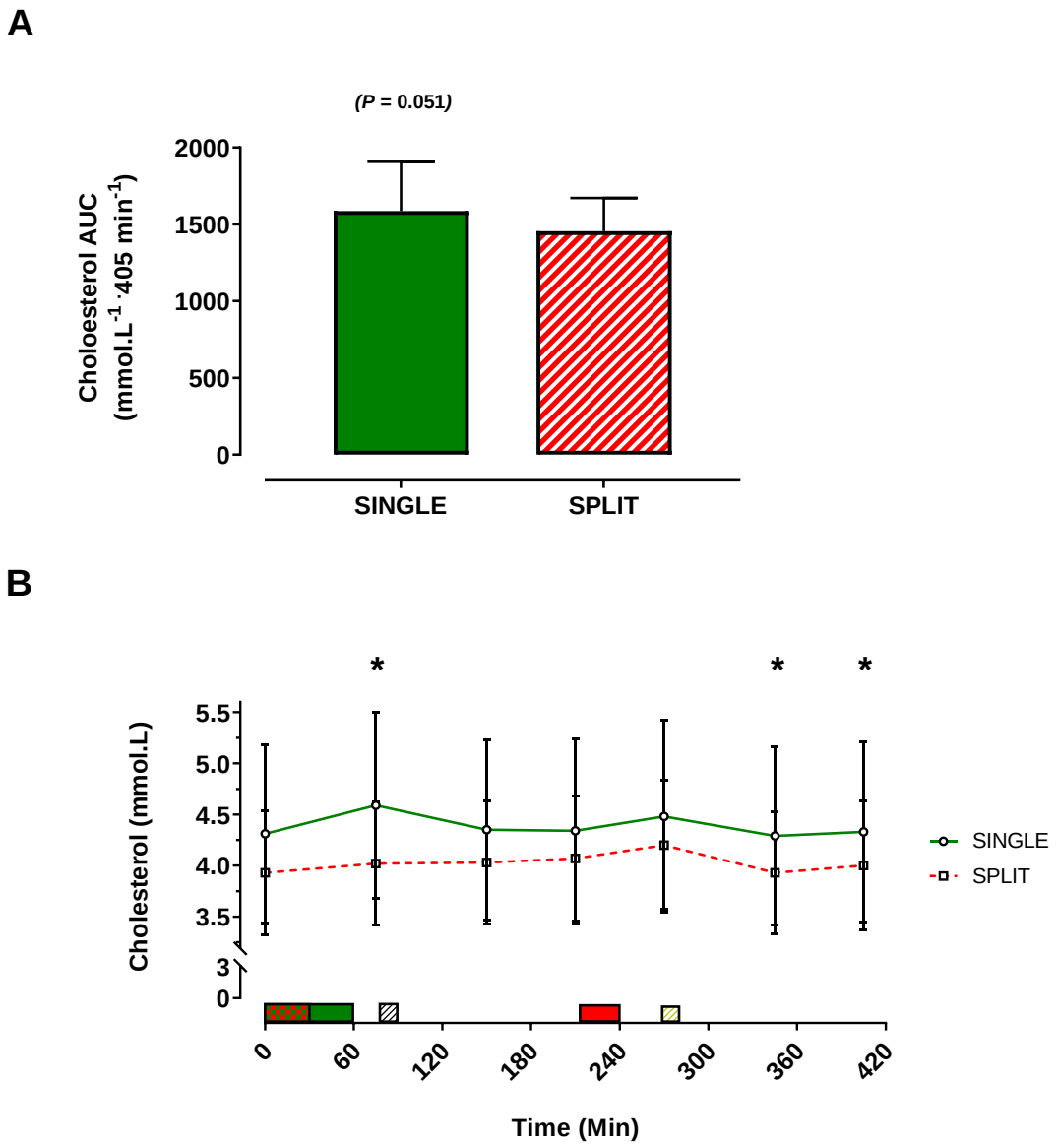

Figure 52: Representive of mean cholesterol AUC (0-405 min) (A) and cholesterol VAS response (B), with vertical error bars display SDs (n = 14). *Chequered red and green rectangle* indicates 30-min exercise period; *Filled green rectangle* indicates the remaining 30-min exercise period of the 60-min single bout; *Hashed black rectangle* indicates 30% ETEE milk-drink. *Red rectangle* indicates remaining 30-min exercise period for split exercise; *Hashed yellow rectangle* indicates ingestion of 70% ETEE semi-solid meal. There was no main effect observed for cholesterol AUC ($p < 0.05$) examined by a one-way repeated-measured ANOVA. There was a main trial, time and a main interaction effect observed for cholesterol ($p < 0.05$) examined by a two-way repeated-measured ANOVA. * Indicates post-hoc tests were significantly different between SINGLE vs SPLIT, determined by Bonferroni adjusted paired *t-test* ($p < 0.05$). AUC, area under curve.

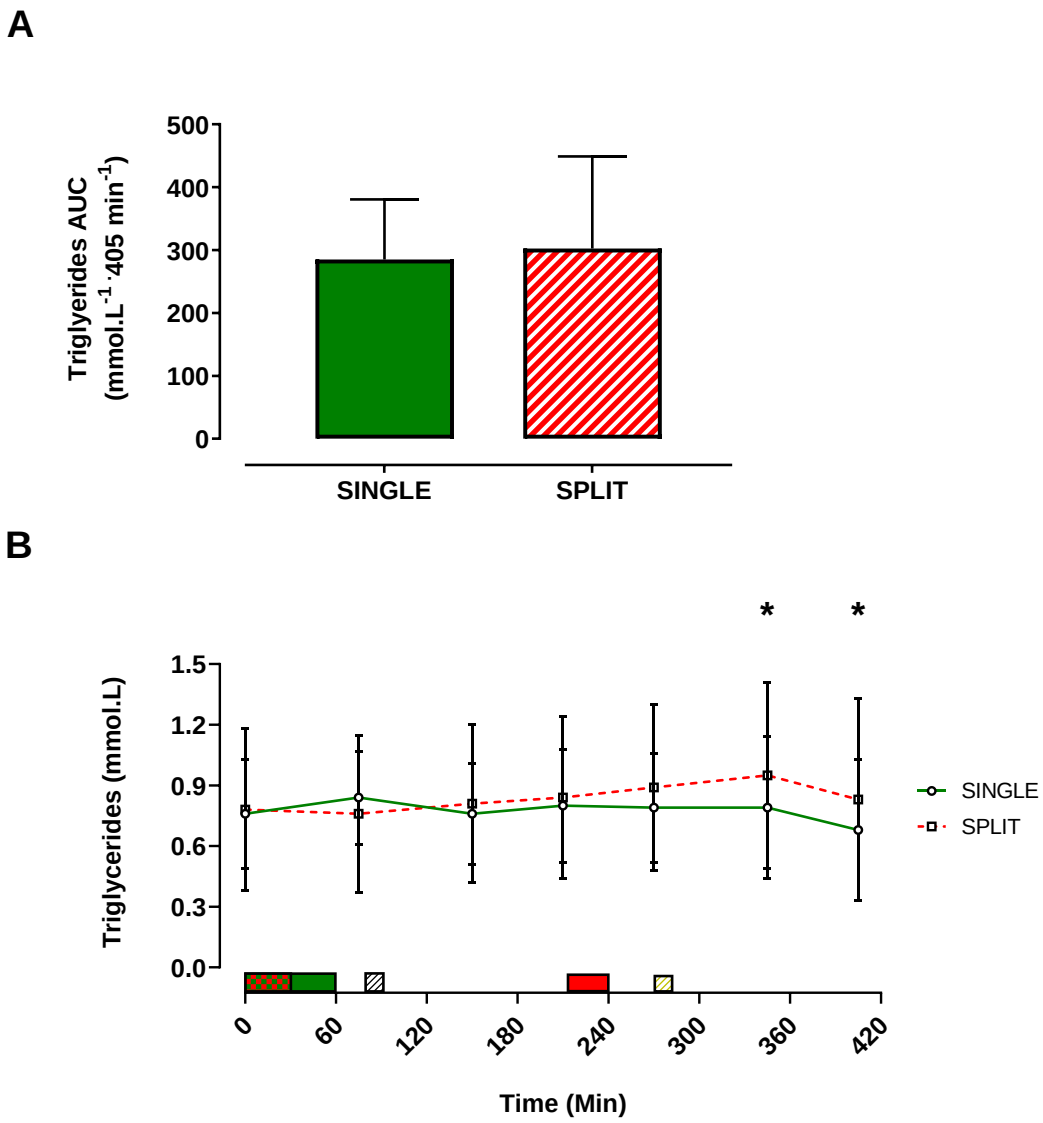

Figure 53: Representive of mean triglycerid AUC (0-405 min) (A) and triglyceride VAS response (B), with vertical error bars display SDs (n = 14). *Chequered red and green rectangle* indicates 30-min exercise period; *Filled green rectangle* indicates the remaining 30-min exercise period of the 60-min single bout; *Hashed black rectangle* indicates 30% ETEE milk-drink. *Red rectangle* indicates remaining 30-min exercise period for split exercise; *Hashed yellow rectangle* indicates ingestion of 70% ETEE semi-solid meal. There was no main effect observed for triglyceride AUC ($p < 0.05$) examined by a one-way repeated-measured ANOVA. There was no main effect for trial ($p > 0.05$). However, there was a main effect for time and a main interaction effect observed for triglyceride ($p < 0.05$) examined by a two-way repeated-measured ANOVA. * Indicates post-hoc tests were significantly different between SINGLE vs SPLIT, determined by Bonferroni adjusted paired *t-test* ($p < 0.05$). AUC, area under curve.

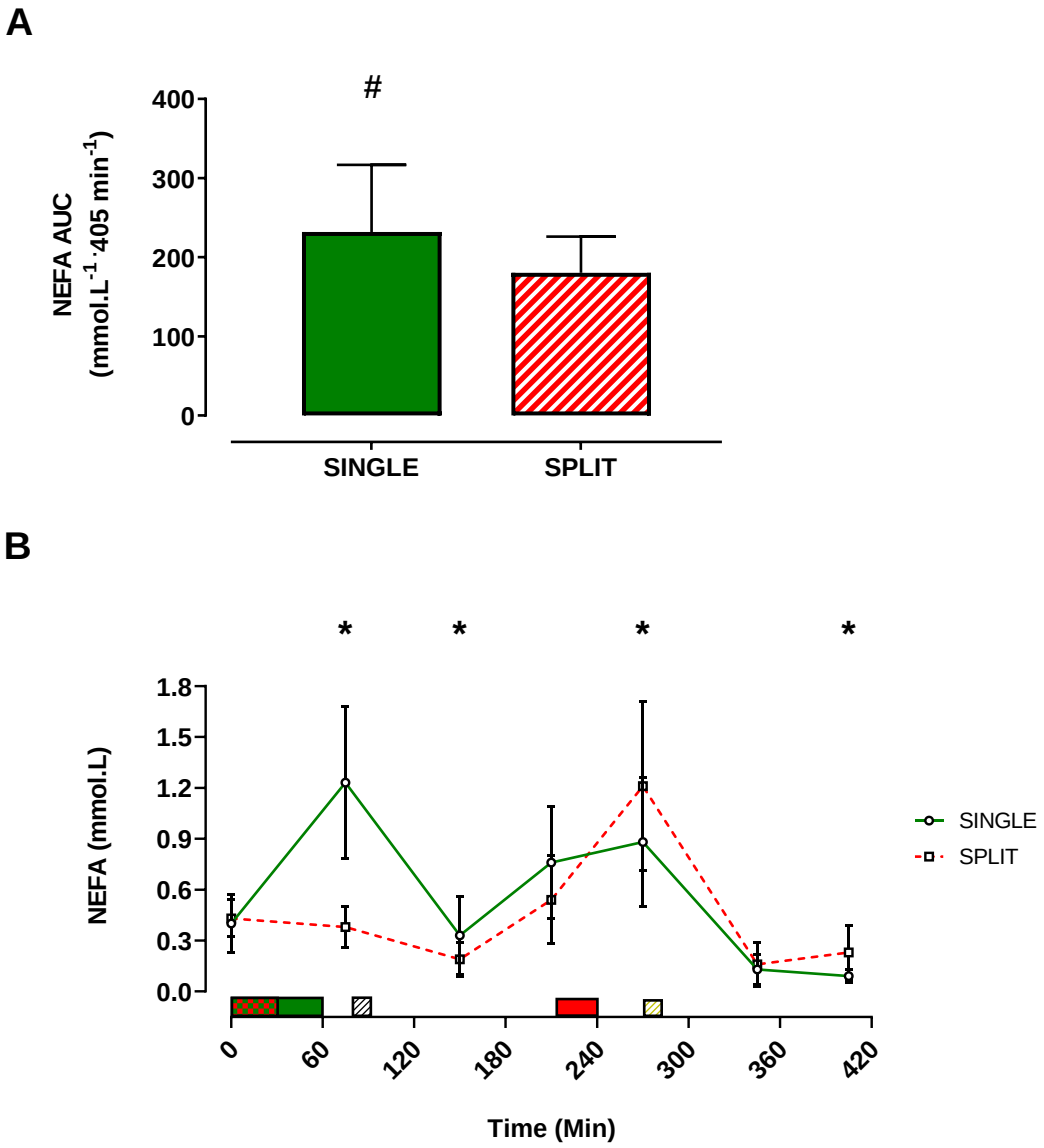

Figure 54: Representive of mean NEFA AUC (0-405 min) (A) and NEFA VAS response (B), with vertical error bars display SDs (n = 14). *Chequered red and green rectangle* indicates 30-min exercise period; *Filled green rectangle* indicates the remaining 30-min exercise period of the 60-min single bout; *Hashed black rectangle* indicates 30% ETEE milk-drink. *Red rectangle* indicates remaining 30-min exercise period for split exercise; *Hashed yellow rectangle* indicates ingestion of 70% ETEE semi-solid meal. There was a main effect observed for NEFA AUC ($p < 0.05$) examined by a one-way repeated-measured ANOVA. There was no main effect for trial ($p > 0.05$). However, there was a main effect for time and a main interaction effect observed for NEFA ($p < 0.05$) examined by a two-way repeated-measured ANOVA. *, # Indicates post-hoc tests were significantly different between SINGLE vs SPLIT, determined by Bonferroni adjusted paired *t-test* ($p < 0.05$). AUC, area under curve; NEFA, non-esterified fatty acid.

6.3.8. Gut Hormones

6.3.8.1. Active Ghrelin

Active ghrelin responded similarly post exercise at 75 min (pre-breakfast) as SINGLE and SPLIT decreased compared to baseline. Active ghrelin then gradually increased throughout the first recovery phase post-breakfast until in both trials active ghrelin peaked at 270 min (pre-lunch). Active ghrelin then decreased post lunch, before beginning to rise in both trials at 405 min. A main effect of time ($p < 0.001$, $\eta^2p = 0.546$), trial ($p = 0.017$, $\eta^2p = 0.392$) and interaction effect was observed for ghrelin ($p = 0.030$, $\eta^2p = 0.251$, Figure 55B). Post-hoc tests revealed SINGLE significantly decreased at 75 min (pre-breakfast) compared to SPLIT (138± 49 vs. 166± 67 pg.mL: p =0.024, d = 0.50, 95% CI = -34.6–26.1 pg.mL). A post-hoc test also revelled SPLIT was significantly increased at 405 min compared to SINGLE (223± 82 vs. 151± 87 pg.mL: p =0.017, d = 0.88, 95% CI = -42.0–46.4 pg.mL).

There was a main effect for active ghrelin AUC as SPLIT was significantly increased compared to SINGLE (61866 ± 21333 vs. 56896 ± 20008 pg.mL^{-1}. 405 min^{-1}: $p = 0.043$, d = 0.25, 95% CI = −11174–10480 pg.mL^{-1}. 405 min^{-1}, Figure 55A).

6.3.8.2. GLP-1

GLP-1 responded similarly post exercise at 75 min (pre-breakfast) as SINGLE and SPLIT decreased compared to baseline. Both trials then increased post-breakfast (150 min), before SINGLE began to decline until 270 min. However, SPLIT increased once more at 150-min to 210 min before also declining at 270 min. GLP-1 increased post lunch in both trials at 345 min before decreasing at similar rates at 405 min. A main effect of time (p =0.001, $\eta^2p = 0.367$) was observed for GLP-1. However, there were no trial ($p = 0.740$, $\eta^2p = 0.009$) nor main interaction effects observed ($p = 0.544$, $\eta^2p = 0.059$, Figure 56B). There was no main effect for GLP-1 AUC (p = 0.795, η^2p = 0.006; Figure 56A).

6.3.8.3. PYY

PYY responded differently post exercise at 75 min (pre-breakfast) as SINGLE increased and SPLIT decreased compared to baseline. Both trials then increased post-breakfast (150 min). SPLIT then remained elevated above SINGLE for the remainder of the study. A main effect of time (p =0.003, $\eta^2p = 0.452$) was observed for PYY.

However, there were no trial (p = 0.295, η^2p = 0.136) nor main interaction effects observed (p = 0.098, η^2p = 0.193, Figure 57B). There was no main effect for PYY AUC (p = 0.355, η^2p = 0.108; Figure 57A).

6.3.8.4. PP

PP increased in both trials from baseline until 270 min (pre-lunch). PP responded similarly across both trials in the 2h post lunch recovery phase. A main effect of time (p =0.002, η^2p = 0.470) was observed for PP. However, there were no trial (p = 0.838, η^2p = 0.004) nor main interaction effects observed (p = 0.102, η^2p = 0.163, Figure 58B). There was no main effect for PP AUC (p = 0.643, η^2p = 0.018; Figure 58A).

6.3.8.5. Insulin

Insulin responded differently post exercise at 75 min (pre-breakfast) as SINGLE increased and SPLIT decreased compared to baseline. Insulin then responded similarly as both trials increased at 150 min (60 min post breakfast), before declining gradually until 270 min (pre-lunch). Insulin then increased once again post-lunch in both trials where SINGLE remained elevated above SPLIT at 345 and 405 min. A main effect of time (p <0.001, η^2p = 0.799) and interaction effect (p = 0.021, η^2p = 0.366) was observed for insulin. However, there were no trial effects for insulin (p = 0.090, η^2p = 0.317; Figure 59B). A post-hoc test revealed SINGLE was significantly increased at 405 min compared to SPLIT (1056 ± 514 vs. 547± 356 pg.mL: p =0.011, d = 1.22, 95% CI = -334.5–233.8 pg.mL). There was no main effect for insulin AUC (p = 0.052, η^2p = 0.239; Figure 59A).

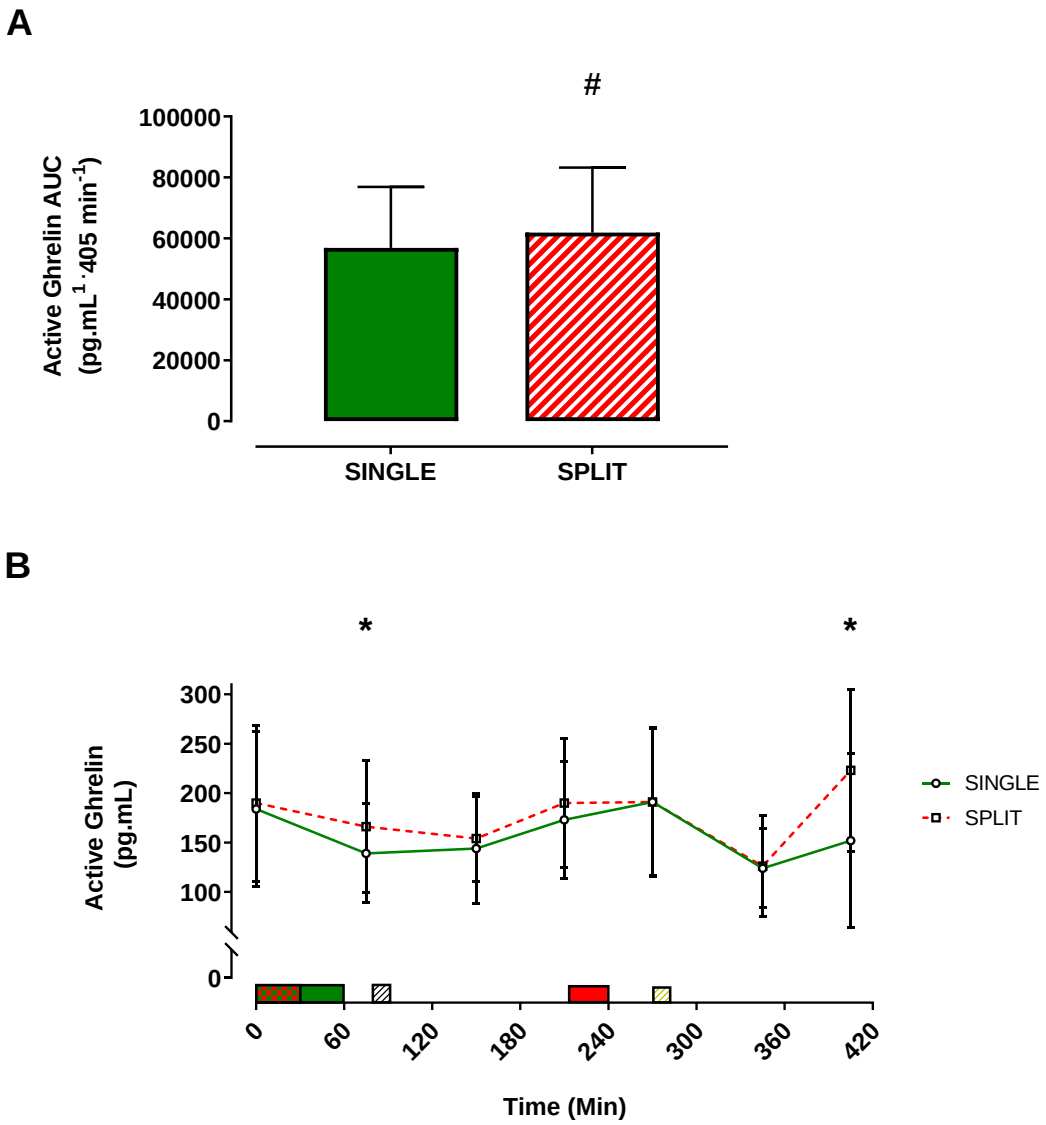

Figure 55: Representive of mean active ghrelinAUC (0-405 min) (A) and active ghrelin VAS response (B), with vertical error bars display SDs (n = 14). *Chequered red and green rectangle* indicates 30-min exercise period; *Filled green rectangle* indicates the remaining 30-min exercise period of the 60-min single bout; *Hashed black rectangle* indicates 30% ETEE milk-drink. *Red rectangle* indicates remaining 30-min exercise period for split exercise; *Hashed yellow rectangle* indicates ingestion of 70% ETEE semi-solid meal. There was a main effect observed for active ghrelin ($p < 0.05$) examined by a one-way repeated-measured ANOVA. There was a main trial, time and a main interaction effect observed for active ghrelin ($p < 0.05$) examined by a two-way repeated-measured ANOVA. *, # Indicates post-hoc tests were significantly different between SINGLE vs SPLIT, determined by Bonferroni adjusted paired *t-test* ($p < 0.05$). AUC, area under curve.

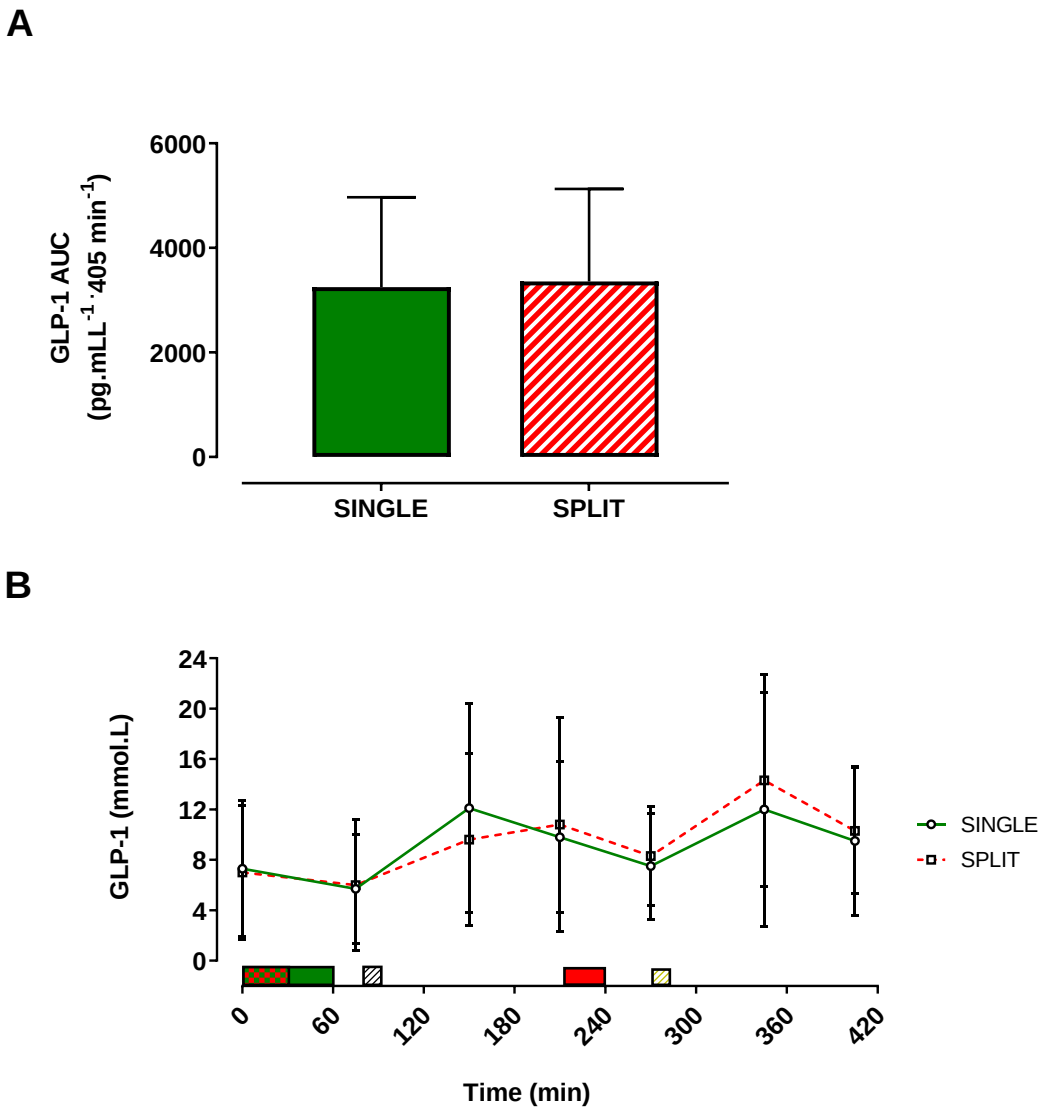

Figure 56: Representive of mean GLP-1AUC (0-405 min) (A) and GLP-1 VAS response (B), with vertical error bars display SDs (n = 14). *Chequered red and green rectangle* indicates 30-min exercise period; *Filled green rectangle* indicates the remaining 30-min exercise period of the 60-min single bout; *Hashed black rectangle* indicates 30% ETEE milk-drink. *Red rectangle* indicates remaining 30-min exercise period for split exercise; *Hashed yellow rectangle* indicates ingestion of 70% ETEE semi-solid meal. There was a main effect observed for GLP-1AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial nor main interaction effect observed for GLP-1 ($p > 0.05$). However, there was an effect for time ($p < 0.05$) examined by a two-way repeated-measured ANOVA. AUC, area under curve; GLP-1, glucagon-like peptide-1.

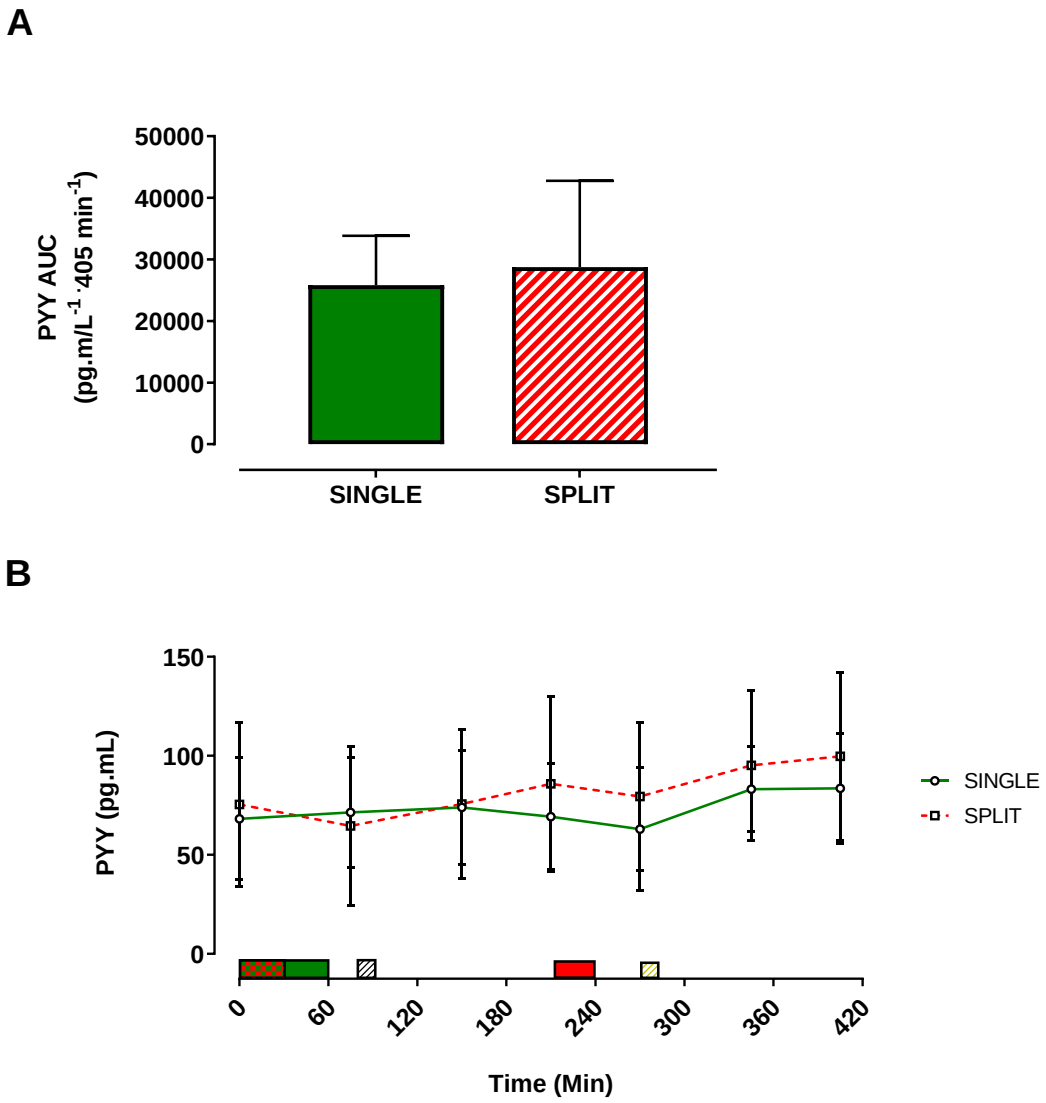

Figure 57: Representive of mean PYY AUC (0-405 min) (A) and PYY VAS response (B), with vertical error bars display SDs (n = 9). *Chequered red and green rectangle* indicates 30-min exercise period; *Filled green rectangle* indicates the remaining 30-min exercise period of the 60-min single bout; *Hashed black rectangle* indicates 30% ETEE milk-drink. *Red rectangle* indicates remaining 30-min exercise period for split exercise; *Hashed yellow rectangle* indicates ingestion of 70% ETEE semi-solid meal. There was a main effect observed for PYY AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial nor main interaction effect observed for PYY ($p > 0.05$). However, there was an effect for time ($p < 0.05$) examined by a two-way repeated-measured ANOVA. AUC, area under curve; PYY, peptide-YY.

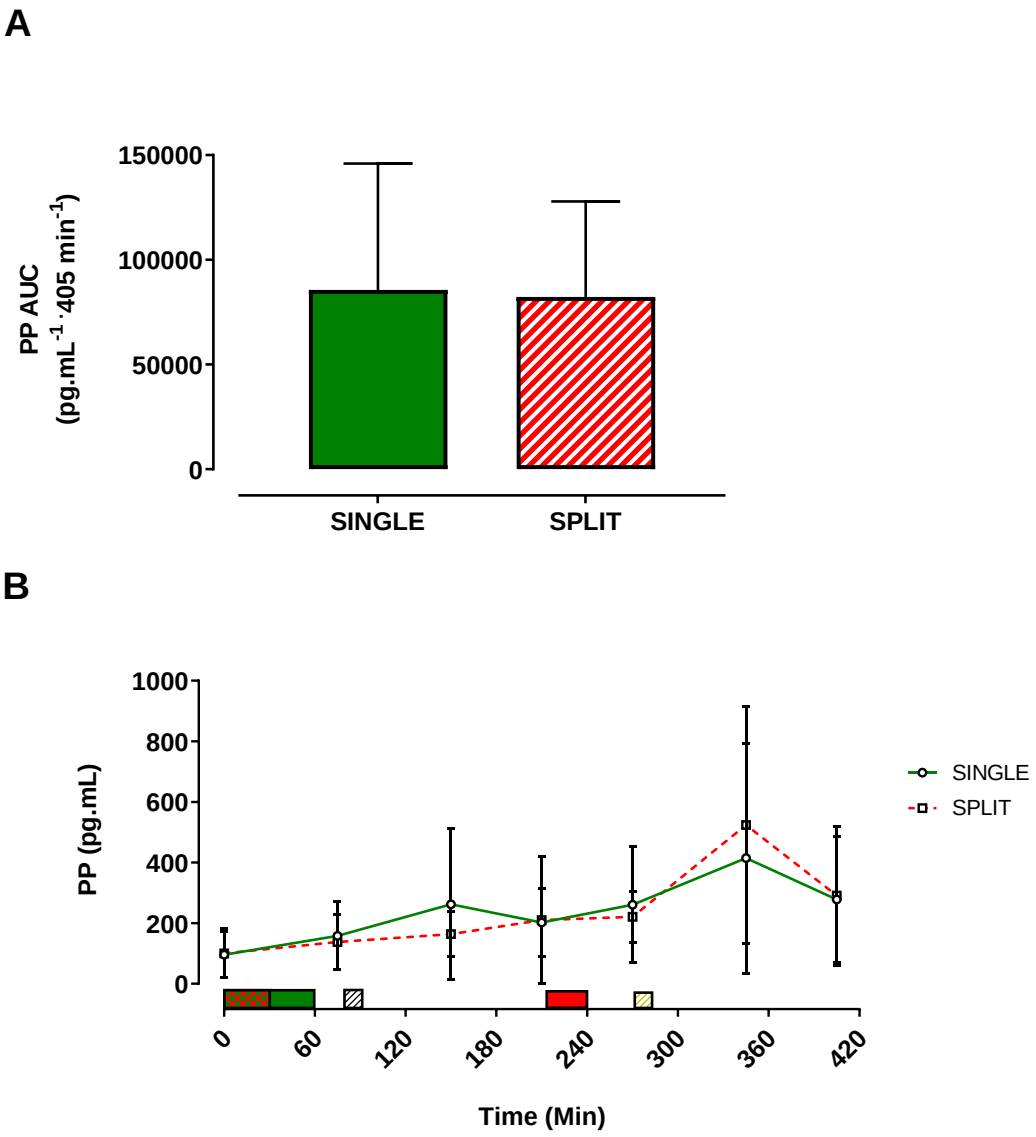

Figure 58: Representive of mean PP AUC (0-405 min) (A) and PP VAS response (B), with vertical error bars display SDs (n = 14). *Chequered red and green rectangle* indicates 30-min exercise period; *Filled green rectangle* indicates the remaining 30-min exercise period of the 60-min single bout; *Hashed black rectangle* indicates 30% ETEE milk-drink. *Red rectangle* indicates remaining 30-min exercise period for split exercise; *Hashed yellow rectangle* indicates ingestion of 70% ETEE semi-solid meal. There was a main effect observed for PP AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There were no main trial nor main interaction effects observed for PP ($p > 0.05$). However, there was an effect for time ($p < 0.05$) examined by a two-way repeated-measured ANOVA. AUC, area under curve; PP, pancreatic polypeptide.

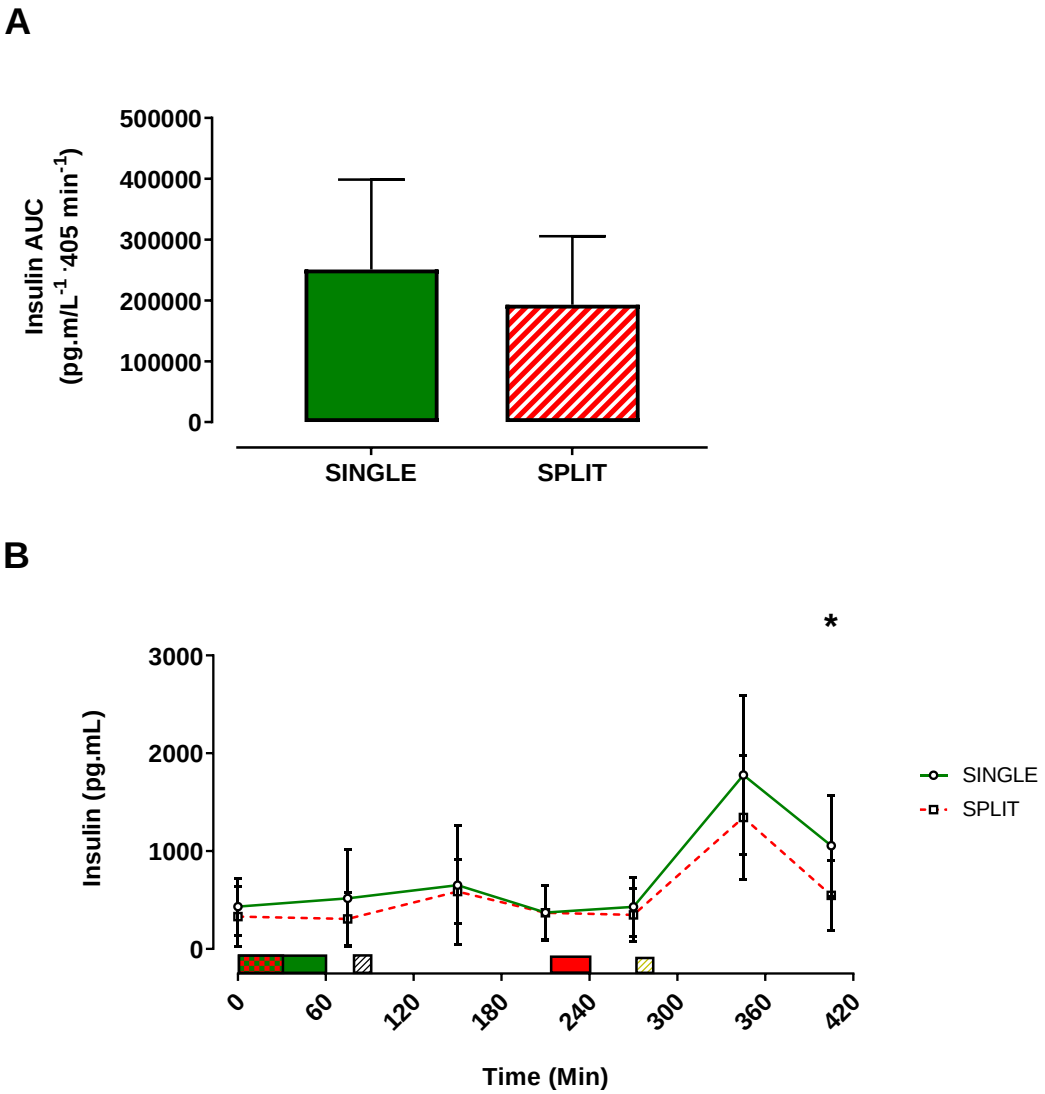

Figure 59: Representive of mean insulin AUC (0-405 min) (A) and insulin VAS response (B), with vertical error bars display SDs (n = 9). *Chequered red and green rectangle* indicates 30-min exercise period; *Filled green rectangle* indicates the remaining 30-min exercise period of the 60-min single bout; *Hashed black rectangle* indicates 30% ETEE milk-drink. *Red rectangle* indicates remaining 30-min exercise period for split exercise; *Hashed yellow rectangle* indicates ingestion of 70% ETEE semi-solid meal. There was a main effect observed for insulin AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial effect observed for insulin ($p > 0.05$). However, there was an effect for time and a main interaction effect ($p < 0.05$) examined by a two-way repeated-measured ANOVA. * Indicates post-hoc tests were significantly different between SINGLE vs SPLIT, determined by Bonferroni adjusted paired *t-test* ($p < 0.05$). AUC, area under curve.

6.3.9. Substrate Utilisation

6.3.9.1. Carbohydrate Oxidation

Carbohydrate oxidation AUC was significantly lower for SINGLE compared to SPLIT (175 ± 35 vs 200 ± 43 g/min^{-1} 405 min^{-1}, *P* =0.022, d = 0.66, 95% CI =-21.86–19.00 g/min^{-1} 405 min^{-1}, Figure 60). There was a main effect of time (*P* <0.001, η^2p = 0.951), trial (*P* = 0.024, η^2p = 0.333) and interaction (*P* <0.001, η^2p = 0.896) effects for carbohydrate oxidation.

Post-hoc tests revealed SINGLE was significantly lower at 30-min (2.52 ± 0.60 vs 3.05 ± 0.80 g/min: *P* = 0.020, d = 0.78, 95% CI =0.36–1.09 g/min) and unsurprisingly higher at 45-min (2.30 ± 0.63 vs 0.17 ± 0.13 g/min: *P* <0.001, d = 4.86, 95% CI =4.53–4.93 g/min) and 60min (2.21 ± 0.51 vs 0.10 ± 0.10 g/min: *P* <0.001, d = 5.92, 95% CI =5.65–5.97 g/min) compared to SPLIT. Subsequently, carbohydrate oxidation was higher 2-h post breakfast (210-min) during SPLIT compared to SINGLE (0.16 ± 0.07 g/min vs 0.11 ± 0.07 g/min: *P* = 0.003, d = 0.74, 95% CI =0.70–0.78 g/min). SPLIT exercise was also significantly higher at 225-min (2.67 ± 0.62 vs 0.16 ± 0.07 g/min: *P* <0.01, d = 5.90, 95% CI =5.58–5.94 g/min) and 240-min (2.52 ± 0.73 vs 0.09 ± 0.10 g/min: *P* <0.01, d = 4.84, 95% CI =4.46–4.89 g/min), which was during exercise compared to rest in the SINGLE trial (Figure 61A).

6.3.9.2. Fat Oxidation

Fat oxidation AUC was significantly higher for SINGLE compared to SPLIT (51 ± 18 vs 42 ± 12 g/min^{-1} 405 min^{-1}, *P* =0.036, d = 0.61, 95% CI =-8.82–6.90 g/min^{-1} 405 min^{-1}, Figure 60). There was a main effect of time (*P* <0.001, η^2p = 0.527) and interaction (*P* <0.001, η^2p = 0.480), but no main effect of trial (*P* = 0.055, η^2p = 0.255) for fat oxidation**.**

Post-hoc tests revealed SINGLE was significantly higher at 45-min (0.36 ± 0.24 g/min vs 0.14 ± 0.05 g/min: *P* = 0.004, d = 1.32, 95% CI =1.19-1.34 g/min) and 60-min (0.42 ± 0.23 g/min vs 0.14 ± 0.05 g/min: *P* = 0.001, d = 1.75, 95% CI =1.63-1.77 g/min). During the first recovery period post breakfast fat oxidation was higher during SINGLE at 180-min (0.13 ± 0.04 g/min vs 0.09 ± 0.02 g/min: *P* = 0.001, d = 1.31, 95% CI =1.29-1.32 g/min) and 210-min (0.15 ± 0.02 g/min vs 0.10 ± 0.02 g/min: *P* = 0.001, d = 2.59, 95% CI =2.58-2.60 g/min) compared to SPLIT. Therefore, during

the second exercise bout SPLIT was unsurprisingly higher at 225-min (0.24 ± 0.15 g/min vs 0.12 ± 0.03 g/min: *P* = 0.009, d = 1.15, 95% CI =1.07-1.17 g/min) and 240-min (0.34 ± 0.16 g/min vs 0.13 ± 0.05 g/min: *P* = 0.001, d = 1.84, 95% CI =1.75-1.86 g/min) compared to the SINGLE trial rest period. Within the second recovery period post lunch SINGLE was significantly higher 60-min post-lunch (345-min) compared to SPLIT (0.09 ± 0.03 g/min vs 0.06 ± 0.03 g/min: *P* = 0.015, d = 1.04, 95% CI =1.02-1.05 g/min, Figure 61B)

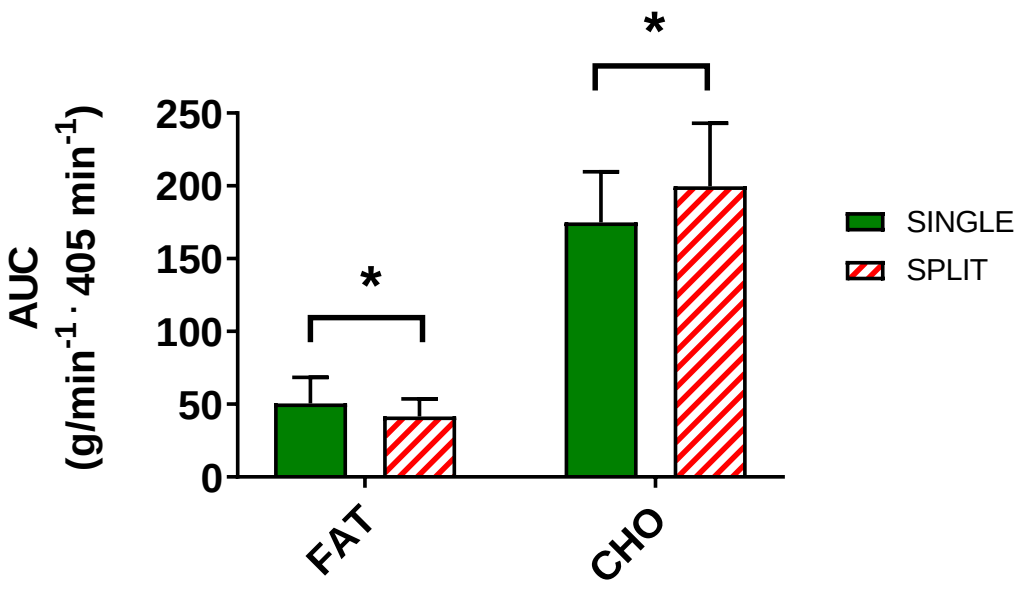

Figure 60: Representative of mean fat and carbohydrate oxidation AUC (0-405 min^{-1}), with vertical error bars display SDs (n = 14). There was a main effect observed for fat and carbohydrate oxidation AUC ($p < 0.05$) examined by a one-way repeated-measured ANOVA. *Indicates SPLIT was significantly different from SINGLE, determined by Bonferroni adjusted paired *t*-test ($p < 0.05$). AUC, area under curve; CHO, carbohydrate.

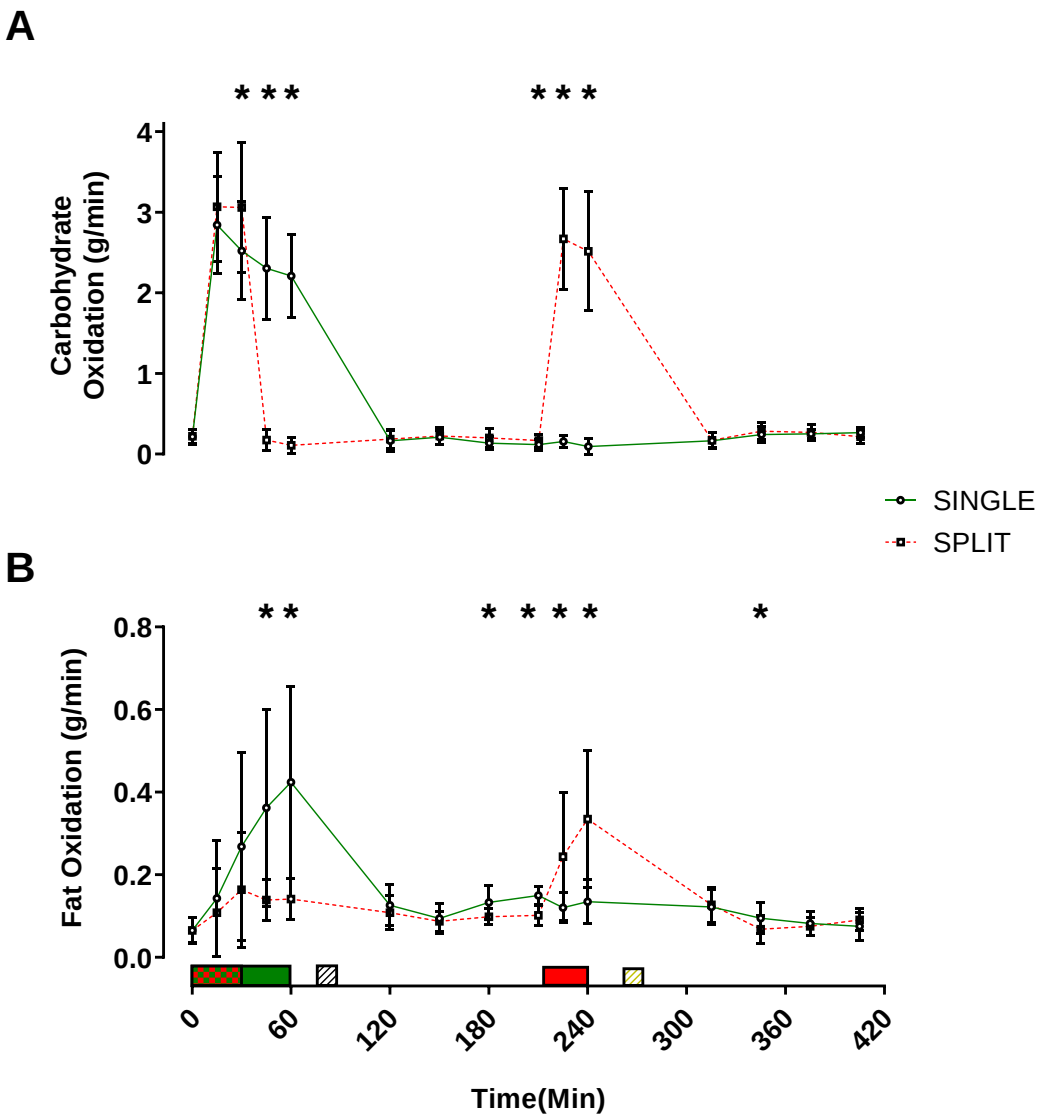

Figure 61: Representative of mean carbohydrate oxidation (g/min) (A) and fat oxidation (g/min) (B), with vertical error bars display SDs (n = 14). *Chequered red and green rectangle* indicates 30-min exercise period; *Filled green rectangle* indicates the remaining 30-min exercise period of the 60-min single bout; *Hashed black rectangle* indicates 30% ETEE milk-drink. *Red rectangle* indicates remaining 30-min exercise period for split exercise; *Hashed yellow rectangle* indicates ingestion of 70% ETEE semi-solid meal. There was no main trial effect for fat oxidation ($p > 0.05$) although there was for carbohydrate oxidation ($p < 0.05$). Furthermore, there was a main effect for time and a main interaction effect ($p < 0.05$) for both fat and carbohydrate oxidation, examined by a three-way repeated-measured ANOVA. * Indicates SINGLE vs SPLIT was significantly different, determined by Bonferroni adjusted paired *t-test* ($p < 0.05$).

6.4. Discussion

The primary aim of the current study was to compare the effects of twice-a-day (two high intensity continuous exercise bouts for 30 min separated by 2 h recovery) vs single bout (one off 60 min high intensity continuous exercise bout) of exercise on hormonal and subjective appetite regulation, metabolic responses, GER, and EI in the subsequent 24 h. The main findings were SINGLE exercise caused a transient elevation in fat utilisation by ~17 % overall, which was possibly directly related to exercising in a fasted state for 60 min. Nevertheless, this led to a suppression in hunger (subjective appetite) by ~17% and strengthened by molecular data, as acylated ghrelin also reduced by ~16% post-first exercise bout (75 min) compared to SPLIT exercise. In addition, there was no difference in hormonal nor subjective appetite regulation within the recovery period after a breakfast meal. Subsequently, GER of a semi-solid lunch meal was significantly accelerated within the SPLIT compared to SINGLE exercise leading to a raised acylated ghrelin concentration 2 h post lunch at 420 min, which might account for the ~29% increase in EI in the subsequent 24-h after twice-a-day exercise. These results suggest that a single bout of continuous exercise completed at a high intensity, might be superior at controlling appetite and EI within healthy men, and whether these findings extend to different populations (lean/overweight men or females) is currently unknown.

To the author's knowledge, this is the only study to examine GER of a semi-solid meal after completing twice-a-day vs. one off exercise bout. Previous studies have examined how different nutritional solutions or meals effect GER within the post-exercise period. A number of investigations have found an accelerated emptying rate (Clayton et al., 2014, Evans et al., 2018a); no change between emptying rate (Evans et al., 2016, Mattin et al., 2018b, McIver et al., 2018); and a time of day difference in emptying rate after exercise (McIver et al., 2019). Within the current study GER of a semi-solid meal was accelerated, indicated by a ~17% faster maximal emptying time (T_{lag}) and a ~20% faster half emptying time (T_{half}) after the SPLIT compared to the SINGLE exercise trial. Consequently, the majority of previous literature investigating the effects of exercise intensity on GER during exercise have suggested GER is impeded at intensity >75% $\dot{V}O_{2max}$ (Neufer et al., 1989) and slower

GER is detected after intermittent sprint exercise compared to moderately intense continuous exercise (Leiper et al., 2001a).

Therefore, it is apparent that exercising at a high intensity disrupts gastric emptying more so during exercise than within the post-prandial period. To emphasise this point data from Chapter 5 (Mattin et al., 2020a), identified the emptying rate of a semi-solid meal was unaffected 30 min after intermittent exercise at a low (40% $\dot{V}O_{2peak}$) and a moderate intensity (60% $\dot{V}O_{2peak}$), compared to an energy matched continuous exercise bout at the same low and moderate intensity. Data from the current investigation indicated that a semi-solid meal is released from the stomach at a faster rate when a meal in consumed 30 min after a bout of exercise in excess of >60% $\dot{V}O_{2peak}$. This may suggest if sufficient time is allocated after high intensity strenuous exercise the body's ability to recover and deliver nutrients to the small intestine is increased to match the demands of exercise delivered. Nevertheless, Jeukendrup (2017b), advocates that the GI system is highly adaptable and gastric emptying as well as the stomach can be 'trained'. With limited research, directly focusing on measuring GER, this statement is bold and warrants further investigation to understand if highly trained individuals' or athletes' GER responds similarly to that of an untrained population as for an athlete an accelerated GER post-exercise may be seen as an advantage. Although, whether an accelerated GER is beneficial for health-related changes requires further investigation.

One potential mechanism for changes in GER is gut permeability as increased I-$FABP_2$ have been shown to correlate with splanchnic hypoperfusion (reduced blood flow to the abdominal region), post strenuous exercise (van Wijck et al., 2011). Kashima et al, (2017) supports these findings as I-$FABP_2$ concentration increased after intermittent supramaximal cycling and GER was delayed post-exercise compared to a control. The delay in GER was attributed to the increase in I-$FABP_2$ and therefore suggests small intestinal mucosal damage triggered a delay in GER. Nevertheless, within the current investigation an increase in I-$FABP_2$ was observed for both trials after the first exercise bout, although no differences in I-$FABP_2$ were documented between conditions.

Previous studies have reported that healthy individuals express a suppression in appetite immediately after exercise at an intensity >60% $\dot{V}O_{2max}$ (Martins et al.,

2007a, Becker et al., 2012, King et al., 2013b). Consistent with the current study, participants reported a ~17% decrease in hunger after 60 min of continuous exercise at 70% $\dot{V}O_{2peak}$ (SINGLE) but surprisingly, no difference was established after the SPLIT exercise trial operated at the same intensity for 30 min. It must be noted, the difference between subjective hunger may not have been related to the duration of exercise, but instead, to the time difference between finishing exercise at 30 min during SPLIT and 60 min for SINGLE before completing a VAS questionnaire at 75 min pre-breakfast. Holliday and Blannin, (2017c) found no suppression in appetite immediately after completing an exercise bout for 15, 30 and 45 min at ~80% $\dot{V}O_{2peak}$ showing high intensity continuous exercise does not result in further decreases in appetite, regardless of the difference in duration. However, they did discover that after as little as 20 min after exercise subjective appetite began to increase from immediate post-exercise values (Holliday and Blannin., 2017c). Deighton and Stenel et al, (2014) are in agreement, that appetite values tend to return to control values within 30 min of the cessation of exercise; reinforcing the point appetite suppression experienced after a high intensity exercise bout is short lived. Despite this decrease in perceived hunger post exercise, subjective appetite sensations were not significantly different between trials, after a relatively small breakfast drink (semi-skimmed milk). This suggests that subjective appetite can be offset by a small breakfast meal, independent of the changes observed after exercise. No differences were seen for the remaining appetite related questions (fullness, PFC, satisfaction, bloat nor nausea), and no further differences were observed during the remainder of trial.

Despite appetite responding similarly within the postprandial period after lunch in the current study, EI was ~29% (733 KCal) higher in the subsequent 24 h after the SPLIT exercise trial; although the majority of the available literature states exercise does not stimulate a change in EI > 20 h after exercise (King et al., 1997, King et al., 2010a, Hanlon et al., 2012). Controversially, within Chapter 5 (Mattin et al., 2020a), revealed moderate, intense, intermittent exercise stimulated an 723 KCal increase in EI in the subsequent 24 h, when compared directly to an energy matched continuous exercise bout. Disappointingly, the mechanism for this increase in EI within Chapter 5 was not directly clear as limitations within the protocol, prevented the collection of metabolic and hormonal markers. Nevertheless, within the current

study, metabolic and hormonal markers were collected as concentrations of the orexigenic hormone acylated ghrelin and the anorexigenic hormone GLP-1, which are thought to respond to fluctuations in energy balance (Cummings, 2006, Holst, 2007, Clayton et al., 2015). Therefore, the crosstalk between the gut and the brain might be critical in controlling energy balance, as many of the gut hormones secreted from enteroendocrine cells signal through the vagus nerve; following meals, ingestion cells in the GI tract secrete a number of satiety-inducing hormones; CCK, GLP-1 and PYY (Cork, 2018).

One potential mechanism is the role of GLP-1 in the mechanical distension of the stomach, signalling to the brain that the recipient is content. Evidence has emerged within mice models, that GLP-1R are highly expressed on vagal afferent terminals in the hepatic vein (Vahl et al., 2007) and brainstem (Williams et al., 2016). Furthermore, these findings suggest that GLP-1R are also highly predominant in the afferent vagus nerve and are not found in great numbers near to the intestinal villi close to GLP-1 secreting cells but are largely expressed in stomach muscle. This theory is further supported by Krieger et al, (2016) as they demonstrated that knockdown GLP-1R in the vagal afferents of rates increased meal size and meal duration. For this reason, within the current study, each meal period was standardised for volume, regardless of the difference in EI between participants, to prevent the change in stomach size between meals affecting satiety-inducing hormones. GLP-1 and PYY did increase after both meal periods; however, there was very little difference between conditions.

Acylated ghrelin positively correlates with the feeling of hunger, with concentrations being high before and decreasing after eating (Cummings et al., 2004, Clayton et al., 2016, Hazell et al., 2016, McIver et al., 2018). However, acylated ghrelin has demonstrated large reductions in concentrations ~60%, compared to pre-exercise or resting controls, after completing high to moderate intense, aerobic exercise <70% $\dot{V}O_{2peak}$ (Broom et al., 2009, King et al., 2011a, Balaguera-Cortes et al., 2011, Becker et al., 2012, Wasse et al., 2012, Wasse et al., 2013b, Kawano et al., 2013, Broom et al., 2017). Consistent with the current study, acylated ghrelin decreased from fasting baseline values by ~24% after SINGLE and by ~12% after SPLIT. Subsequently, acylated ghrelin concentrations remained relatively stable during the remainder of the trial, until the final sample at 420 min where acylated

ghrelin concentrations increased by ~31% during the SPLIT trial compared to the SINGLE. The mechanism for this increase in acylated ghrelin 2 h after lunch is not directly clear, although this increase might be an indicator of the body's response to restore energy balance homeostasis. A number of factors might account for the increase in acylated ghrelin, which in turn might strengthen the fact EI was ~29% high after the SPLIT trial. This indirect increase in appetite during the hours after leaving the laboratory, might be accounted for in response to the different acylated ghrelin concentration before leaving.

Research suggests that increased blood glucose (Shiiya et al., 2002, Sim et al., 2014) and insulin concentrations (Flanagan et al., 2003, Broglio et al., 2004) are associated with decreased ghrelin levels. This has been well established within animal models as injecting insulin into rat stomach cells was found to impair ghrelin (Gagnon and Anini, 2012). Iwakura et al, (2010) also found that ghrelin producing cells express insulin receptors, which potentially explains why insulin might inhibit ghrelin after consuming nutrient dense substances. To further discuss the changes discovered in the present study, overall glucose AUC was greater and overall insulin AUC tended to be greater ($P = 0.052$) during SINGLE, suggesting glycaemic control was impaired after a single bout of exercise, compared to a split exercise bout. As we know from previous studies, any person with higher blood glucose after feeding is at an increased risk of cardiovascular disease (Pekkanen, 1999, Ning et al., 2012) or becoming insulin resistant (Pöykkö et al., 2003, Purnell et al., 2003). To further emphasise this point, within the current study, glucose and insulin values were significantly lower 2 h post lunch (420 min) during SPLIT, and the reason for this may be explained by a number of different deviations throughout the trial. In particular, Gonzalez et al, (2013) suggested plasma glucose concentrations are elevated after exercise by pre-exercise feeding. Although, within the present study participants within the SINGLE trial consumed breakfast ~270 min prior to the increases seen in glucose at 60- and 120-min post lunch. Therefore, exercising 2 h after consuming a liquid breakfast (30% ETEE), potentially stimulated postprandial insulin sensitivity from a semi-solid lunch meal (70% ETEE).

One potential theory for the difference seen in glucose and insulin, might be explained by limited glucose availability during exercise because of glycogen

depletion (Coyle and Coggan, 1984). Plasma glucose during exercise when fasting is primarily derived from hepatic sources, suggesting a potential role for liver carbohydrate status in the regulation of postexercise energy balance (Edinburgh et al., 2019). However, carbohydrate in the form of muscle glycogen has been shown to be the predominant substrate used during prolonged exercise above ~65% $V02_{Max}$ (van Loon et al., 2001, Romijn et al., 1993). Edinburgh et al, (2019) observed that plasma glucose disposal rates during a fasted exercise bout represented the use of glucose from hepatically derived sources, as no carbohydrate was ingested before or during a fasted exercise trial. Eating breakfast is encouraged in the literature to maximize carbohydrate stores before exercise performance (Williams and Serratosa, 2006), as liver glycogen stores have been shown to decrease substantially after an overnight fast, resulting in a greater decrease in blood glucose concentration during a prolonged exercise bout (Taylor et al., 1996, Learsi et al., 2019). We know from work carried out within our own laboratory, fasting prior to exercise reduces carbohydrate utilisation during exercise (McIver et al., 2018, McIver et al., 2019), and these results are consistent with the current study as fat utilisation increased and carbohydrate utilisation decreased during 60 min of exercise at 70% $\dot{V}O_{2peak}$ in a fasted state. Overall Fat oxidation AUC was greater during SINGLE by ~17%, suggesting overall lipolysis may have been increased. Although, this result should not be misinterpreted, as the increase in fat oxidation AUC data between trials was most likely a result of exercising for 60 min fasted during the SINGLE trial compared to 30 min during the SPLIT trial. This can further be supported by the fact that fat oxidation did increase in the second exercise bout within the SPLIT trial 2 h after breakfast, although this was not at the same extent as exercising in the morning after a ~10 h fast.

In brief, these differences observed 2 h post-lunch might further be supported by an increase in NEFA and triglycerides within the SPLIT trial compared to the SINGLE, suggesting a switch in energy utilisation before the end of the trial at 420 min. It could be considered that the increase in acylated ghrelin, NEFA and triglycerides and the decrease in glucose and insulin between trials may support the reason why EI was higher 24 h after the SPLIT trial. Although, additional work must consider how changes in the metabolic effects after exercise might further effect EI, and appetite in the days following strenuous exercise.

6.4.1. Limitations

Limitations within the current study must be acknowledged, as any research that investigates substrate utilisation during high intensity exercise may be brought into question. The assumptions on which indirect calorimetry is based can be more problematic during intensity >75% $\dot{V}O_{2Max}$ and the validity of using gas exchange measurements to calculate substrate oxidation above this intensity must be questioned (Jeukendrup and Wallis, 2005). However, if researchers acknowledge that indirect calorimetry under various experimental conditions might have relatively high variation between participants, when exercise >60% $\dot{V}O_{2Max}$, gas exchange measurements can be a very useful tool for the measurement of substrate oxidation. Future studies should further examine the differences in post-exercise energy demands after consuming food, as the energy demands in the recovery period must be considered, as any increase in fat oxidation during exercise may be mitigated by consuming food post-exercise (Melanson et al., 2002).

There might be some extenuating factors contributing to the reason why I-$FABP_2$ was increased but not different between exercise conditions. To begin with, both trials were conducted at the same high exercise intensity 70% $\dot{V}O_{2peak}$, suggesting 30 min was sufficient to cause an increased intestinal cellular injury which caused cell membrane integrity to be compromised following strenuous exercise to the same extent as 60 min. According to March et al, (2017) 20 min of running at 80% $\dot{V}O_{2peak}$ was enough to cause an increase in I-$FABP_2$ within a control group. Secondly, no further increases were observed after the second exercise bout (SPLIT only) at 270 min. This may be related to the fact these samples were not collected in a fasted condition, and any further increase in I-$FABP_2$ might have been affected by the role of I-$FABP_2$ in cellular uptake and metabolism of fatty acids (Ockner and Manning, 1974, Lowe et al., 1987, Zimmerman et al., 2001). It must be added, the large proportion of literature which has identified an increase in I-$FABP_2$ post exercise after continuous exercise (van Wijck et al., 2011), resistance exercise (van Wijck et al., 2013) and an intermittent mode of exercise (Pugh et al., 2017, Kashima et al., 2017), has directly compared the increase to a non-exercise control group. Therefore, it may be considered that without a non-exercise control group to directly compare the basal state, it is unclear if the increase in I-$FABP_2$ seen after exercise, and the accelerated

GER after lunch, is a response to the exercise stimulus, or the environment the study was conducted in, and without control data, these types of conclusions may be invalid.

6.4.2. Conclusions

The results of this study demonstrate that exercising twice-a-day at a high intensity (70% $\dot{V}O_{2peak}$), compared to a one-off single bout of exercise matched at the same intensity, accelerated GER. Despite this, no differences were observed during the short 2 h monitoring period after lunch, for subjective appetite in response to the accelerated GER within the twice-a-day trial. However, an increase in acylated ghrelin was reported at the end of the trial after twice-a-day exercise, but surprisingly not after the single bout. This suggests a potential rationale for the reason why EI was elevated 24 h after twice-a-day exercise. These findings may have important implications for current exercise prescription guidelines, as performing multiple exercise bouts within the same day, separated by a short recovery phase (2 h) is possibly less effective at controlling EI in response to high intensity physical activity energy expenditure, when meal periods are balanced for energy in, to energy out. If increased EI persisted over a longer period, this might eventually lead to weight gain and long-term health concerns. Going forward, it is important to understand that if structured exercise is completed in multiple bouts throughout the day to reflect a normal working schedule, it will also affect appetite regulatory hormones and EI. This would require lengthening the time between exercise bouts and providing participants with a breakfast period, which reflects an adequate calorie consumption during this meal period. Conversely, promoting the simple act of providing a more substantial breakfast meal could theoretically compensate for the changes seen when exercising twice-a-day rather than once.

Chapter 7

How Does Exercising Twice-a-Day Affect Gastrointestinal Function, Appetite, and Metabolic Response after Consuming a Standardised Breakfast and Lunch Respectively after each Exercise Bout in Healthy Males: A Randomised Cross Over Trial[1]

[6] The data from this study contained within this chapter was going to be presented at Europhysiology. However, this event was cancelled due to COVID-19. "Mattin LR, Ishihara K, McIver LR, Yau AMW, James LJ, Evans GH (2020). How does a 'twice-a-day approach' to a daily exercise routine, effect gastrointestinal hormonal, appetite regulation and metabolic response after consuming a standardised breakfast and lunch respectively after each exercise bout in healthy males. Europhysiology. ESTREL, Congress centre, Berlin, Germany."

7.1 Introduction

What we know from the literature is that ghrelin commonly decreases immediately after moderate or high intensity exercise (60-75% $\dot{V}O_{2max}$) (Broom et al., 2007, Broom et al., 2009, King et al., 2010a, Becker et al., 2012, Wasse et al., 2013b, Holliday and Blannin, 2017c). However, investigations have predominantly focused on continuous exercise bouts, suggesting further investigations are needed to improve understanding of the appetite response after an acute bout of high intensity intermittent exercise. As discussed previously, one potential mechanism for adaptation in GER after exercise is potentially a modification in gut hormone response, as even a small alteration in the secretion of gut hormones may have important implications in the regulation of GER, and consequently EI (Little et al., 2007, Meneguetti et al., 2019). With exercise intensity possibly being more important for appetite regulation than exercise duration (Hazell et al., 2016).

In recent years the health effects of interval/ intermittent training on cardio-metabolic outcomes have been strongly investigated (Atakan et al., 2021). Larger groups of adult males and females are engaging in HIIT based training programmes to improve health and fitness (Thompson, 2021). Adaptations within the general population such as improvements in ṾO2max, body composition, mitochondrial biogenesis and fat oxidation during exercise are just a small potential benefit of intermittent exercise (Andrade-Souza et al., 2020, Bishop et al., 2019, Wewege et al., 2017). Increase in whole-body fat oxidation and reduction in carbohydrate utilisation during exercise and rest is a regular adaptation observed in populations who commit to regular endurance training (Hurley et al., 1986, Hargreaves and Spriet, 2020).

Therefore, limited research has identified GER as a potential mechanism responsible for an altered appetite response or substrate utilisation in the hours and days following exercise or particularly intermittent exercise. Higher fat oxidation capacity is associated with better metabolic health (Kelley et al., 1999, Thompson et al., 2001). To date, this is the first investigation to examine whether intermittent exercise will affect appetite regulatory hormones, GER and the association between EI after exercise.

Food is an excellent anorexic agent known to reduce hunger and to suppress eating for a period after administration. Although, as the desire to eat begins to increase the feeling of hunger is mitigated, leading to nutritional and calorie intake once more (Blundell, 1991). One major disadvantage of consuming food to reduce the feelings of hunger, is the increase in weight gain if not balanced with energy expenditure (Paravidino et al., 2020). It must be noted within Chapter 4 (Mattin et al., 2018a), Chapter 5 (Mattin et al., 2020a) and Chapter 6 of this thesis; participant's subjective appetite responses were unaffected in the short 2 h monitoring period after exercise, which is consistent with the majority of investigations that have studied appetite response after exercise (Burns et al., 2007, Martins et al., 2007a, Becker et al., 2012, Gonzalez et al., 2013). Subsequently, EI was significantly higher 24 h after completing a moderate intensity intermittent exercise bout (Chapter 5) and after multiple exercise bouts separated by 2 h recovery (Chapter 6). Interestingly Notable, Deighton and Stensel et al, (2014) suggest that exercising at high intensities >60% $\dot{V}O_{2max}$, have consistently been found to supress appetite via changes in appetite-regulating hormones, known as 'exercise-induced anorexia' which subsequently restricts EI immediately after exercise. Despite these transient effects in appetite, it is unlikely that overall EI is influenced as many investigations have failed to discover any changes in EI >24 h after exercise (King et al., 1997, Pomerleau et al., 2004, Hanlon et al., 2012, King et al., 2013a). For this reason, it is unknown if the unanticipated increase in EI was governed by the intermittent nature of exercise or the separation of exercise bouts throughout the day.

It must be noted that endurance trained athletes have several different methods of training. The primary focus is to improve performances, by intensifying the productivity of the human body and potentially increasing the number of mitochondria within a cell, these generate cellular energy via oxidative phosphorylation (Bishop et al., 2019, Ghiarone et al., 2019). It has been suggested that performing regular intermittent exercise increases $\dot{V}O_{2Max}$ and shifts substrate metabolism from carbohydrate to lipid oxidation, leading to showing a reduction in fat mass by increased exercise induced energy expenditure (Kiens et al., 1993, Hazell et al., 2012, Skelly et al., 2014, Townsend et al., 2014). A potential mechanism for this adaptation is the stimulation of cytoplasmic and nuclear proteins post endurance exercise, which

increase mitochondrial biogenesis, which is the generation of new mitochondrial components leading to increased mitochondrial content and respiratory functions (Ghiarone et al., 2019, Granata et al., 2018, Andrade-Souza et al., 2020).

A number of investigations using athletes have suggested initiating training sessions with reduced muscle glycogen stores, by using a 'train-low' approach (Burke et al., 2021, Lane et al., 2015, Hulston et al., 2010, Yeo et al., 2008a, Yeo et al., 2008b); this might better improve training induced adaptations in markers of oxidative metabolism when compared with exercise sessions with a normal muscle glycogen stores (Morton et al., 2009, Hulston et al., 2010). Recently, one exercise design has had increased attention within the athlete population, known as the 'twice-a-day' approach (Hansen et al., 2005, Cochran et al., 2015, Ghiarone et al., 2019, Andrade-Souza et al., 2020). Participants usually perform an exercise bout for 30 to 90 min at >50% $\dot{V}O_{2max}$, followed by a second exercise session 1-3h later with reduced muscle glycogen (Ghiarone et al., 2019). This method has been shown to improve aerobic fitness ($\dot{V}O_{2peak}$ and lactate thresholds) and was sufficient within 3 weeks to reduce body mass and body fat (Andrade-Souza et al., 2020). Nevertheless, the primary attention of most of the literature within this area of research is skeletal muscle adaptations and gene expression. To date, no study has investigated whether exercising 'twice-a-day' will disrupt appetite regulation within a healthy population leading to similar body fat responses found within athletes.

To our knowledge, no investigation has compared whether the timing or routine of intermittent exercise influences appetite and GER response, leading to a change in metabolic and hormonal regulation within healthy males. It was hypothesised that performing intermittent exercise within the morning would reveal a reduction in appetite, and acylated ghrelin post exercise, leading to compensatory increases in EI. Hence, the null hypothesis was that there would be no differences between the conditions, regardless of whether an intermittent bout of exercise was performed within the morning or afternoon.

Therefore, the aim of this study was to explore whether performing intermittent exercise during the morning [10 × 1 min high intensity bouts at PPO, separated by 2 min recovery], followed by a 30 min low intensity (50% $\dot{V}O_{2peak}$) continuous exercise bout separated by 3 h recovery, compared to an intermittent session within the

afternoon [continuous exercise during the morning followed by intermittent exercise 3 h later within the afternoon] would affect: 1) Hormonal regulation of appetite, 2) GER following a post-lunch semi-solid meal, and 3) Whether the timing of intermittent exercise affects subjective feelings of wellbeing, leading to overall alterations in EI 24 h following acute bouts of exercise?

7.2 Methods

7.2.1. Participants

Twelve healthy men were recruited from central Manchester and undertook experimental trials between September 2019 and March 2020 in the physiology laboratories at Manchester Metropolitan University, UK (Table 16). Verbal and written explanations of the experimental procedures were provided before the start of the trials and written informed consent was obtained. An a priori calculation was conducted using data from Chapter 6 focusing on GER and appetite data. An effect size η^2p = 0.240 from a repeated measures ANOVA model, attributing GER as the primary outcome measure, and using an α of 0.05 and a statistical power of 0.8 determined that $\geq$ 12 participants would be required to reject the null hypothesis (G*Power 3.0.10, Heinrich Hein Universitat, Dusseldorf, Düsseldorf, Germany). This chapter was registered at clinicaltrials.gov online, a resource provided by the U.S. National Library of medicine. Identifier: NCT04122209; Does the timing of when High Intensity Intermittent exercise is undertaken matter?

Table 16: S-4, Baseline Subject Characteristics[1]

	Males (*n*=12)
Age, (y)	26 ± 6
Height, (m)	1.82 ± 0.04
Weight, (Kg)	81.8 ± 12.8
BMI, (kg/m²)	24.6 ± 4
Body fat, (%)	20.4 ± 6.7
Systolic BP, (mmHg)	134 ± 8
Diastolic BP, (mmHg)	75 ± 11
$\dot{V}O_{2peak}$, (ml/kg/min)	48 ± 10
Continuous, (W)	116 ± 20
HIIE, (W)	295 ± 28
ETEE, (KCal)	628 ± 35

[1] Values are means ± SDs
ETEE; Estimated trial energy expenditure

7.2.2. Experimental Trials

Participants reported to the laboratory on two occasions to complete each ~8 h (0-450min) trial. Experimental trials commenced between 0700 and 0800 following an overnight fast from 22:00, except for plain water consumption. In addition to the pre-trial familiarisation outlined in general methods, each participant was also required to standardise their diet and physical activity before each trial (see section 3.7. Pre-trial Standardisation).

Upon arrival at the laboratory, participants were asked to completely empty their bladder before pre-trial body mass was obtained. A heart rate monitor (Polar H10, Kemple, Finland) was then strapped around each participant's chest so heart rate (HR) could be observed during exercise periods. Participants then rested for 15 min in a semi-supine position whilst a cannula was inserted into the antecubital vein to enable baseline (0 min) venous blood samples to be collected. Following this, VAS appetite and wellbeing questionnaires were completed prior to expired gas samples being collected for 15 min in a semi-supine position on a bed using a breath-by-breath gas

analyser (Metalyzer 3b, Cortex, Leipzig, Germany). All expired gas samples during this study were collected during rest (0, 30, 135, 195, 255, 285, 390 and 450 min). The procedures for these baseline measurements are outlined in general methods.

Participants then completed a 30-min intermittent cycle (INT-AM) at peak power output (PPO) consisting of 10 × 1 min exercise bouts interspersed by a 2 min rest period or a 30 min continuous cycle (INT-PM) at 50% VO_{2peak}, from 0-30 min. In the afternoon, participants completed the second 30 min exercise bout in the opposite mode, INT-AM continuous exercise or INT-PM intermittent exercise from 255-285 min. Details of exercise intensity are documented in general methods. HRs were recorded every 5 min during the continuous exercise morning bout (0, 5, 10, 15, 20, 25 and 30 min) and afternoon bout (255, 260, 265, 270, 275, 280 and 285 min); and RPE (Borg, 1973) was recorded every 10 min during the morning bout (0, 10, 20 and 30 min) and afternoon bout (255, 265, 275 and 285 min). HR was also collected during intermittent exercise at the end of each 1 min interval during the morning at (0, 1, 4, 7, 10, 13, 16, 19, 22, 25 and 28 min) and afternoon (255, 256, 259, 262, 265, 268, 271, 274, 277, 280 and 283 min). RPE was recorded at regular intervals during intermittent exercise in the morning (0, 4, 10, 16, 22 and 28 min) and afternoon (255, 259, 265, 271, 277 and 283 min). HR and RPE data are reported as; individual points during exercise period (Figure 64) and average heart rate across the total completed 60 min exercise period (Table 17).

At 30 min (post-exercise) participants removed themselves from the cycle ergometer and laid in a semi-supine position for 15 min to collect expired gas sample. A further blood sample, appetite VAS and wellbeing questionnaires were collected during this rest period. At 60 min (pre-breakfast) a further blood sample and appetite VAS were collected before participants were provided with a standardised breakfast meal (see section 3.6. Meal Details) in which they had 15 min to consume the meal and instructed to consume it as quickly as they were able. Time taken to eat the breakfast meal was recorded. Participants then entered their first rest period, in which they rested in the laboratory for 3-h (75-255 minutes). During this period, participants were free to work, read or watch DVDs; expired air samples, appetite VAS and blood samples were collected every 60-min (135, 195 and 255 min) and a wellbeing questionnaire at 255 min. Furthermore, during the first recovery phase, each

participant was provided with a standardised amount of water (125ml) every 15-min from (135-180 min) resulting in a total of 500 ml being consumed.

At 255-min the start of the second exercise period, participants completed the exercise mode that they did not undertake within the morning exercise period for the remaining 30 min exercise period (255-285 min). At the end of the second exercise period (285 min) participants removed themselves from the cycle ergometer and laid in a semi-supine position for 15 min to collect expired gas sample, blood, appetite VAS and wellbeing questionnaire. Following these post exercise measurements participants were given 15 min to shower and change their clothes, before a further blood sample, appetite VAS and baseline gastric emptying sample were collected at 315 min (pre-lunch). Participants were then provided with meal two (Lunch), a bowl of Heinz vegetable soup (see section 3.6. Meal Details). Each participant was given a maximum of 15 minutes to consume the standardised meal and instructed to consume it as quickly as they were able. Time taken to eat this meal was recorded.

Participants remained in a semi-supine position throughout the remaining 2h sampling procedure (second recovery period) 330-450 min. Gastric emptying samples were obtained pre-meal ingestion (315 min) and every 15 min post-meal (345, 360, 375, 390, 405, 420, 435 and 450 min). Additional expired air samples, VAS and blood samples were collected at 60-min intervals (390 and 450 min) and wellbeing questionnaires were collected at 450 min. Following the collection of the final samples, participants were provided with a 500 ml bottle of water and were free to leave the laboratory at 465 min and start their post-trial 24 h weighed dietary intake, and final wellbeing questionnaire, which was to be completed 24-h after the final blood sample had been withdrawn. The measurements within this study are described in general methods. A schematic diagram of the experimental protocol is presented in (Figure 62).

7.2.3. Exercise Intensity

Exercise intensity was calculated in a similar manner as described within Chapter 4. Workload was then calculated using the liner trend line equation to evoke a work rate equivalent to 50% $\dot{V}O_{2Peak}$ (Moderate intensity) for the continuous section and for the intermittent section intensity was set at ~100% $\dot{V}O_{2Peak}$ (Very high intensity) and peak power output (PPO) were calculated. Each result was then

calculated, with account taken for ramp exercise exchange, which resulted in two-thirds of the ramp rate being deducted from the calculated work rate (Whipp et al., 1981).

7.2.4. Biochemical Analysis

Blood sample analysis is described in general methods. Gut hormone analysis was performed on 180 duplicate samples. Corresponding intra-assay CVs for active ghrelin, GLP-1, insulin, PP and PYY were 9%, 17%, 8%, 8% and 11% respectively. Inter-assay CVs were determined from concentrations across seven different assays for active ghrelin, GLP-1, insulin, PP and PYY were 9.6%, 7.8%, 9%, 9.6 and 15.8% respectively.

Metabolite analysis was performed on 240 duplicate samples. Corresponding intra-assay CVs for glucose, triglycerides, cholesterol and NEFA were 1%, 1.2%, 1.2% and 4.3% respectively. Inter-assay CVs were determined from concentrations across six verification runs for glucose, triglycerides, cholesterol and NEFA were 5.4%, 5.2%, 3.9% and 6.5% respectively.

7.2.5. Statistical Analysis

All data are presented as mean ± standard deviation (SD) unless otherwise stated. Data was tested for normality of distribution using the Shapiro-Wilk test. Differences in standardisation measurements, pre-trial body mass, time to consume breakfast and lunch, weight of soup consumed, pre and post-EI, proportion of macronutrient, average HR, gastric emptying T_{lag}, $T_{1/2}$, and all AUC results were analysed using paired student's t-Test. Two-way repeated ANOVA were used to examine differences in; HR, RPE, carbohydrate and fat oxidation, gastric emptying DOB and cumulative dose $\%^{13}C0_2$ values, gut hormones concentrations, metabolites concentrations, appetite VAS scores and wellbeing. Sphericity for repeated measures was assessed and Greenhouse-Geisser epsilon < 0.75 and the Huynh-Feldt correction adopted for less severe differences to correct for violations. Significant main effects were followed by paired student's t-Test or one-way repeated ANOVA with Bonferroni adjusted pairwise comparisons as appropriate. Effect size was documented as partial eta squared (η^2_p) or Cohen's (d) and 95% confidence intervals (CI), see section (3.19.2. Post-Statistics Effect Size).

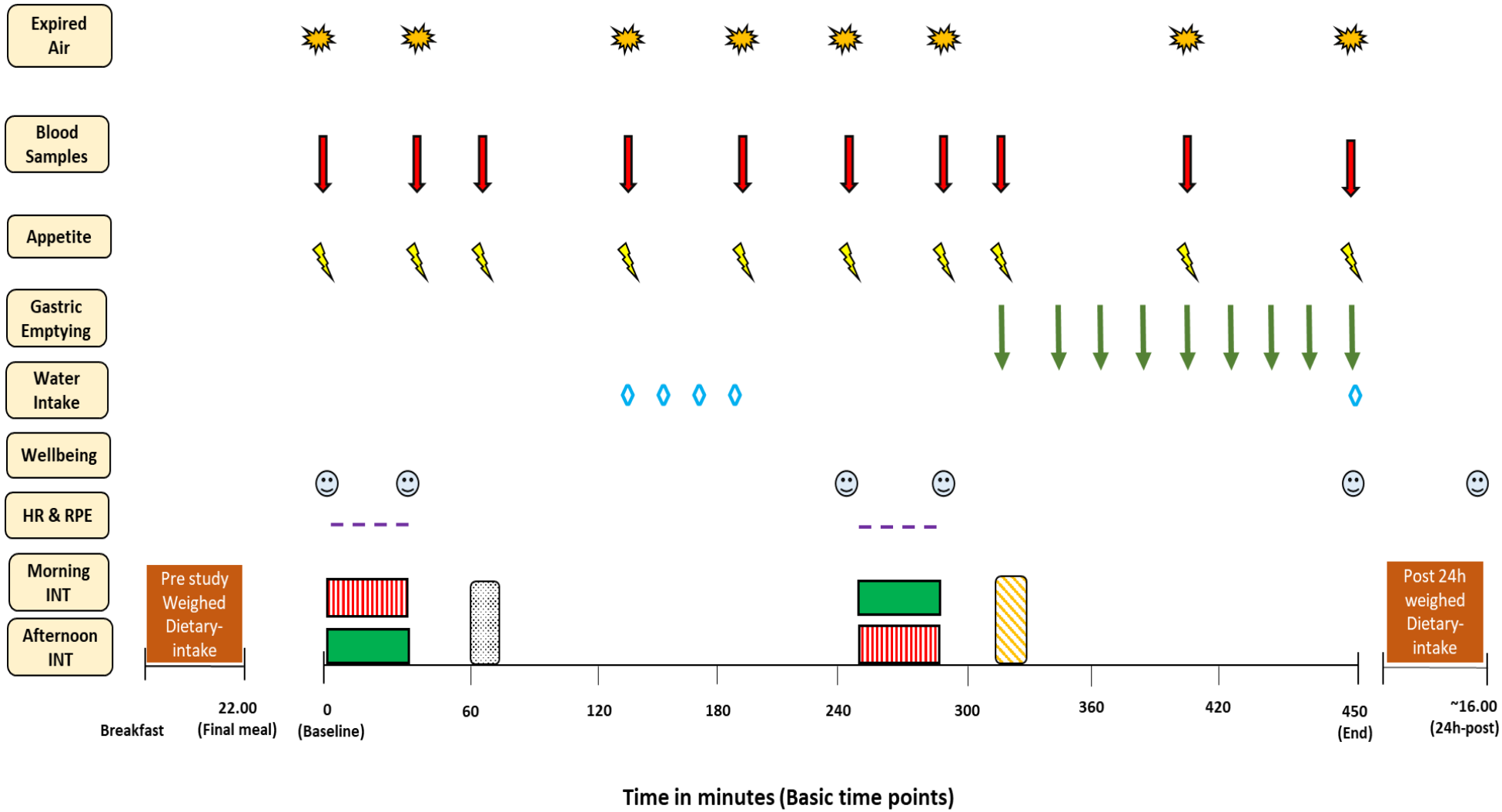

Figure 62: Schematic diagram of the experimental trial protocol S-4. Green rectangle represents 30 min continuous exercise bout. Red lined rectangle represents 30 min intermittent exercise bout. Black dotted rectangle represents 15 min breakfast period. Yellow lined rectangle represents 15 min standardised semi-solid meal period. HR, heart rate. RPE, rating of perceived exertion. Appetite, visual analogy scale questionnaire. Expired Air, (Substrate Utilisation). Morning INT, (INT-AM). Afternoon INT, (INT-PM)

7.3 Results

7.3.1. Standardisation Measurements

Pre-trial body mass was not significantly different between INT-AM compared to INT-PM (P = 0.448, η^2p = 0.053). Average HR over the total 60-min exercise period was not significantly different between INT-AM compared to INT-PM (P = 0.533, η^2p = 0.036). Furthermore, there were no differences between time to consume breakfast (P = 0.835, η^2p = 0.004) nor lunch (P = 0.586, η^2p = 0.028) and no difference between the weight of soup consumed (P = 0.350, η^2p = 0.080; Table 17).

Table 17: S4, Standardisation Measurements During Laboratory Visit

	INT-AM	**INT-PM**	***P*=value**
	Pre-Trial Measurements		
Body Mass (Kg)	81.7 ± 12.6	81.8 ± 12.7	0.448
	Exercise Measurement		
Average HR (60-min)	143 ± 11	142 ± 12	0.533
	Breakfast Measurements		
Time to eat breakfast (s)	445 ± 118	452 ± 109	0.835
	Lunch Measurements		
Weight of soup consumed (g)	782 ± 9	778 ± 9	0.350
Time to eat soup (s)	441 ± 136	456 ± 155	0.586

Data are means ± SD. Values are significant P<0.05

7.3.2 Energy and Macronutrients Intake

Pre-trial energy intake was not significantly different between INT-AM compared to INT-PM respectively (2921 ± 571 Kcal vs 2888 ± 620 Kcal, P =0.357, η^2p = 0.078, Figure 63A). This led to a similar proportion of energy from carbohydrates (P = 0.625, η^2_p=*0.022*), fats (P = 0.772, η^2_p=*0.008*), protein (P = 0.841, η^2_p=*0.004*) and fibre (P = 0.910, η^2_p=*0.001*). In addition, fluid consumption before was also not significantly different between trials (P = 0.848, η^2_p=*0.003*, Table 18).

24-hour post-trial energy intake revealed that INT-PM was significantly higher compared to INT-AM (3511 ± 879 vs 2829 ± 614 Kcal: *P* =0.007, d = 0.94, 95% CI = -496.39–348.34 KCal, Figure 63B). Individual data indicated 9 out of 12 participants had a higher energy intake 24-h after the INT-PM trial compared to the INT-AM. This led to a different proportion of macronutrients in the 24-h after the trial. There was no difference between; Fat (P = 0.140, η^2_p=*0.187*), protein (P = 0.163, η^2_p=*0.169*), fibre (P = 0.061, η^2_p=*0.284*) and water (P = 0.577, η^2_p=*0.029*). However, participants did consume considerably more carbohydrate 24-h after completing INT-PM trial compared to INT-AM (*P* =0.018, d = 1.10, 95% CI = -142.04–243.26, Table 18)

Table 18: Pre and post-trial macronutrient content (KCal) from total energy intake for INT-AM and INT-PM trial.

Macronutrients	**INT-AM**	**INT-PM**	***P*=value**
Pre-Trial Macronutrients (KCal)			
Carbohydrate	1313 ± 385	1290 ± 409	0.625
Protein	502 ± 231	508 ± 217	0.841
Fat	1048 ± 336	1030 ± 361	0.772
Fibre	58 ± 40	59 ± 33	0.910
Water (g)	2251 ± 1667	2216 ± 1428	0.848
Post-Trial Macronutrients (KCal)			
Carbohydrate	1227 ± 428	*1593 ± 464	**0.018**
Protein	466 ± 134	553 ± 253	0.163
Fat	1026 ± 388	1254 ± 557	0.140
Fibre	81 ± 54	95 ± 50	0.061
Water (g)	1971 ± 1430	2063 ± 1148	0.577

Data are means ± SD. Values are significant *P*<0.05
* Indicates INT-PM is significantly larger than INT-AM.

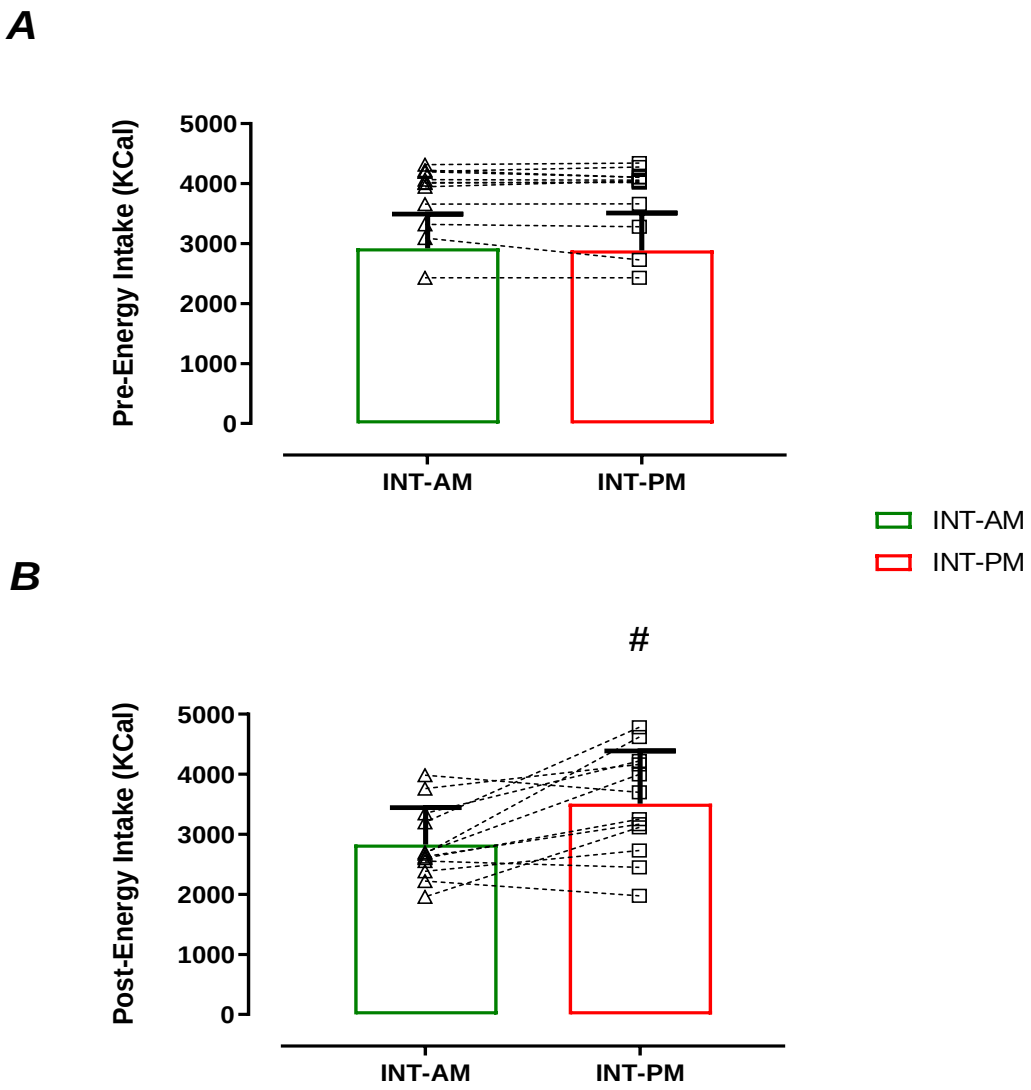

Figure 63: Representative of mean values for pre-energy intake (KCal) (A) and post-energy intake (KCal) (B) with vertical error bars displaying SDs (n=12). Individual data is represented as (Δ) for INT-AM and (□) represents INT-PM. There was no main effect observed for pre-energy intake (p >0.05) examined by a one-way repeated-measured ANOVA. However, there was a main effect observed for post-energy intake (p <0.05) examined by a one-way repeated-measured ANOVA. # Indicates INT-PM was significantly different from INT-AM determined by Bonferroni adjusted paired t-test (p <0.05).

7.3.3. RPE and Heart Rate

A main effect for time (p <0.001, η^2p = 0.866) and trial x time interaction (p <0.001, η^2p = 0.878) was observed. However, there was no effect of trial (p =0.867, η^2p = 0.003) on RPE. Post-hoc test revealed no differences between intermittent exercise bouts, as a main trial (p =0.408, η^2p = 0.063) and trial x time interaction effect (p =0.203, η^2p = 0.123). However, there was an effect of time (p <0.001, η^2p = 0.933).

Furthermore, continuous exercise bouts did show a main trial (p =0.034, η^2p = 0.348) and time effect (p <0.001, η^2p = 0.866). However, there was no trial x time interaction effect (p =0.427, η^2p = 0.076) observed. Further post hoc tests revealed no between-trial differences.

There were no differences found within the first exercise period regardless of exercise mode as a main trial (p <0.001, η^2p = 0.919) and time effect (p <0.001, η^2p = 0.728) was observed, but no trial x time interaction effect (p =0.461, η^2p = 0.061). Further post-hoc tests revealed no between-trial differences. This result was mimicked for the second exercise period as a main trial (p <0.001, η^2p = 0.702) and time effect (p <0.001, η^2p = 0.727) was observed, but no effect for trial x time interaction effect (p =0.051, η^2p = 0.273, Figure 64). Further post-hoc tests revealed no between-trial differences.

A main effect for time (p <0.001, η^2p = 0.945) and trial x time interaction effect (p <0.001, η^2p = 0.951) was observed for heart rate. However, there was no effect of trial (p =0.389, η^2p = 0.068). Post-hoc test revealed there were no significant differences at baseline (0 min) for INT-AM vs INT-PM (65 ± 9 vs. 68 ± 12 BPM: P =0.383, d = 0.30, 95% CI = -6.49–5.39). There was however an effect observed pre-second exercise bout (255 min) (79 ± 9 vs. 66 ± 8 BPM: P <0.001, d = 1.08, 95% CI = -4.01–7.87).

There were no differences between the first (0 min- 30 min) and second exercise bouts (255 min- 285 min) for intermittent exercise, as a main trial (p =0.419, η^2p = 0.060) and trial x time interaction effect (p =0.333, η^2p = 0.095). However, there was a main effect of time (p <0.001, η^2p = 0.975). Although, there was an effect found between the first and second exercise bouts for continuous exercise, as there was a main trial (p =0.015, η^2p = 0.427), time (p <0.001, η^2p = 0.952) and trial x time interaction effect (p =0.017, η^2p = 0.202) observed. Further analysis revealed INT-AM was significantly higher than INT-PM at 280 min (126 ± 13 vs. 118 ± 10 BPM: P <0.002, d = 0.72, 95% CI = -6.63–6.38) and 285 min (128 ± 13 vs. 121 ± 11 BPM: P <0.007, d = 0.61, 95% CI = -6.75–6.83).

There were differences found within the first and second exercise periods regardless of exercise mode between INT-AM vs INT-PM. A main effect for trial (p

<0.001, η^2p = 0.889; p <0.001, η^2p = 0.921), time (p <0.001, η^2p = 0.980; p <0.001, η^2p = 0.964) and trial x time interaction effect (p <0.001, η^2p = 0.909; p <0.001, η^2p = 0.879) was observed respectively. Post-hoc tests revealed all points between 1 min – 30 min (p<0.001) and 256 min – 285 min (p<0.001; Figure 64) were highly significant.

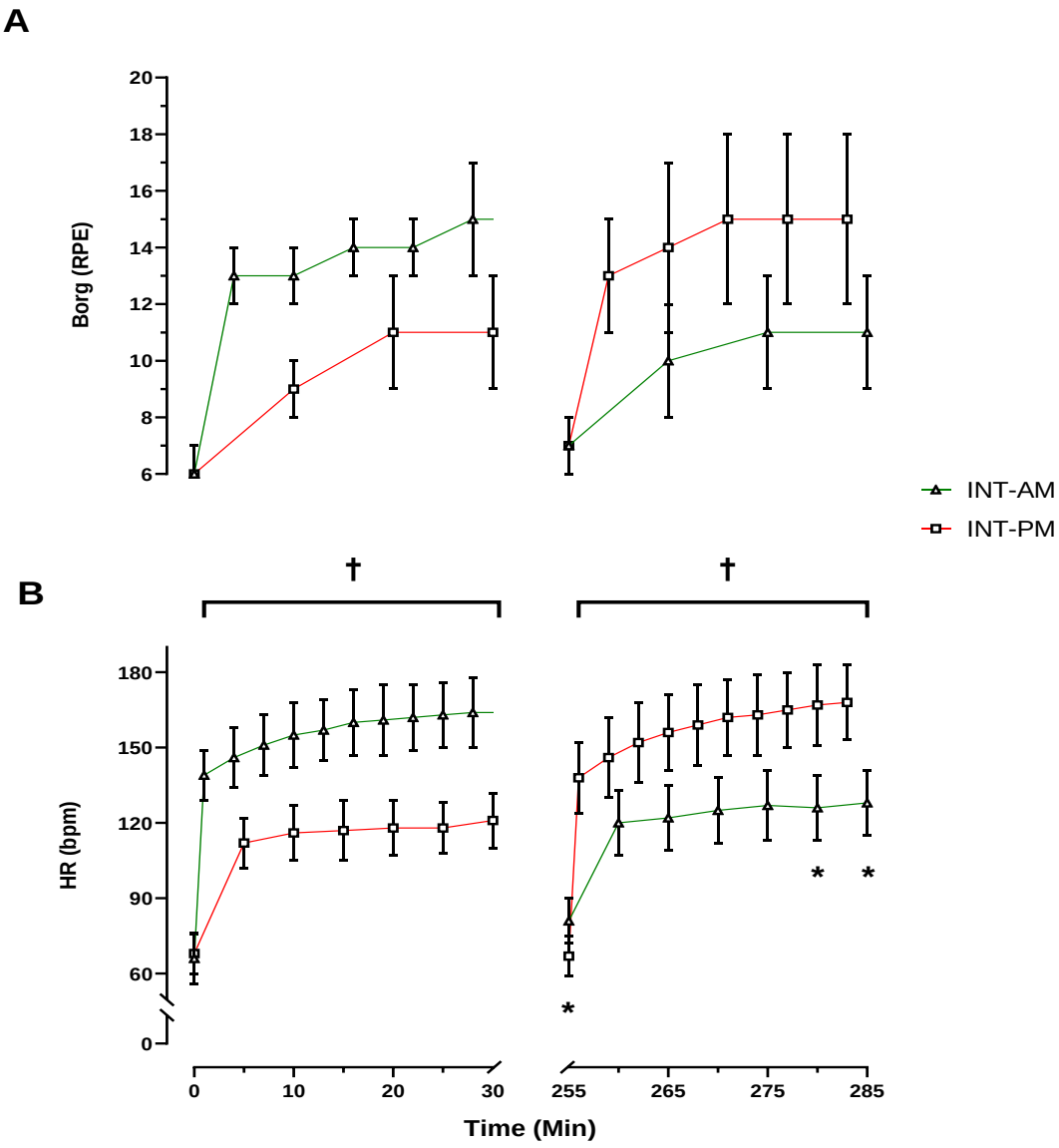

Figure 64: Representative of mean RPE (A) and HR (B), with vertical error bars displaying SDs. (n = 12). There was a main effect for time and a main interaction effect (p <0.05) for HR and RPE during exercise. However, no trial effect observed for HR nor RPE (p >0.05) examined by a two-way repeated-measured ANOVA. † Indicates INT-AM vs INT-PM were significantly high at each time point (p < 0.001). * Indicates INT-AM vs INT-PM was significantly different (p < 0.05). Post-hoc tests were, determined by Bonferroni adjusted paired *t-test*. RPE; rate of perceived exertion. HR, heart rate; bpm, beats per minute.

7.3.4. Gastric Emptying

There was no main trial (P = 0.208, η^2_p=*0.140*) nor main interaction effect (P = 0.068, η^2_p=*0.184*) observed for DOB. However, there was a main effect of time (P <0.001, η^2_p=*0.956*). Post-hoc test revelled both trials increased at every time point from baseline (P<0.001, Figure 66B).

There was no main effect observed for DOB AUC (p = 0.202, η^2p = 0.143, Figure 66A). No main trial (P = 0.117, η^2_p=*0.208*) nor main interaction effect (P = 0.084, η^2_p=*0.234*) observed for percent $^{13}C0_2$. However, there was a main effect of time (P <0.001, η^2_p=*0.992*, Figure 67).

The time taken to empty half of the soup from the stomach (T_{half}) amounted to 151 ± 111 min and 99 ± 24 min for INT-AM vs INT-PM respectively (P = 0.171, η^2_p=*0.163*). The time of maximal emptying rate (T_{lag}) amounted to 62 ± 11 min and 57 ± 8 min for INT-AM vs INT-PM, respectively (P = 0.139, η^2_p=*0.188*; Figure 65)

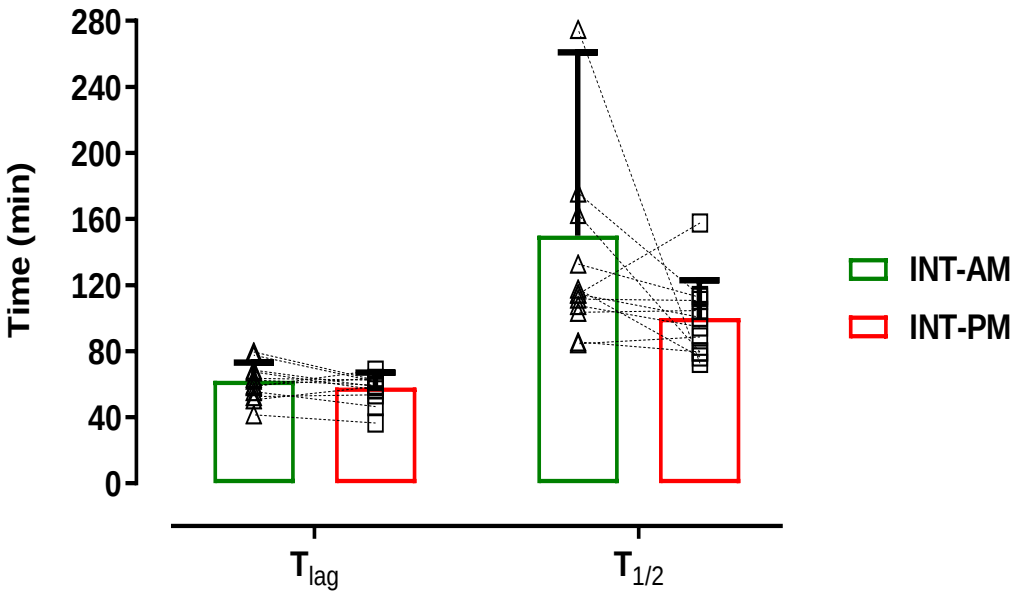

Figure 65: Representative of mean T_{lag} and $T_{1/2}$ with vertical error bars display SDs (n = 12). Individual data is represented as (Δ) for INT-AM and (□) represents INT-PM. There was no main effect observed for T_{lag} and $T_{1/2}$ (p >0.05) examined by a one-way repeated-measured ANOVA. T_{lag}, Time of maximal emptying rate; $T_{1/2}$, Half emptying time.

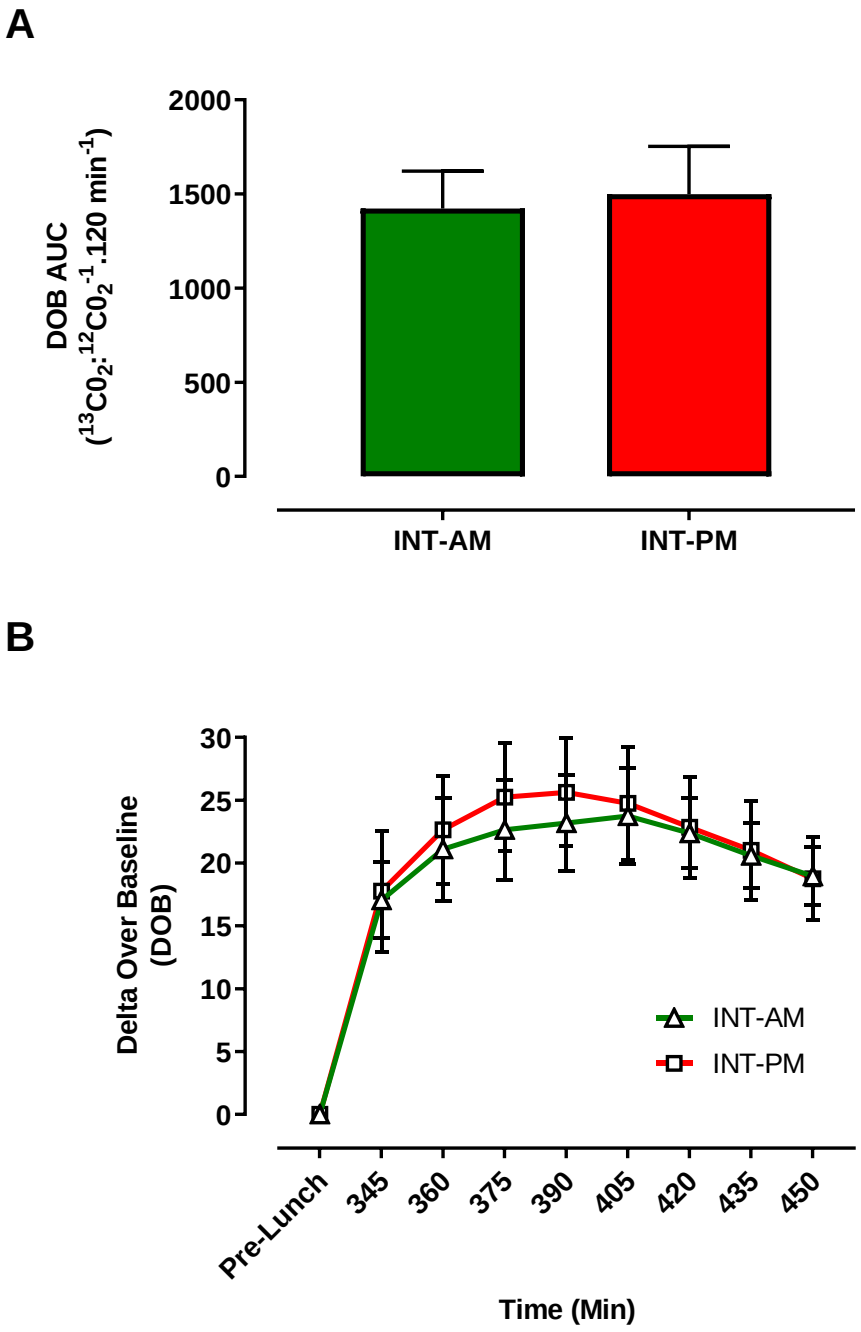

Figure 66: Representative of mean DOB AUC (0-120 min^{-1}) (A) and DOB response (B), with vertical error bars displaying SDs (n = 12). There was no main effect observed for DOB AUC ($p > 0.05$) examined by a two-way repeated-measured ANOVA. There was no main effect for trial nor interaction ($p > 0.05$). However, there was a main effect for time observed for DOB ($p < 0.05$), examined by a two-way repeated-measured ANOVA. DOB, delta over baseline; AUC, area under curve.

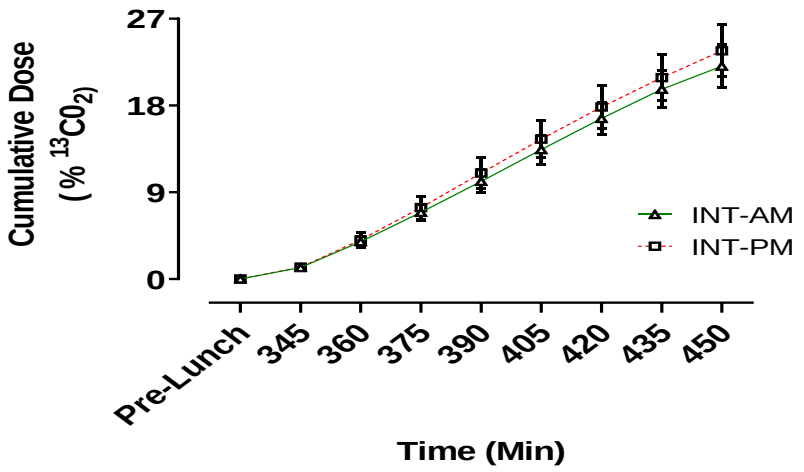

Figure 67: Representative of mean cumulative dose of % $^{13}CO_2$, with vertical error bars displaying SDs (n = 12) There was no main effect for trial, nor main interaction effect observed (p >0.05). However, there was a main effect for time (p <0.05), examined by a one-way repeated-measured ANOVA.

7.3.5. Appetite

7.3.5.1 Hunger

A main effect of time (p < 0.001, η^2p = 0.447) and main interaction effect (p <0.001, η^2p = 0.356) was observed for hunger. However, there was no trial effect (p = 0.103; η^2p = 0.224; Figure 68B). Post-hoc tests revealed INT-PM was significantly increased post first exercise bout (30 min) compared to INT-AM (72 ± 20 vs. 39 ± 22 mm: p = 0.002, d = 1.64, 95% CI = −9.68–14.09 mm). Moreover, hunger remained increased during INT-PM pre-breakfast (60 min), despite hunger beginning to rise during INT-AM (79 ± 25 vs. 51 ± 26 mm: p = 0.016, d = 1.15, 95% CI = −13.56–15.29 mm). Both trials decreased in hunger after food ingestion (breakfast) before gradually rising throughout recovery. Post second exercise bout (285 min), INT-PM decreased, before both trials followed the same pattern, until the end of the trial at 450 min showing no further significance. No differences in AUC were observed (p = 0.118, η^2p = 0.207; Figure 68A)

7.3.5.2. Fullness

A main effect of time ($p < 0.001$, $\eta^2p = 0.581$) and interaction effect ($p = 0.016$, $\eta^2p = 0.180$) was observed for fullness. However, there was no trial effect for fullness ($p = 0.926$, $\eta^2p = 0.001$, Figure 69B). Post-hoc tests revealed INT-AM was significantly higher pre-breakfast (60 min) compared to INT-PM (27 ± 19 vs. 7 ± 7 mm: $p = 0.007$, d = 1.46, 95% CI = −9.29–5.42 mm). Fullness remained relatively stable in both trials until post second exercise bout (285 min) and pre-lunch (315 min) were INT-PM increased compared to INT-AM, although these results were not significant. There was no main effect observed for AUC ($p = 0.926$, $\eta^2p = 0.001$; Figure 69A).

7.3.5.3. PFC

A main effect of time ($p < 0.001$, $\eta^2p = 0.494$) and interaction effect ($p = 0.023$, $\eta^2p = 0.216$) was observed for PFC. However, there was no trial effect ($p = 0.500$, $\eta^2p = 0.042$, Figure 70B). Post-hoc tests revealed INT-PM was significantly higher pre-breakfast (60 min) compared to INT-AM (87 ± 12 vs. 62 ± 24 mm: $p = 0.012$, d = 1.38, 95% CI = −5.41–14.96 mm). PFC remained relatively stable in both trials until post second exercise bout (285 min) where INT-PM was lower compared to INT-AM, although these results were not significant. There was no main effect for AUC ($p = 0.498$, $\eta^2p = 0.043$; Figure 70A).

7.3.5.4. Satisfaction

A main effect of time ($p < 0.001$, $\eta^2p = 0.574$) and interaction effect ($p = 0.040$, $\eta^2p = 0.200$) was observed for satisfaction. However, there was no trial effect ($p = 0.102$, $\eta^2p = 0.0224$, Figure 71B). Post-hoc tests revealed INT-AM was significantly higher post first exercise bout (30 min) compared to INT-PM (30 ± 17 vs. 20 ± 13 mm: $p = 0.006$, d = 0.69, 95% CI = −8.93–8.05 mm). Moreover, satisfaction remained increased during INT-AM pre-breakfast (60 min) as INT-PM rapidly decreased (29 ± 19 vs. 8 ± 6 mm: $p = 0.003$, d = 1.53, 95% CI = −9.22–5.49 mm). Satisfaction remained relatively stable in both trials until post second exercise bout (285 min) where INT-PM was higher compared to INT-AM, although these results were not significant. There was no main effect for AUC ($p = 0.181$, $\eta^2p = 0.156$; Figure 71A).

7.3.5.5. Bloat

There was no main effect of time (p =0.056, η^2p = 0.216), trial (p = 0.793, η^2p = 0.007) nor main interaction effect observed for bloat (p = 0.681, η^2p = 0.046, Figure 72B). There was no main effect for AUC (p = 0.757, η^2p = 0.009; Figure 72A).

7.3.5.6. Nausea

There was a main effect of time (p =0.042, η^2p = 0.252), trial (p = 0.033, η^2p = 0.352) and main interaction effect observed for nausea (p = 0.005, η^2p = 0.302, Figure 73B). Post-hoc tests revealed INT-AM was significantly higher post first exercise bout (30 min) compared to INT-PM (19 ± 16 vs. 6 ± 6 mm: p = 0.003, d = 2.26, 95% CI = −1.13–5.66 mm). Both trials then remained relatively similar until 450 min showing no further differences for nausea. There was no main effect for AUC (p = 0.123, η^2p = 0.203; Figure *73*A).

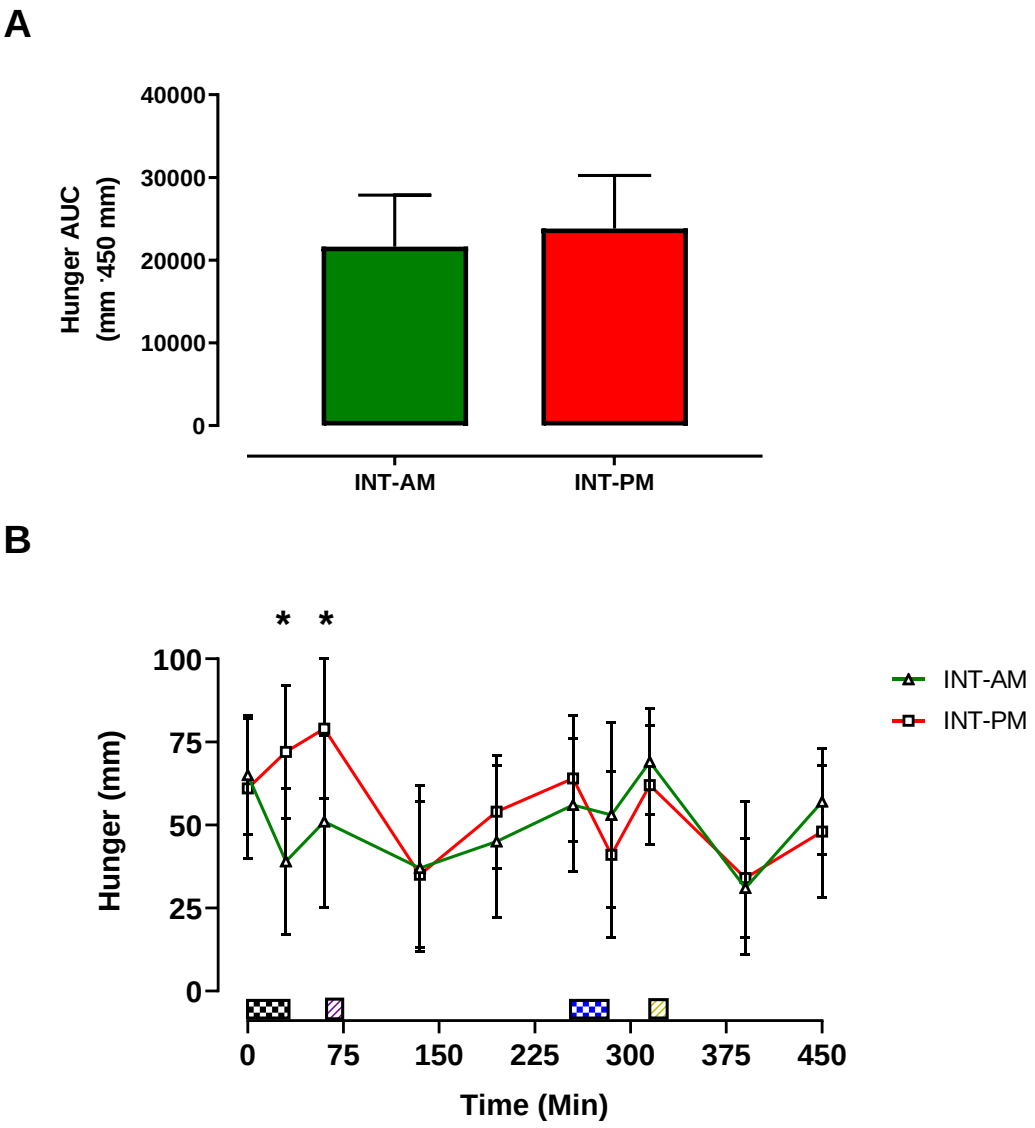

Figure 68: Representive of mean hunger AUC (0-450 min) (A) and hunger VAS response (B), with vertical error bars displaying SDs (n = 12). *Chequered black and white rectangle* indicates first 30-min exercise bout; *Hashed purple rectangle* indicates breakfast meal. *Chequered blue and white rectangle* indicates second 30-min exercise bout; *Hashed yellow rectangle* indicates ingestion of semi-solid lunch meal. There was no main effect observed for hunger AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial effect observed for hunger ($p > 0.05$). However, there was a main effect for time and a main interaction effect ($p < 0.05$) examined by a two-way repeated-measured ANOVA. * Indicates post-hoc tests were significantly different between INT-AM vs INT-PM, determined by Bonferroni adjusted paired t-test ($p < 0.05$). AUC, area under curve.

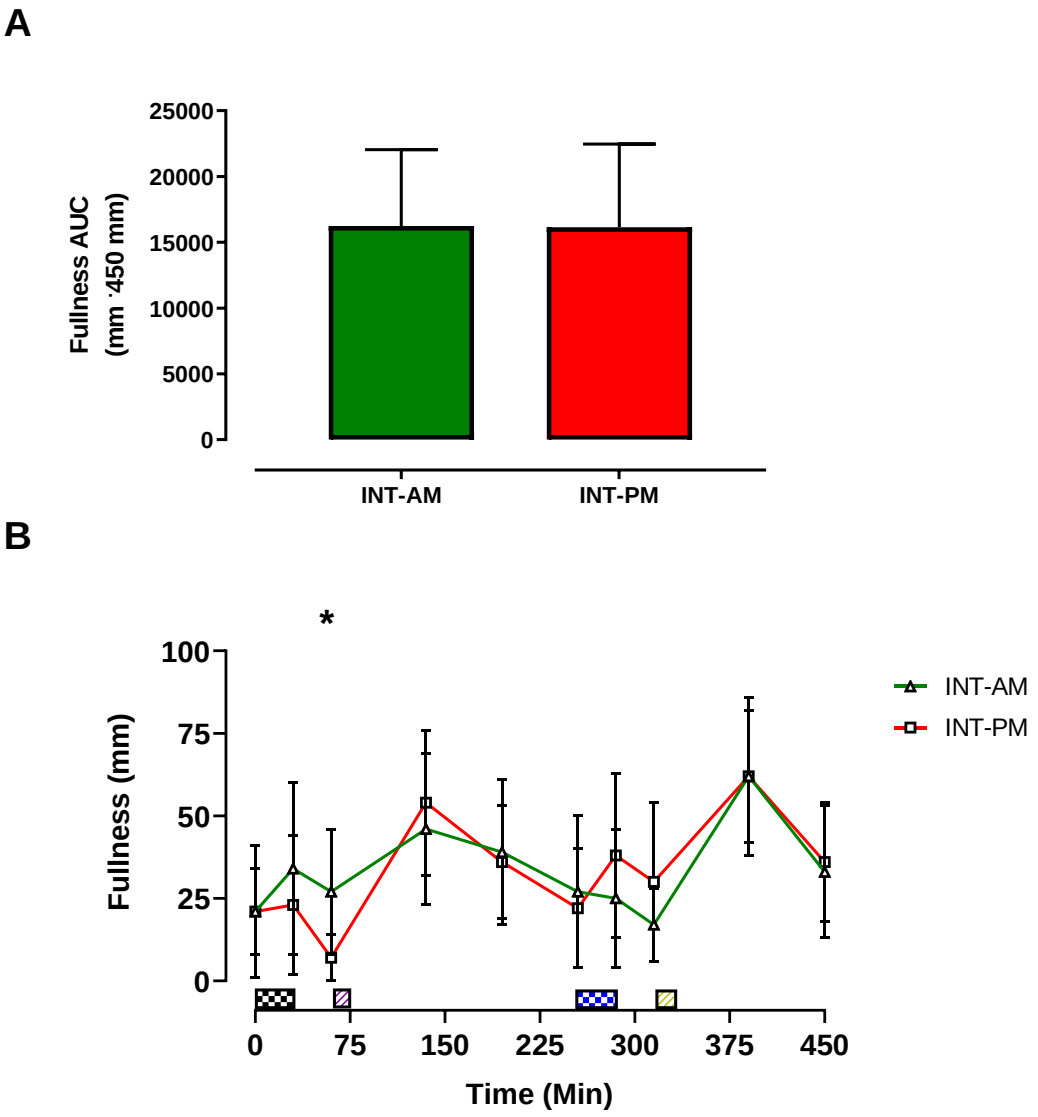

Figure 69: Representive of mean fullness AUC (0-450 min) (A) and fullness VAS response (B), with vertical error bars displaying SDs (n = 12). *Chequered black and white rectangle* indicates first 30-min exercise bout; *Hashed purple rectangle* indicates breakfast meal. *Chequered blue and white rectangle* indicates second 30-min exercise bout; *Hashed yellow rectangle* indicates ingestion of semi-solid lunch meal. There was no main effect observed for fullness AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial effect observed for fullness ($p > 0.05$). However, there was a main effect for time and a main interaction effect (<0.05) examined by a two-way repeated-measured ANOVA. * Indicates post-hoc tests were significantly different between INT-AM vs INT-PM, determined by Bonferroni adjusted paired *t-test* ($p < 0.05$). AUC, area under curve.

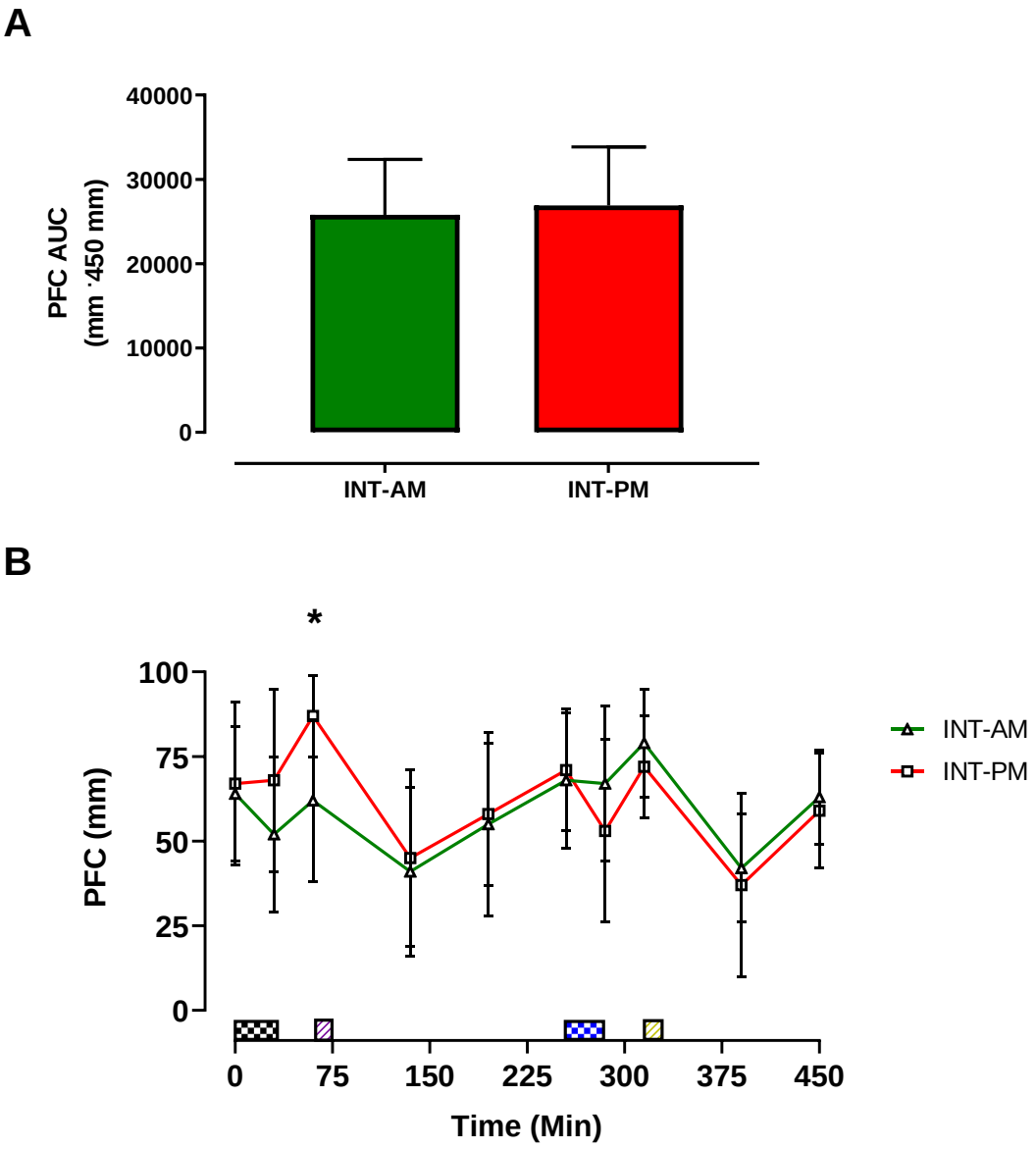

Figure 70: Representive of mean PFC AUC (0-450 min) (A) and PFC VAS response (B), with vertical error bars displaying SDs (n = 12). *Chequered black and white rectangle* indicates first 30-min exercise bout; *Hashed purple rectangle* indicates breakfast meal. *Chequered blue and white rectangle* indicates second 30-min exercise bout; *Hashed yellow rectangle* indicates ingestion of semi-solid lunch meal. There was no main effect observed for PFC AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial effect observed for PFC ($p > 0.05$). However, there was a main effect for time and a main interaction effect ($p < 0.05$) examined by a two-way repeated-measured ANOVA. * Indicates post-hoc tests were significantly different between INT-AM vs INT-PM, determined by Bonferroni adjusted paired *t-test* ($p < 0.05$). AUC, area under curve; PFC, prospective food consumption.

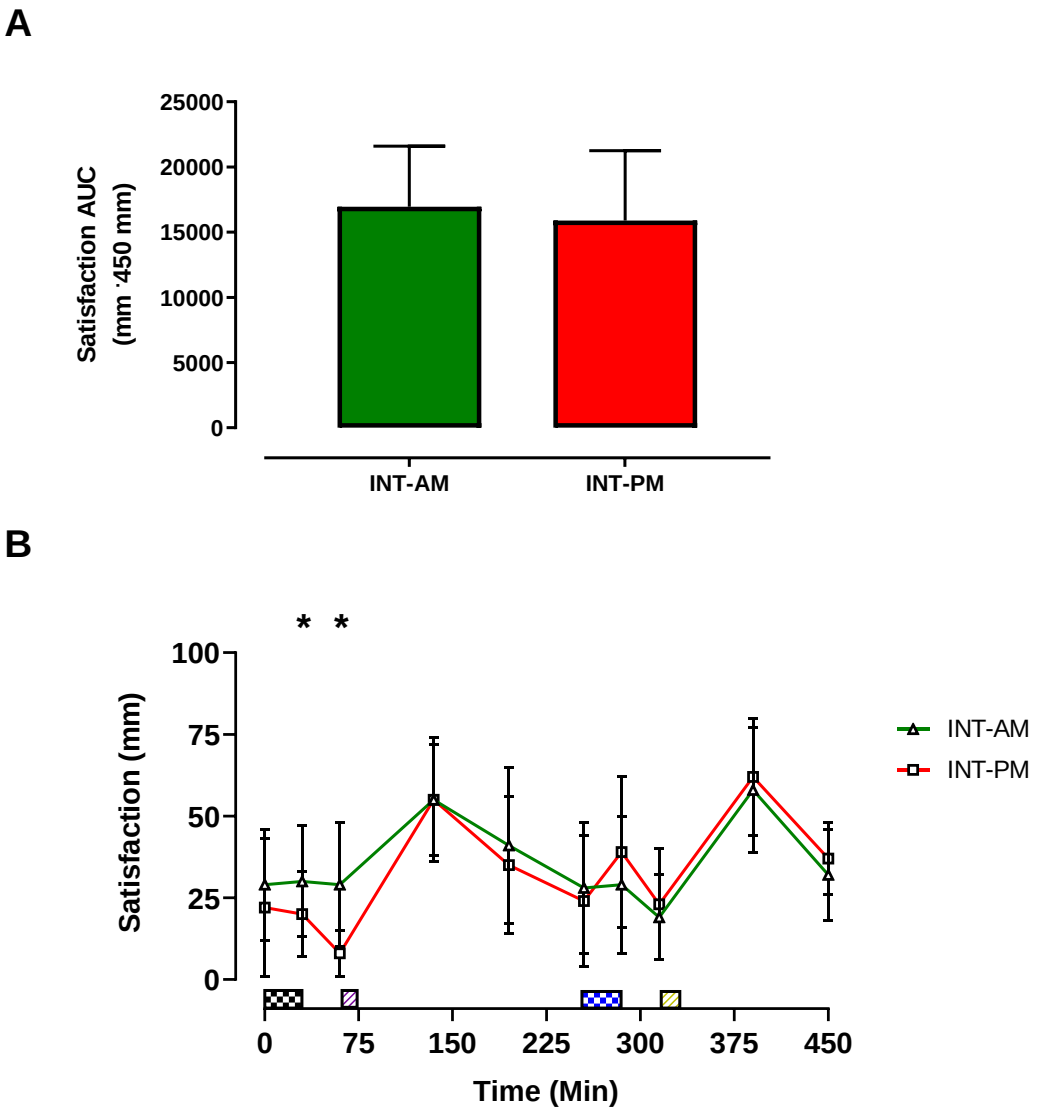

Figure 71: Representive of mean satisfaction AUC (0-450 min) (A) and satisfaction VAS response (B), with vertical error bars displaying SDs (n = 12). *Chequered black and white rectangle* indicates first 30-min exercise bout; *Hashed purple rectangle* indicates breakfast meal. *Chequered blue and white rectangle* indicates second 30-min exercise bout; *Hashed yellow rectangle* indicates ingestion of semi-solid lunch meal. There was no main effect observed for satisfaction AUC. ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial effect observed for satisfaction ($p > 0.05$). However, there was a main effect for time and a main interaction effect ($p < 0.05$) examined by a two-way repeated-measured ANOVA. * Indicates post-hoc tests were significantly different between INT-AM vs INT-PM, determined by Bonferroni adjusted paired *t-test* ($p < 0.05$). AUC, area under curve.

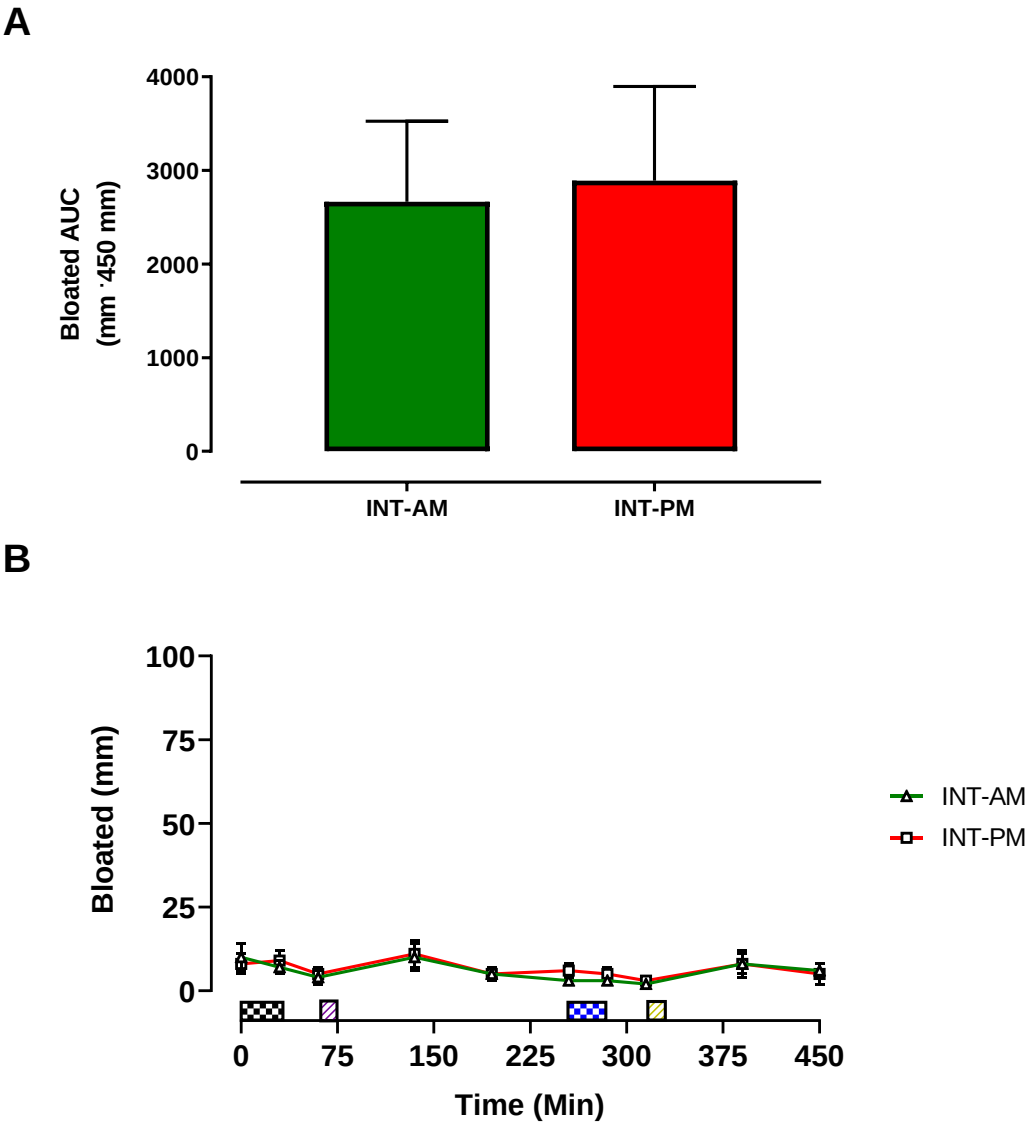

Figure 72: Representive of mean bloat AUC (0-450 min) (A) and bloat VAS response (B), with vertical error bars displaying SDs (n = 12). *Chequered black and white rectangle* indicates first 30-min exercise bout; *Hashed purple rectangle* indicates breakfast meal. *Chequered blue and white rectangle* indicates second 30-min exercise bout; *Hashed yellow rectangle* indicates ingestion of semi-solid lunch meal. There was no main effect observed for bloat AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial, time nor main interaction effect observed for bloat ($p > 0.05$) examined by a two-way repeated-measured ANOVA. AUC, area under curve

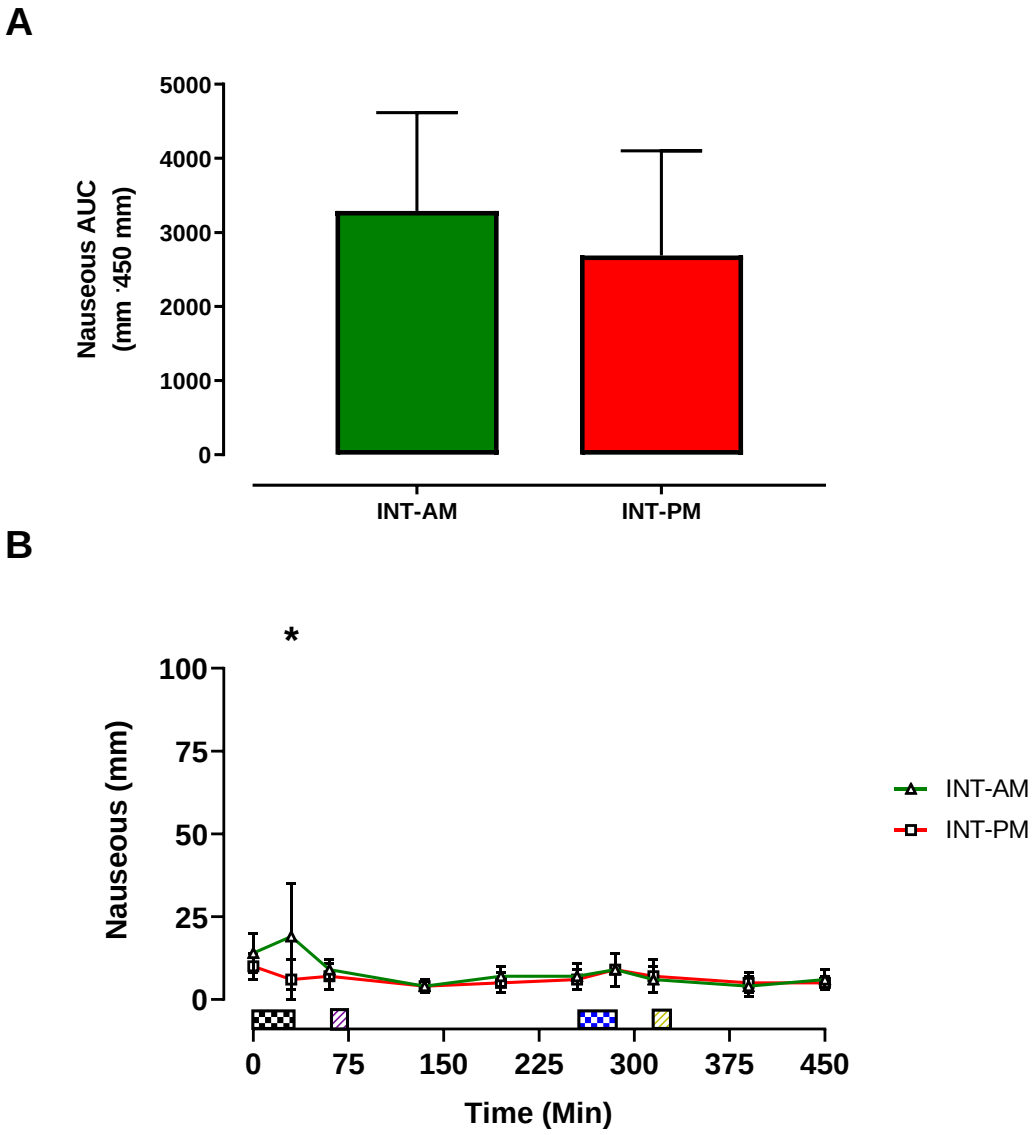

Figure 73: Representive of mean nausea AUC (0-450 min) (A) and nausea VAS response (B), with vertical error bars displaying SDs (n = 12). *Chequered black and white rectangle* indicates first 30-min exercise bout; *Hashed purple rectangle* indicates breakfast meal. *Chequered blue and white rectangle* indicates second 30-min exercise bout; *Hashed yellow rectangle* indicates ingestion of semi-solid lunch meal. There was no main effect observed for nausea AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was a main trial, time and interaction effect observed for nausea ($p < 0.05$) examined by a two-way repeated-measured ANOVA. * Indicates post-hoc tests were significantly different between INT-AM vs INT-PM, determined by Bonferroni adjusted paired *t-test* ($p < 0.05$). AUC, area under curve.

7.3.6. Blood Metabolites

7.3.6.1. Glucose

A main effect of time ($p < 0.001$, $\eta^2p = 0.431$) and a main interaction effect ($p < 0.001$, $\eta^2p = 0.406$) was observed for glucose. However, no main trial effect was observed ($p = 0.067$, $\eta^2p = 0.273$, Figure 74B). Post-hoc tests revealed INT-AM was significantly increased post first exercise bout (30 min) compared to INT-PM (5.07 ± 0.43 vs. 4.70 ± 0.25 mmol.L: $p = 0.017$, d = 1.10, 95% CI = 0.86–1.24 mmol.L). INT-PM then remained marginally elevated post breakfast until 1h post lunch (390 min) where INT-AM was significantly elevated compared to INT-PM (6.27 ± 0.91 vs. 5.22 ± 0.69 mmol.L: $p = 0.001$, d = 1.36, 95% CI = 0.84–1.75 mmol.L) and remained higher at 450 min (5.09 ± 0.5 vs. 4.51 ± 0.36 mmol.L: $p = 0.004$, d = 1.39, 95% CI = 1.11–1.59 mmol.L). No differences in AUC were observed ($p = 0.093$, $\eta^2p = 0.235$; Figure 74A)

7.3.6.2. Cholesterol

A main effect of time ($p < 0.001$, $\eta^2p = 0.858$) and a main interaction effect ($p < 0.001$, $\eta^2p = 0.512$) was observed for cholesterol. However, no main trial effect was observed ($p = 0.423$, $\eta^2p = 0.059$, Figure 75B). Cholesterol responded similarly across the duration of the whole study in both INT-AM and INT-PM trials, as post-hoc tests revealed no further significance ($p > 0.05$). There was no main effect for AUC ($p = 0.386$, $\eta^2p = 0.069$, Figure 75A).

7.3.6.3. Triglycerides

A main effect of time ($p = 0.006$, $\eta^2p = 0.405$) was observed for triglycerides. However, no main trial ($p = 0.220$, $\eta^2p = 0.133$) nor main interaction effect ($p = 0.082$, $\eta^2p = 0.221$, Figure 76) was observed. There was no main effect for AUC ($p = 0.210$, $\eta^2p = 0.139$, Figure 76A).

7.3.6.4. NEFA

There was a main effect of time ($p < 0.001$, $\eta^2p = 0.624$), trial ($p = 0.029$, $\eta^2p = 0.366$) and main interaction effect observed for NEFA ($p = 0.005$, $\eta^2p = 0.433$, Figure 77B). Post-hoc tests revealed INT-PM was significantly increased post first exercise bout (30 min) compared to INT-AM (0.490 ± 0.328 vs. 0.240 ± 0.070 mmol.L: $p =$

0.020, d = 1.10, 95% CI = 0.91–1.14 mmol.L) and pre breakfast (60 min) (0.495 ± 0.329 vs. 0.235 ± 0.077 mmol.L: p = 0.018, d = 1.14, 95% CI = 0.95–1.18 mmol.L). NEFA remained elevated during the first recovery period; 1h post breakfast (135 min) (0.495 ± 0.328 vs. 0.240 ± 0.075 mmol.L: p = 0.018, d = 1.12, 95% CI = 0.93–1.16 mmol.L), 2h post breakfast (195 min) (0.462 ± 0.240 vs. 0.265 ± 0.095 mmol.L: p = 0.012, d = 1.13, 95% CI = 0.99–1.18 mmol.L) and 3h post breakfast (255 min) (0.441 ± 0.240 vs. 0.255 ± 0.098 mmol.L: p = 0.018, d = 1.06, 95% CI = 0.92–1.12 mmol.L). Moreover, NEFA remained increased during INT-PM compared to INT-AM post second exercise bout (285 min) (0.453 ± 0.240 vs. 0.262 ± 0.098 mmol.L: p = 0.01, d = 1.09, 95% CI = 0.95–1.14 mmol.L). There was no further significance pre-lunch (315 min) until the end of the trial at 450 min.

There was a main effect for NEFA AUC as INT-PM was significantly increased compared to INT-AM (159 ± 81 vs. 94 ± 29 mmol.L^{-1}. 450 min^{-1}: p = 0.022, d = 1.12, 95% CI = −44.71–17.52 mmol.L^{-1}. 450 min^{-1}, Figure 77A).

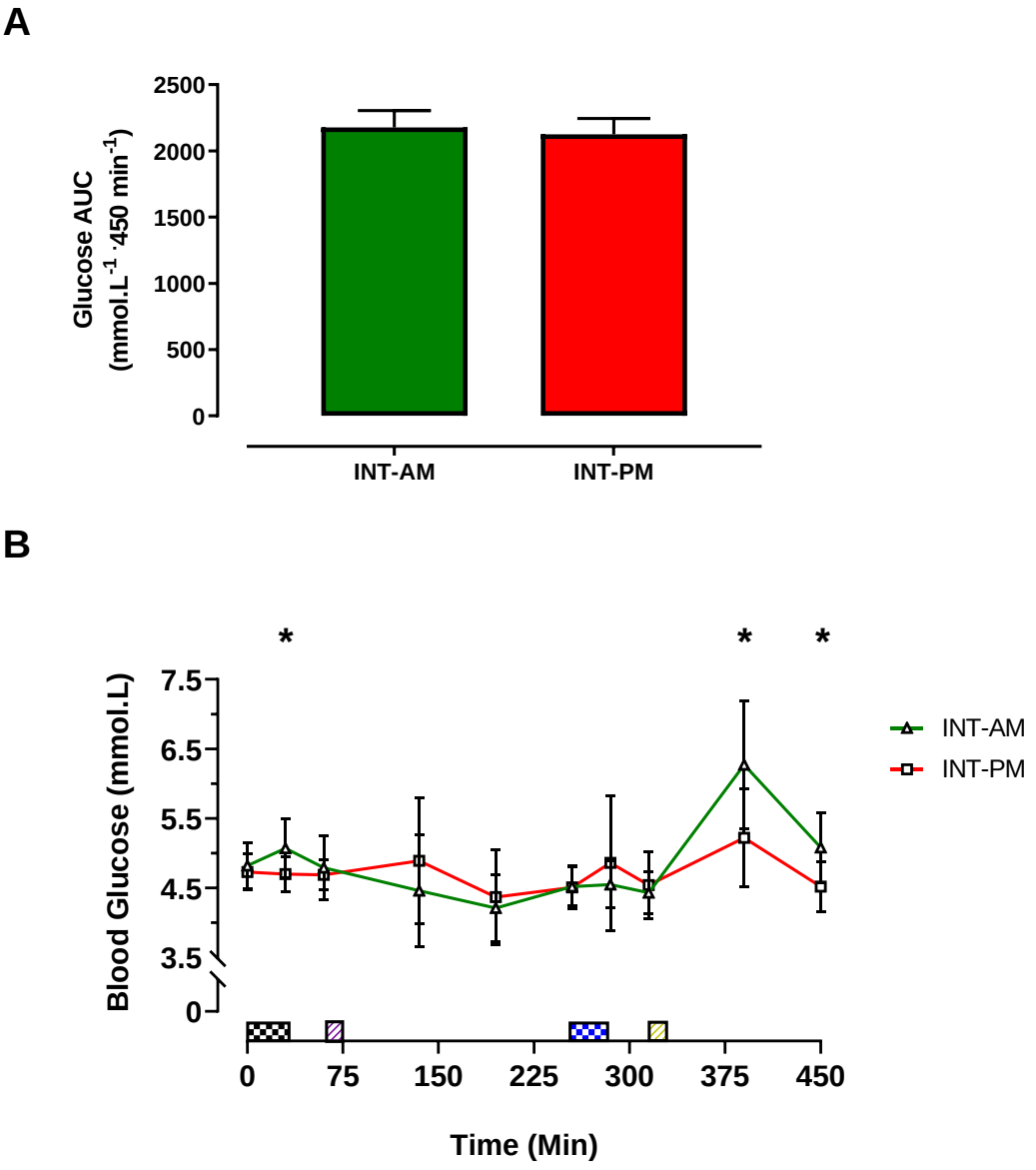

Figure 74: Representive of mean glucose AUC (0-450 min) (A) and glucose VAS response (B), with vertical error bars displaying SDs (n = 12). *Chequered black and white rectangle* indicates first 30-min exercise bout; *Hashed purple rectangle* indicates breakfast meal. *Chequered blue and white rectangle* indicates second 30-min exercise bout; *Hashed yellow rectangle* indicates ingestion of semi-solid lunch meal. There was no main effect observed for glucose AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial effect observed for glucose ($p > 0.05$). However, there was a main effect for time and a main interaction effect ($p < 0.05$) examined by a two-way repeated-measured ANOVA. * Indicates post-hoc tests were significantly different between INT-AM vs INT-PM, determined by Bonferroni adjusted paired *t-test* ($p < 0.05$). AUC, area under curve.

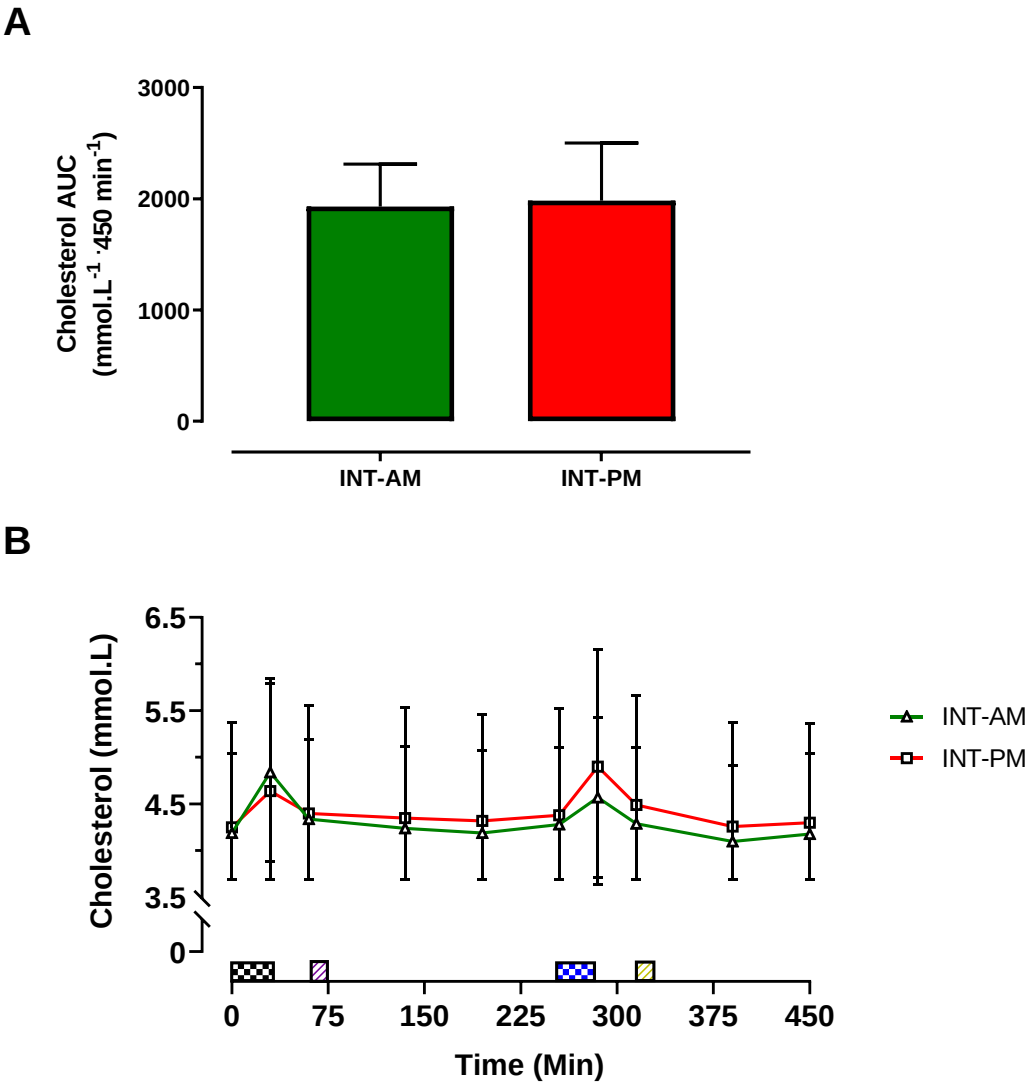

Figure 75*:* Representive of mean cholesterol AUC (0-450 min) (A) and cholesterol VAS response (B), with vertical error bars displaying SDs (n = 12). *Chequered black and white rectangle* indicates first 30-min exercise bout; *Hashed purple rectangle* indicates breakfast meal. *Chequered blue and white rectangle* indicates second 30-min exercise bout; *Hashed yellow rectangle* indicates ingestion of semi-solid lunch meal. There was no main effect observed for cholesterol AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial effect observed for cholesterol ($p > 0.05$). However, there was a main effect for time and a main interaction effect ($p < 0.05$) examined by a two-way repeated-measured ANOVA. Post-hoc tests revealed no further significance between INT-AM vs INT-PM, determined by Bonferroni adjusted paired *t-test* ($p > 0.05$). AUC, area under curve.

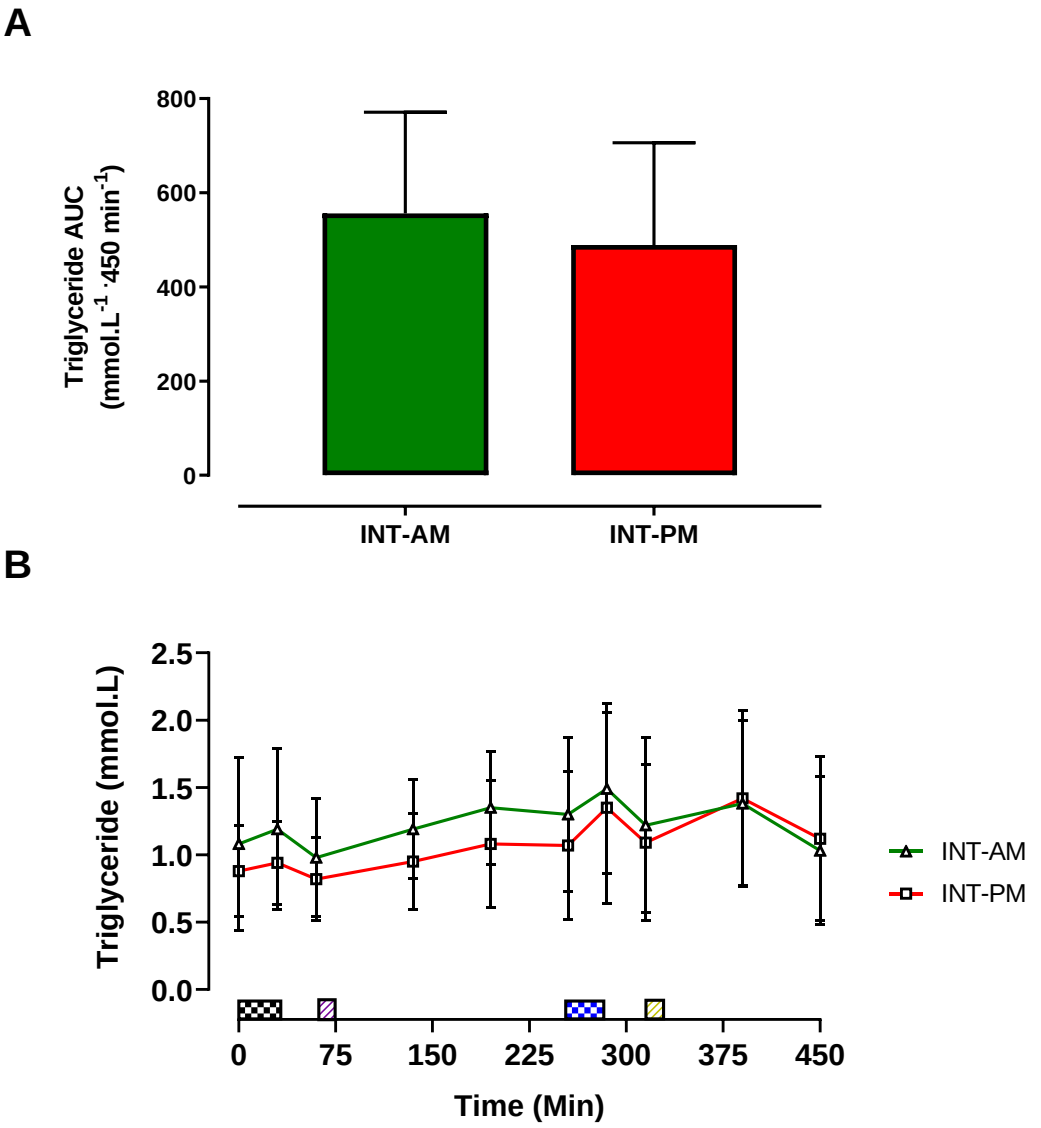

*Figure 76:*Representive of mean triglyceride AUC (0-450 min) (A) and triglyceride VAS response (B), with vertical error bars displaying SDs (n = 12). *Chequered black and white rectangle* indicates first 30-min exercise bout; *Hashed purple rectangle* indicates breakfast meal. *Chequered blue and white rectangle* indicates second 30-min exercise bout; *Hashed yellow rectangle* indicates ingestion of semi-solid lunch meal. There was no main effect observed for triglyceride AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial nor interaction effect observed for triglyceride ($p > 0.05$). However, there was a main effect for time ($p < 0.05$) examined by a two-way repeated-measured ANOVA. Post-hoc tests revealed no further significance between INT-AM vs INT-PM, determined by Bonferroni adjusted paired *t-test* ($p > 0.05$). AUC, area under curve.

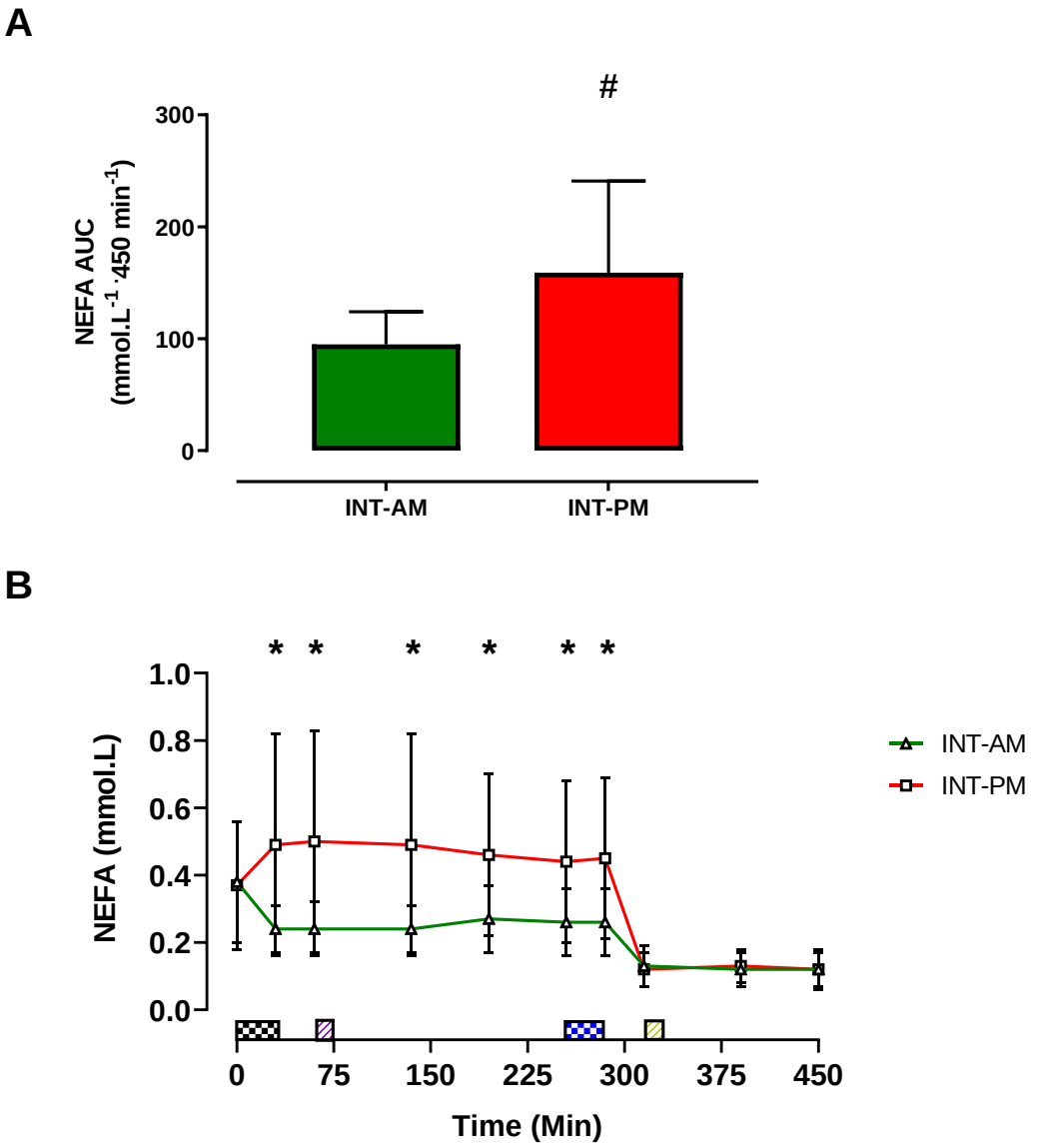

Figure 77: Representive of mean NEFA AUC (0-450 min) (A) and NEFA VAS response (B), with vertical error bars displaying SDs (n = 12). *Chequered black and white rectangle* indicates first 30-min exercise bout; *Hashed purple rectangle* indicates breakfast meal. *Chequered blue and white rectangle* indicates second 30-min exercise bout; *Hashed yellow rectangle* indicates ingestion of semi-solid lunch meal. There was a main effect observed for NEFA AUC ($p < 0.05$) examined by a one-way repeated-measured ANOVA. There was a main effect for trial, time and a main interaction effect observed for NEFA ($p < 0.05$). examined by a two-way repeated-measured ANOVA. *, # Indicates post-hoc tests were significantly different between INT-AM vs INT-PM, determined by Bonferroni adjusted paired t-test ($p < 0.05$). AUC, area under curve; NEFA, non-esterified fatty acid.

7.3.7. Gut Hormones

7.3.7.1. Active ghrelin

A main effect of time (p = 0.001, η^2p = 0.018) and a main interaction effect (p =0.001, η^2p = 0.379) was observed for active ghrelin. However, no main trial effect was observed (p = 0.665, η^2p = 0.018, Figure 78B). Post-hoc tests revealed INT-PM was significantly increased pre breakfast (60 min) compared to INT-AM (435 ± 236 vs. 267 ± 211 pg.mL: p = 0.001, d = 0.80, 95% CI = -132.73–120.18 pg.mL). Active ghrelin then decreased post breakfast, before beginning to rise in both throughout the first recovery phase. Post second exercise bout (285 min) INT-PM significantly decreased compared to INT-AM (267 ± 167 vs. 585 ± 419 pg.mL: p = 0.003, d =1.04, 95% CI = -236.03–95.53 pg.mL) and remained lower pre-lunch (315 min) (361 ± 223 vs. 519 ± 357 pg.mL: p = 0.022, d = 0.55, 95% CI = -201.43–126.73 pg.mL). There was no further significance post lunch until the end of the trial at 450 min. Furthermore, no main effect for AUC (p = 0.773, η^2p = 0.008, Figure 78A).

7.3.7.2. GLP-1

A main effect of time (p < 0.001, η^2p = 0.458) was observed for GLP-1. However, no main trial (p = 0.973, η^2p = 0.001) nor main interaction effect (p =0.442, η^2p = 0.112, Figure 79) was observed. Despite there being no further differences, GLP-1 did spike 1h after breakfast (135 min) and lunch (390 min). There was no main effect for AUC (p = 0.934, η^2p = 0.001, Figure 79A).

7.3.7.3. PYY

There was no main effect of time (p =0.510, η^2p = 0.083), trial (p = 0.731, η^2p = 0.014) nor main interaction effect observed for PYY (p = 0.313, η^2p = 0.121,Figure 80B). Despite there being no significant difference, PYY was marginally elevated during INT-PM pre-breakfast (60 min) PYY then responded similarly across the remainder of the whole study in both INT-AM and INT-PM. There was no main effect for AUC (p = 0.546, η^2p = 0.042; Figure 80A).

7.3.7.4. PP

A main effect of time (p = 0.004, η^2p = 0.500) was observed for PP. However, no main trial (p = 0.951, η^2p = 0.001) nor main interaction effect (p =0.343, η^2p = 0.101,

Figure 81) was observed. Despite there being no further differences, PP did spike in both INT-AM and INT-PM 1h after breakfast (135 min) and lunch (390 min) showing similar responses (p >0.05). There was no main effect for AUC (p = 0.914, η^2p = 0.001, Figure 81A).

7.3.7.5. Insulin

There was no main effect of time (p =0.218, η^2p = 0.128), trial (p = 0.434, η^2p = 0.057) nor main interaction effect observed for insulin (p = 0.755, η^2p = 0.028,Figure 82B). Despite there being no further differences, insulin did show marginal increases in both INT-AM and INT-PM 1h after breakfast (135 min) and lunch (390 min) showing similar non-significant responses (p >0.05). There was no main effect for AUC (p = 0.438, η^2p = 0.056; Figure 82A).

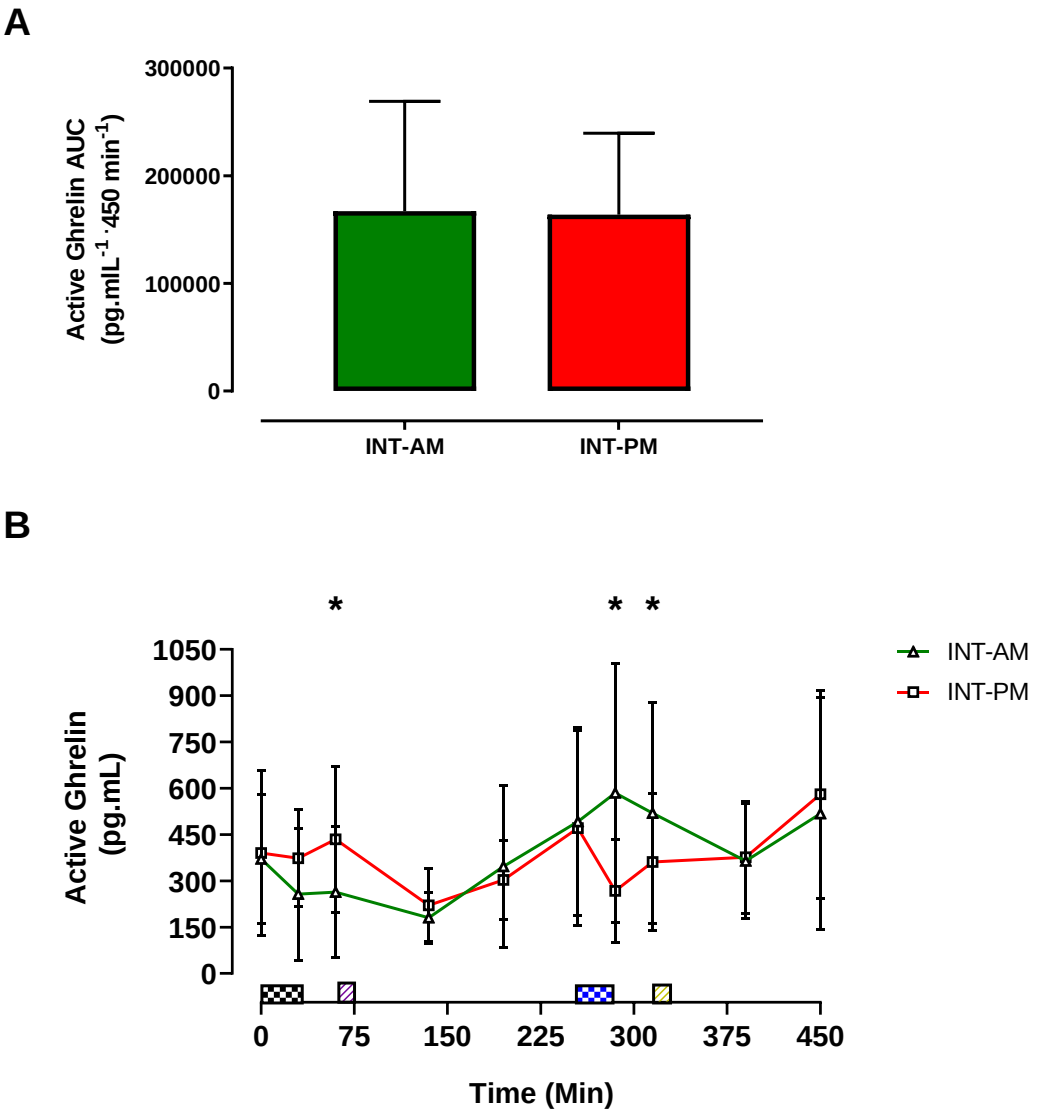

Figure 78: Representive of mean active ghrelin AUC (0-450 min) (A) and active ghrelin VAS response (B), with vertical error bars displaying SDs (n = 12). *Chequered black and white rectangle* indicates first 30-min exercise bout; *Hashed purple rectangle* indicates breakfast meal. *Chequered blue and white rectangle* indicates second 30-min exercise bout; *Hashed yellow rectangle* indicates ingestion of semi-solid lunch meal. There was no main effect observed for active ghrelin AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial effect observed for active ghrelin ($p > 0.05$). However, there was a main effect for time and a main interaction effect ($p < 0.05$) examined by a two-way repeated-measured ANOVA. * Indicates post-hoc tests were significantly different between INT-AM vs INT-PM, determined by Bonferroni adjusted paired *t-test* ($p < 0.05$). AUC, area under curve.

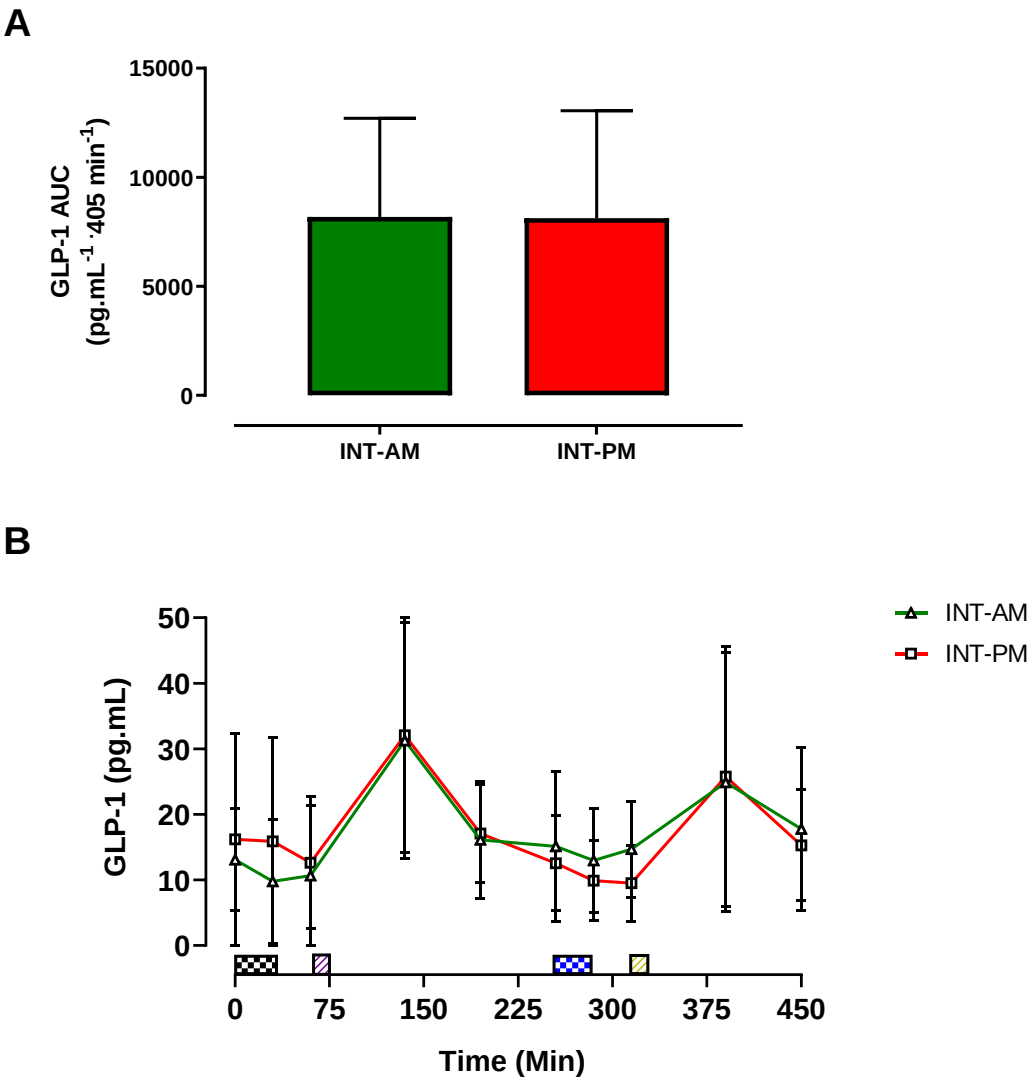

Figure 79: Representive of mean GLP-1 AUC (0-450 min) (A) and GLP-1 VAS response (B), with vertical error bars displaying SDs (n = 9). *Chequered black and white rectangle* indicates first 30-min exercise bout; *Hashed purple rectangle* indicates breakfast meal. *Chequered blue and white rectangle* indicates second 30-min exercise bout; *Hashed yellow rectangle* indicates ingestion of semi-solid lunch meal. There was no main effect observed for GLP-1 AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial nor main interaction effect observed for GLP-1 ($p > 0.05$). However, there was a main effect for time ($p < 0.05$) examined by a two-way repeated-measured ANOVA. AUC, area under curve; GLP-1, glucagon-like peptide-1.

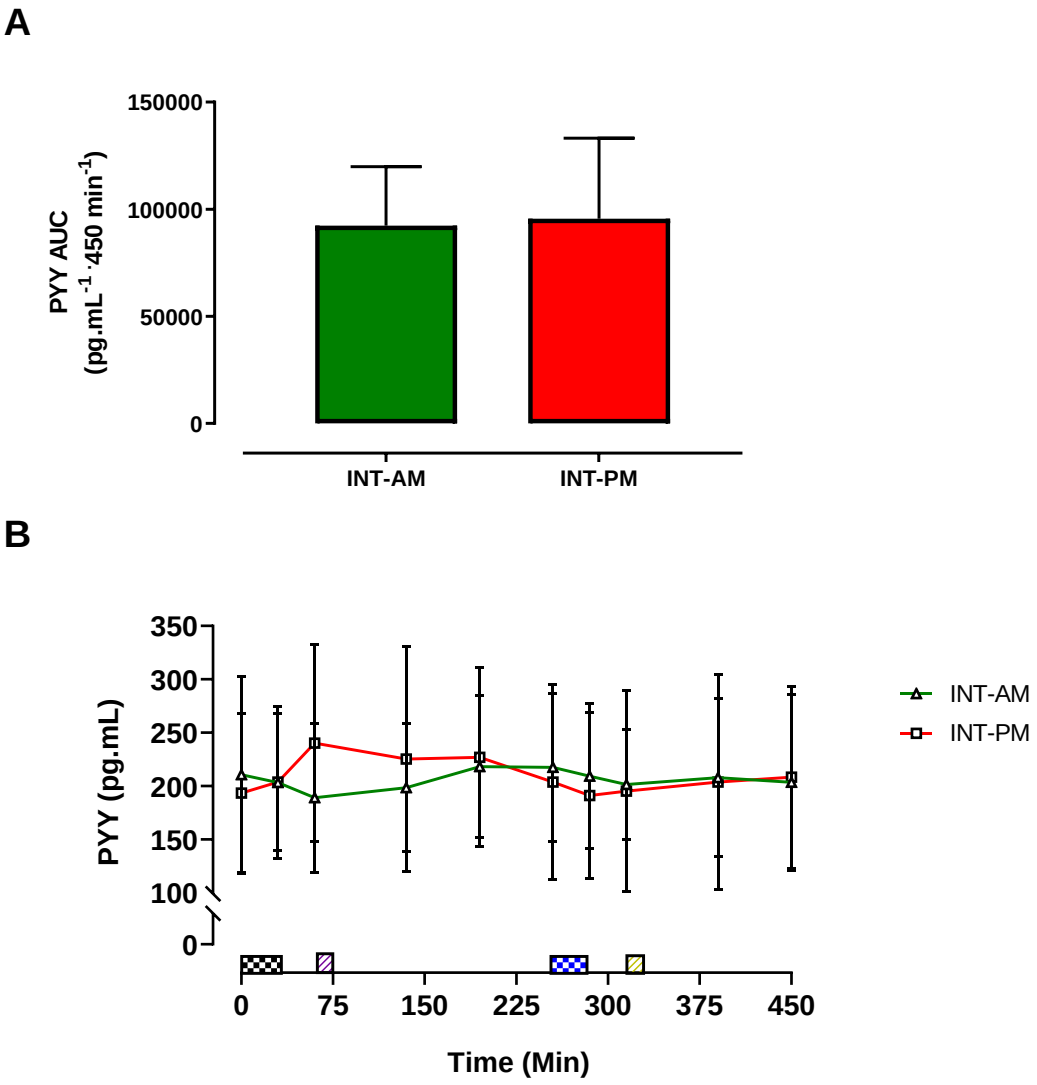

Figure 80: Representive of mean PYY AUC (0-450 min) (A) and PYY VAS response (B), with vertical error bars display SDs (n = 10). *Chequered black and white rectangle* indicates first 30-min exercise bout; *Hashed purple rectangle* indicates breakfast meal. *Chequered blue and white rectangle* indicates second 30-min exercise bout; *Hashed yellow rectangle* indicates ingestion of semi-solid lunch meal. There was no main effect observed for PYY AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial, time nor main interaction effect observed for PYY ($p > 0.05$) examined by a two-way repeated-measured ANOVA. AUC, area under curve; PYY, peptide-YY.

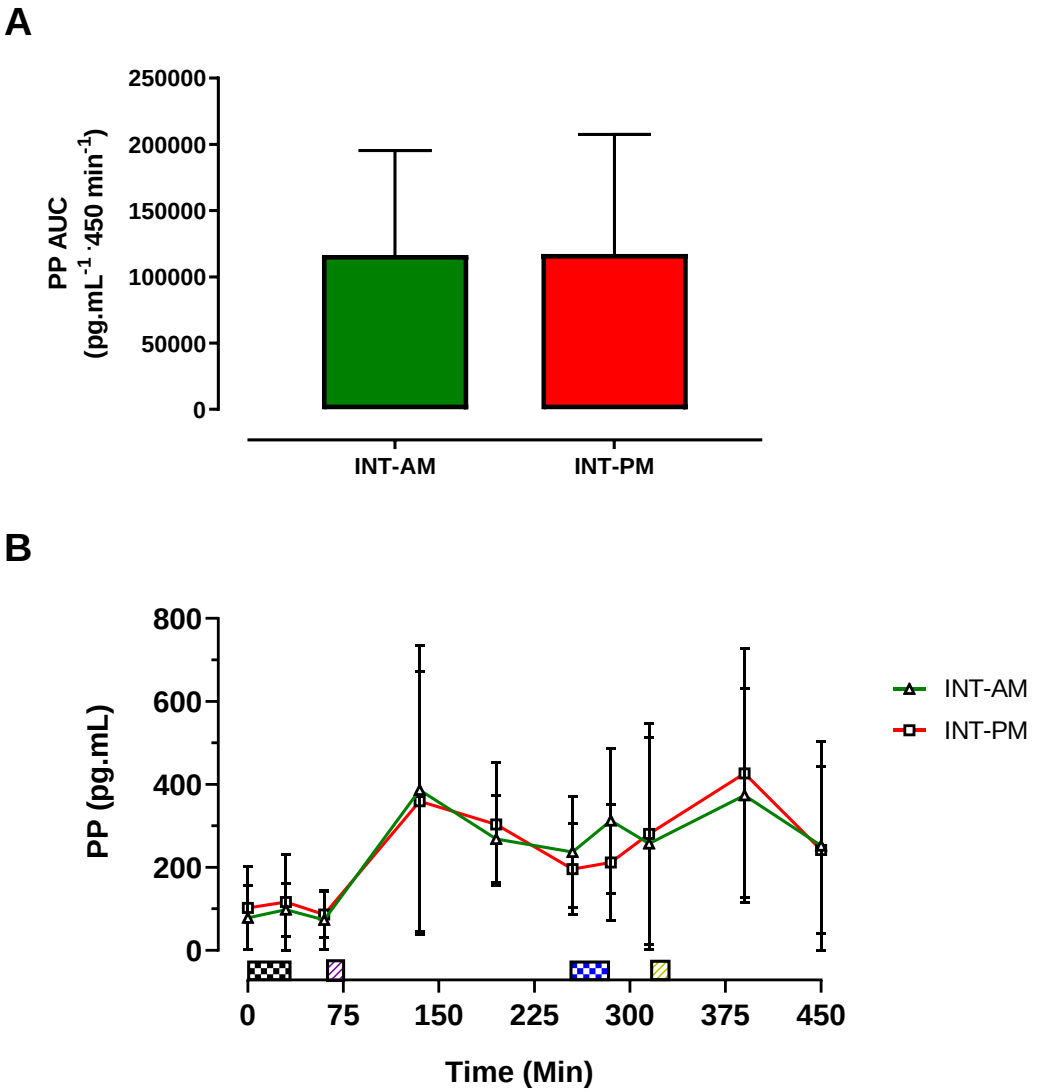

Figure 81: Representive of mean PP AUC (0-450 min) (A) and PP VAS response (B), with vertical error bars displaying SDs (n = 12). *Chequered black and white rectangle* indicates first 30-min exercise bout; *Hashed purple rectangle* indicates breakfast meal. *Chequered blue and white rectangle* indicates second 30-min exercise bout; *Hashed yellow rectangle* indicates ingestion of semi-solid lunch meal. There was no main effect observed for PP AUC ($p > 0.05$) examined by a one-way repeated-measured ANOVA. There was no main trial nor main interaction effect observed for PP ($p > 0.05$). However, there was a main effect for time ($P < 0.05$) examined by a two-way repeated-measured ANOVA. AUC, area under curve; PP, pancreatic polypeptide.

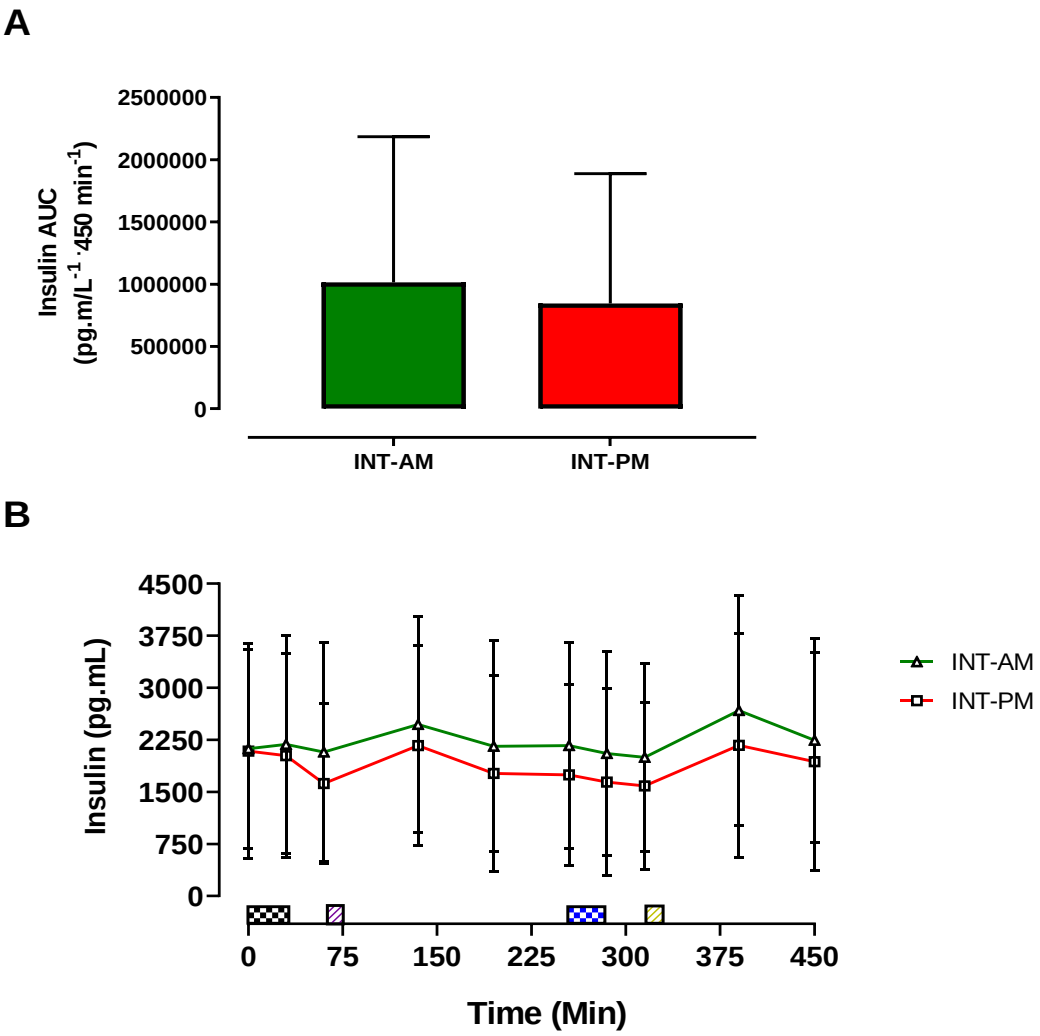

Figure 82: Representive of mean insulin AUC (0-450 min) (A) and insulin VAS response (B), with vertical error bars displaying SDs (n = 12). *Chequered black and white rectangle* indicates first 30-min exercise bout; *Hashed purple rectangle* indicates breakfast meal. *Chequered blue and white rectangle* indicates second 30-min exercise bout; *Hashed yellow rectangle* indicates ingestion of semi-solid lunch meal. There was no main effect observed for insulin AUC (p >0.05) examined by a one-way repeated-measured ANOVA. There was no main trial, time nor main interaction effect observed for insulin (p >0.05) examined by a two-way repeated-measured ANOVA. AUC, area under curve.

7.3.8. Substrate Utilisation

7.3.8.1. Carbohydrate Oxidation

Carbohydrate oxidation AUC was lower for INT-AM compared to INT-PM. Although, this result was not significantly different (128 ± 43 vs 138 ± 37 g/min^{-1} 450 min^{-1}, P =0.425, η^2p = 0.059, Figure 83)

There was a main effect of time (P =0.003, η^2p = 0.240) and main interaction effect (P = 0.017, η^2p = 0.194), but no main trial effect (P =0.460, η^2p = 0.051) for carbohydrate oxidation. Post-hoc tests revealed INT-PM was significantly higher post first exercise bout (30-min) compared to INT-AM (0.355± 0.09 vs 0.261 ± 0.12 g/min: P = 0.045, d = 0.93, 95% CI = 0.87–0.99 g/min, Figure 84A).

7.3.8.2. Fat Oxidation

Fat oxidation AUC was lower for INT-PM compared to INT-AM. Although, this result was not significantly different (33 ± 8 vs 37 ± 12 g/min^{-1} ·450 min^{-1}, P =0.454, η^2p = 0.052, Figure 83).

There was a main effect of time (P =0.018, η^2p = 0.234) and main interaction effect (P <0.001, η^2p = 0.586), but no main trial effect (P =0.465, η^2p = 0.049) for fat oxidation. Post-hoc tests revealed INT-AM was significantly higher post first exercise bout (30-min) compared to INT-PM (0.119 ± 0.048 vs 0.040 ± 0.037 g/min: P < 0.001, d = 1.93, 95% CI = 1.90–1.95 g/min). Post second exercise (285 min), fat oxidation was significantly higher during INT-PM compared to INT-AM (0.119 ± 0.048 vs 0.073 ± 0.042 g/min: P = 0.023, d = 1.07, 95% CI = 1.04–1.09 g/min). One-hour post lunch (390 min) fat oxidation reduced in both INT-AM and INT-PM however, INT-PM remained significantly increased compared to INT-AM (0.087 ± 0.030 vs 0.054 ± 0.027 g/min: P = 0.004, d = 1.21, 95% CI = 1.19–1.22 g/min, Figure *84*B).

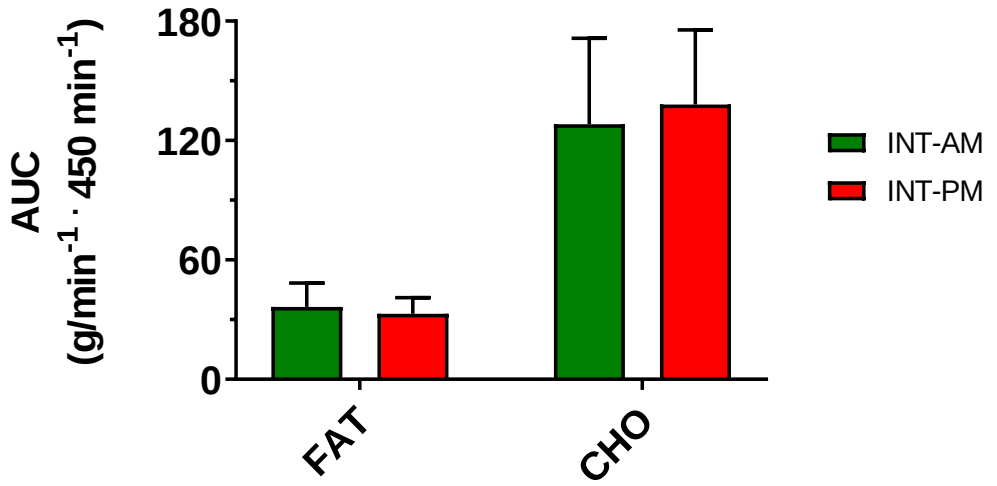

Figure 83: Representative of mean fat and carbohydrate oxidation AUC (0-450 min^{-1}), where vertical error bars display SDs (n = 12). There was no main effect observed for fat and carbohydrate oxidation AUC (p >0.05) examined by a one-way repeated-measured ANOVA. AUC, area under curve; CHO, carbohydrate.

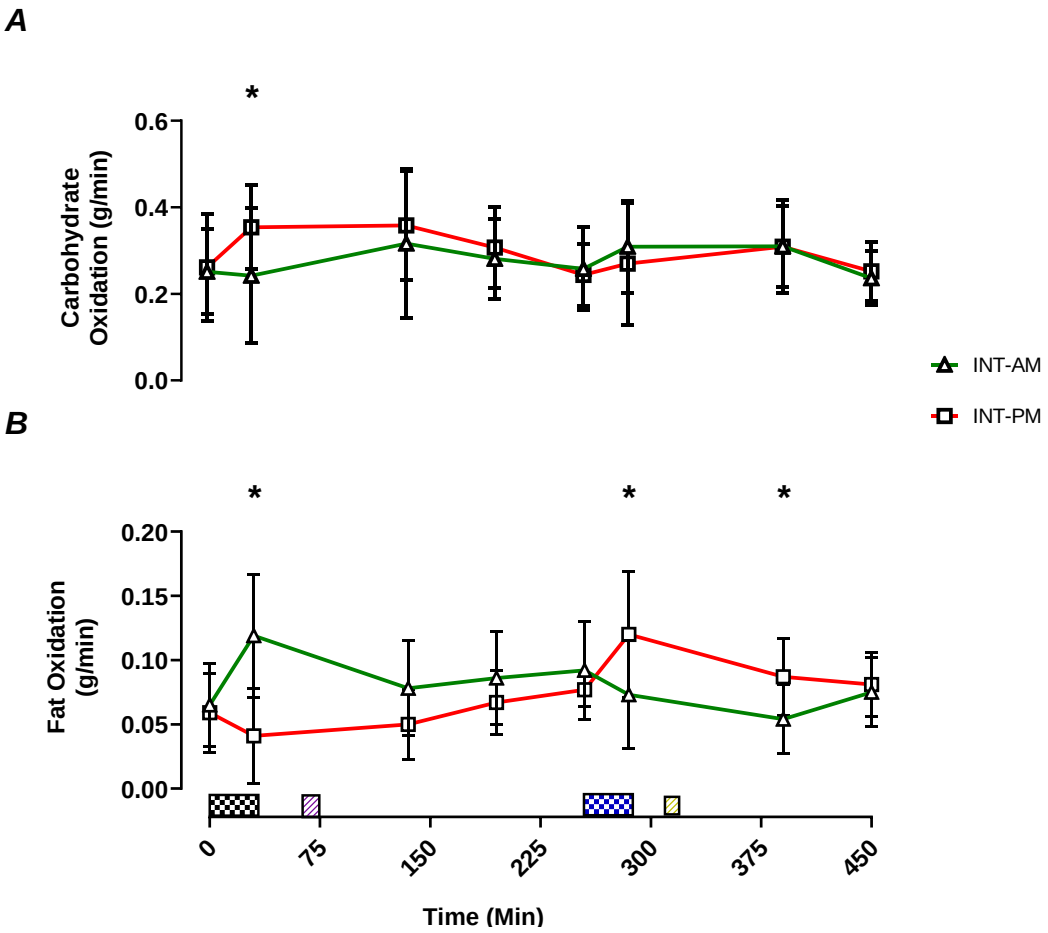

Figure 84: Representative of mean carbohydrate oxidation (g/min) (A) and fat oxidation (g/min) (B), where vertical error bars display SDs (n = 12). *Chequered black and white rectangle* indicates first 30-min exercise bout; *Hashed purple rectangle* indicates breakfast meal. *Chequered blue and white rectangle* indicates second 30-min exercise bout; *Hashed yellow rectangle* indicates ingestion of semi-solid lunch meal. There was no main trial effect for fat and carbohydrate oxidation ($p > 0.05$). Although, there was a main effect for time and a main interaction effect ($p < 0.05$) for both fat and carbohydrate oxidation, examined by a three-way repeated-measured ANOVA. * Indicates INT-AM vs INT-PM was significantly different determined by Bonferroni adjusted paired *t-test* ($p < 0.05$).

7.3.9. Wellbeing

A main effect for time (p <0.001, η^2p = 0.384) and trial x time interaction effect (p =0.001, η^2p = 0.393) was observed. However, there was no effect for trial (p =0.324, η^2p = 0.088) for wellbeing. Post-hoc test revealed INT-AM was significantly higher post first exercise bout (30 min) compared to INT-PM (14 ± 2 vs. 12 ± 2 total: P = 0.018, d = 1.04, 95% CI = -0.09–2.18). Furthermore, INT-PM was significantly higher post second exercise bout (285 min) (14 ± 2 vs. 12 ± 3 total: P = 0.013, d =0.82, 95% CI = -0.31–2.52) and 24 h post trial (13 ± 3 vs. 11 ± 2 total: P = 0.043, d =0.82, 95% CI = -0.88–1.95, Figure 85).

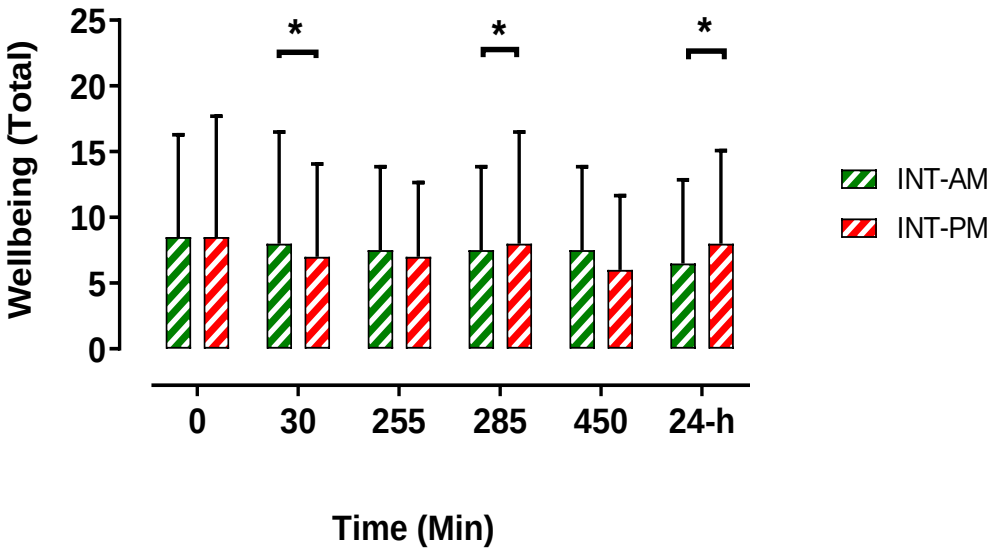

Figure 85: Representative of mean for wellbeing, where vertical error bars display SDs (n = 12). There was a main effect for time and a main interaction effect (p <0.05) for wellbeing. However, no trial effect (p >0.05) examined by a two-way repeated-measured ANOVA. * Indicates INT-AM vs INT-PM were significantly different, determined by Bonferroni adjusted paired *t-test* (p < 0.05).

7.4 Discussion

The primary aim of the current study was to investigate whether mixing the modality of exercise (continuous and intermittent), by switching when intermittent exercise is performed, using a twice-a-day exercise approach, might influence GER, GI hormonal, metabolic response, feelings of wellbeing and EI in the subsequent 24 h following acute bouts of exercise. The main findings were that mixing modality did not cause an elevation in fat nor carbohydrate utilisation. Nevertheless, fat oxidation did peak after both high intensity, intermittent exercise (HIIE), regardless of whether the participants were fed or fasted. Furthermore, the timing of the exercise mode did not cause any compensatory changes in GER. A ~35% decrease in perception of hunger resulted in a ~31% decrease in peripheral acylated ghrelin, and a ~7% increase in circulating glucose immediately after completing HIIE in the morning (30 min). Regardless, that the afternoon values were not significant for hunger and glucose, the results were mimicked, as a ~54% decrease in acylated ghrelin was found immediately after HIIE (285 min), despite participants not being in a fasted state. Moreover, overall wellbeing scores were ~15% higher 24-h post-trial after completing HIIE in the afternoon, which might account for the ~19% increase in EI after INT-PM. These results suggest that the order in which continuous and intermittent exercise are performed does not affect GER of a semi-solid meal nor overall substrate utilisation, and only minor differences in metabolic and hormonal responses can be observed within the same day. Consequently, there might be some differences in the days following mixed exercise bouts, if intermittent exercise is conducted in the second exercise bout during a multiple exercise day, as shown by increases in feeling of wellbeing and EI. Whether these findings extend to different populations is currently unknown, as within the current study healthy men were investigated.

To date, this is the only study to have examined GER of a semi-solid meal after mixing the modality of exercise (continuous and intermittent). Within the current study GER was unaffected by the order of modality. This is not uncommon, as Evans et al, (2016) revealed exercising at 30% (Low) and 100% PPO (High) for 1 min followed by 2 min rest did not result in compensation in postprandial GER. In addition, chapter 4, Mattin et al, (2018a) utilised cycling and McIver et al, (2018) explored walking as a mode of exercise; both studies maintain that GER was unaffected in the

postprandial period after exercise. Conversely, within Chapter 6, GER of a semi-solid meal was accelerated after a twice-a-day (SPLIT) continuous to continuous exercise schedule, compared to a one off (SINGLE) bout of continuous exercise. It is important to consider why GER was accelerated within Chapter 6, and not within the current study (Chapter 7), despite similar study designs. It must be acknowledged there were two noticeable differences; the exercise modality, and secondly the calorific amounts that were provided to participants within the meal periods. Within Chapter 6, resting energy expenditure (REE) and physical activity energy expenditure (PAEE) were calculated to estimate trial energy expenditure (ETEE), which was provided to participants through two meal periods. Although, a number of studies have used total daily energy expenditure (TDEE) to quantify how the human body responds physiologically to nutritional intake after exercise, when the total energy requirements of exercise are replaced (Stephens et al., 2007, Hagobian et al., 2009), neither investigation examined GER as a primary outcome. It must not be forgotten, if sufficient time is allocated after strenuous exercise, the body's ability to recover and deliver nutrients to the small intestine is maintained, regardless of whether HIIE is performed within the morning or afternoon, when undertaking multiple exercise sessions within the same day. The long-term effects of the stomach and the GI system are trainable, remain a desirable outcome and warrant further investigation, to identify if changes in GER relate to differences in metabolic health within a healthy population.

There is strong evidence to suggest that healthy individuals demonstrate a suppression in appetite immediately after exercise at an intensity >60% $\dot{V}O_{2max}$ (Martins et al., 2007a, Becker et al., 2012, King et al., 2013b). Consistent with the current study, participants reported a ~35% decrease in hunger immediately after 30 min of HIIE (INT-AM), and surprisingly, an increase in hunger immediately after 30 min of continuous exercise during INT-PM (50% $\dot{V}O_{2peak}$). Nevertheless, this suppression in appetite was prolonged as hunger remained significantly lower 30 min post-exercise (pre-breakfast consumption) during the INT-AM trial, regardless of Deighton and Stensel et al, (2014) stating that appetite values tend to return to control values within 30 min of the cessation of exercise. The suppression in appetite seen within the current study can be strongly connected to the modality of exercise, for example Mattin et al, (2020a) (Chapter 5) discovered a variance in appetite, as hunger

AUC was significantly lower after exercising intermittently rather than performing a continuous mode of exercise. Despite the fact, exercise bouts were matched for power output (60% $\dot{V}O_{2peak}$). This might potentially suggest that intermittent modes of exercise might acutely affect appetite to a greater extent than continuous exercise. Research has been conducted to further support this statement, as performing exercise at a high intensity (~80% $\dot{V}O_{2peak}$) in a continuous manner has been found by Holliday and Blannin, (2017c) not to affect appetite immediately after continuous exercise, when comparing the duration of exercise. Reinforcing once again the differences between continuous and intermittent modes of exercise, by further suggesting that intermittent modes of exercise might suppress appetite differently, in the periods after exercise, before the consumption of nutrient dense material.

It must be stated, any change in hunger within the current study was not unique, as further VAS questions; prospective food consumption (PFC) responded similarly by decreasing immediately post-exercise, with fullness and satisfaction significantly increasing during INT-AM post-exercise. Although appetite suppression after an intermittent exercise bout is short lived once food is administered, as shown within the current study, subjective appetite sensations were not significantly different between trials after consuming a standardised breakfast meal. This result is consistent with results from Chapter 6 (Mattin et al., 2020a), as a small breakfast drink ~ 334 ml semi-skimmed milk was sufficient to counterbalance the exercise effects on appetite. This suggests, subjective appetite can be offset by a relatively small amount of nutrient dense material after exercise, independent of the changes observed after exercise in a fasted state. Whether the same response would have been seen within the current study if a breakfast drink was consumed, rather than a substantially larger breakfast meal, warrants further investigation. Critically, the larger breakfast meal within the current study resulted in appetite responding similarly for the reminder of the 3h recovery period, before participants commenced in the second exercise bout, although there were no significant differences between trials, it is important to add that appetite trended towards a suppression post-second exercise within the INT-PM trial.

It is important to consider the reasons why EI was elevated by ~19% (681 Kcal) after completing a HIIE bout within the second exercise bout undertaken during the same day (INT-PM). The sole difference between the trials was the order in which

the HIIE bout was performed. Interestingly, an increase in EI has previously been discovered by Mattin et al (2020a) (Chapter 5), which demonstrated an elevated EI by 723 Kcal, 24 h after completing moderate, intense, intermittent exercise. Furthermore, an increase in EI was also discovered within Chapter 6, as exercising twice a day resulted in a 733 KCal increase, compared to a one-off bout. Controversially, the majority of the available literature which has focused on appetite as a major outcome measure has revealed that EI is usually unaffected >20 h after exercise (King et al., 1997, Pomerleau et al., 2004, King et al., 2010a, Hanlon et al., 2012, King et al., 2013a). It is important to consider the reasons why EI has been found to be increased within the research conducted during this thesis. One noticeable difference is the manner in which EI was assessed, as weighed diet recall has potentially high participant variation. Examining EI via a weighed dietary assessment may cause recall bias, and might be one explanation as to why EI was elevated within the current study, although King et al, (1997) and Hanlon et al, (2012) discovered no differences in EI after using a self-reporting measurement of food intake, suggesting their participants could also have been subject to the same reporting bias.

Another theory is the training status of the population recruited, as trained athletes have been shown to experience less inflammation and oxidative stress than untrained individuals, after performing the same bout of exercise (Abbott et al., 2019b). Within the current study the participants were healthy untrained males ($\dot{V}O_{2max}$ = 48 ± 10 ml/kg/min; Body Fat 20.4 ± 6.7 %). In contrast, the voluntary demographic of participants used within appetite research has been shown to be vastly different; King et al, (2013a) used endurance trained males ($\dot{V}O_{2max}$ = 61.5 ± 4.8 ml/kg/min; body Fat 14.9 ± 3.2 %) and King et al, (2010b) used well trained ($\dot{V}O_{2max}$ = 56 ± 1.8 ml/kg/min, Body Fat 19.2 ± 1.2 %). Whether the participants' training state influenced how the body responded to post exercise calorie consumption might be critical for weight management strategies.

One potential mechanism which might explain why post-exercise EI was elevated, is the assessment of subjective wellbeing. It is well established that strenuous exercise can cause damage to the skeletal muscle fibres (Hyldahl and Hubal, 2014). This damage instigates an acute inflammatory response, characterised by the migration of phagocytic immune cells to the affected fibres (Chazaud, 2016).

Nevertheless, limited research has focused on changes in post exercise EI, in relation to recovery from exercise, using a healthy untrained population. Subjective wellbeing was documented as an additional measurement during the current study, to better understand if the unanticipated increase in EI, witnessed within Chapter 5 (Mattin et al., 2020a), and Chapter 6, is related to the body's response to recovery after the physical demands of exercise. Participants reported a relatively minor increase in subjective wellbeing immediately after the first exercise period within the HIIE bout (INT-AM), and this was replicated in the second exercise period, once again subjective wellbeing was elevated within the HIIE bout (INT-PM). Suggesting regardless of the fact that participants performed a HIIE bout within the morning (fasted), or during the afternoon (not fasted), indicating participants may have been in a different metabolic state. Surprisingly, wellbeing was increased 24 h after the INT-PM trial. The mechanisms for the difference in wellbeing score after the INT-PM trial is not well-defined, but might imply a valid reason why EI was similarly increased after INT-PM. Therefore, proposing a low intensity continuous exercise bout (~50% $\dot{V}O_{2peak}$) might be a more effective recovery aid, if performed 3 h after a HIIE bout, rather than before.

Hulston et al, (2010) discovered well-trained cyclists who had reduced muscle glycogen before exercise had enhanced fat metabolism. Morton et al, (2009) suggested this might be related to improved training induced adaptation by markers of oxidative enzyme activity in healthy trained participants. Interestingly, Abbott et al, (2019a) investigated whether supplementing professional football players with tart cherry juice post-match aided muscle recovery. Curiously, 24 h dietary intake was unaffected despite the fact that wellbeing was increased at 12 h and 36 h in both trials, compared to baseline (Pre-Match). This similar response in wellbeing values ≥36 h post-match might support the reason why EI was not different between trials. It must be acknowledged that some studies restrict participants' dietary intake, when the research focused on using polyphenol as a post-exercise recovery aid, by excluding any fruits, vegetables, or foods which are high in polyphenol contents (Bell et al., 2014, Bell et al., 2015, Bell et al., 2016). Yet, within the current study, dietary intake was only restricted for alcohol and caffeine consumption. The rationale in Bell et al, (2014, 2015, 2016) for eliminating foods high in polyphenol, is because there is a growing body of evidence suggesting that functional food-based supplements that contain anti-

inflammatory and/or antioxidant phytonutrients, can attenuate symptoms of exercise-induced muscle damage (EIMD), by accelerating the recovery of muscle function (Myburgh, 2014, Harty et al., 2019). Conversely, participants within the current study consumed ~366 KCal more carbohydrate within the INT-PM trial, which might suggest a higher polyphenol intake, as most fruit and vegetables are carbohydrate dense. Despite this, wellbeing scores were higher within the INT-PM trial. The only true method to understand if polyphenols or total antioxidant status were increased, is to measure individual blood samples, which would help to provide a more comprehensive overview, when combined with the subjective wellbeing and EI data. Unfortunately, no samples were collected during the post 24 h period, and samples that were collected during the ~8 h trial were not used to measure markers of muscle damage nor antioxidant status. Further research is required, to explore the mechanistic effects for the increase in EI and wellbeing, discovered within the present study, when a healthy untrained population is investigated. Whether an increased inflammatory response, and reduced muscle function capacity in the >12 - 48 h following multiple exercise bouts, within the same day is responsible, is unknown.

Within the current study, meal periods (breakfast and lunch) were standardised for volume, weight, and calories, allowing any differences discovered to be attributed to the exercise condition, rather than the food provided. Nevertheless, GLP-1 was not significantly different between trials, although an increase was revealed in both conditions after breakfast (27 ± 19 vs. 26 ± 22 pg.mL) and lunch (22 ± 19 vs. 23 ± 18 pg.mL) during INT-AM vs INT-PM. Consistent with the current study, GLP-1 was reported during Chapter 4 (Mattin et al., 2018b), to respond similarly 30-min after consuming a standardised semi-solid lunch meal (control, 12 ± 10 pg.mL; 40% $\dot{V}O_{2Peak}$, 15 ± 12 pg.mL; and 70% $\dot{V}O_{2Peak}$, 12 ± 10 pg.mL). This trend is further supported within Chapter 6, as GLP-1 spiked 60-min after consuming breakfast (12 ± 8 vs. 10 ± 7 pg.mL) and lunch (12 ± 9 vs. 14 ± 8 pg.mL) during SINGLE vs SPLIT, once again displaying no differences between trials. Evidence has emerged suggesting that GLP-1 concentration can change in response to mechanical distension of the stomach (Vahl et al., 2007, Krieger et al., 2016, Williams et al., 2016), rather than in response to the calorie density of the consumed nutrients. The results within the current study, with support from Chapter 4 (Mattin et al., 2018a), and Chapter 6, might

strengthen the theory GLP-1 reacts to a change in stomach size. Although, without directly measuring changes in muscle activation or movement of the stomach, this theory cannot fully be determined. Further work should focus on better understanding of the communication between the gut and the brain, in relation to appetite control.

Ghrelin has been shown to be reduced after aerobic exercise at moderate to high intensity (>65 $\dot{V}O_{2peak}$), running (Broom et al., 2009, King et al., 2010a, Balaguera-Cortes et al., 2011, King et al., 2011a, Wasse et al., 2012, Wasse et al., 2013b, Broom et al., 2017) and cycling (Becker et al., 2012, Kawano et al., 2013, Wasse et al., 2013b, Mattin et al., 2018a). Although, more recent literature has investigated low/high intermittent exercise (Gholipour et al., 2014, Sim et al., 2014, Martins et al., 2015, Panissa et al., 2016, Holliday and Blannin, 2017d). Consistent with the current study, acylated ghrelin decreased from fasting baseline values by ~31% after INT-AM, and by ~4% after INT-PM immediately post exercise. Additionally, acylated ghrelin values were still ~40% lower pre-breakfast after a HIIE bout (INT-AM), compared to a continuous bout (INT-PM). Subsequently, acylated ghrelin concentrations remained relatively stable during the remainder of the 3h recovery periods post-breakfast, prior to starting exercise bout two, where acylated ghrelin concentrations were ~54% lower immediately after completing a HIIE bout (INT-PM) and remained ~ 30% lower pre-lunch. Regardless of the fact that participants were in a different metabolic state within the second exercise bout, HIIE still caused a substantial reduction in acylated ghrelin concentration, compared to a continuous exercise bout. Nevertheless, not all studies have found a reduction in ghrelin post exercise, as Lardon-Meyer et al, (2012) discovered acylated ghrelin increased after participants walked for 60 min. This can be further supported by Ueda et al, (2009b) who revealed that cycling at 50% $\dot{V}O_{2max}$ increased acylated ghrelin within a control group (normal weight), compared to an obese population. The changes seen in the current study further support the robust evidence that high intensity, intermittent exercise causes a suppression in ghrelin immediately post exercise, and continuous exercise that is at an intensity ≤50 $\dot{V}O_{2peak}$ increases ghrelin, which in turn might result in a surge in hunger.

One particular theory that should be discussed is the link between blood glucose and ghrelin responses. The glucoregulatory actions of ghrelin is well

documented within humans (Broglio et al., 2001, Cummings et al., 2001, Tschöp et al., 2001, Egido et al., 2002, Broglio et al., 2003b) and literature using animal models (Reimer et al., 2003, Dezaki et al., 2004). Nevertheless, during fasting conditions ghrelin increases blood glucose, through stimulating glucagon secretion by acting on the pancreatic islet endocrine α-cell lines to increase glycemia (Chuang et al., 2011). The ability for ghrelin to employ glucagon as a pathway to rise blood glucose is potentially restricted to situations such as an overnight fast (Broglio et al., 2003a, Tack et al., 2006, Tong et al., 2010), and enhancing hepatic glucose production may be an action via the brain (Mani et al., 2019). Interestingly, within the current study, blood glucose increased by 0.25 mmol.L (4.9%) immediately after completing HIIE (INT-AM), compared to a 0.03 mmol.L decrease after a continuous exercise bout (INT-PM). In fact, oral administration of macronutrients such as amino acids and fatty acids can each suppress ghrelin, with glucose in particular causing a rapid and profound suppression in ghrelin in humans, when administered orally (Djurhuus et al., 2002, Shiiya et al., 2002). Although, within the current study, participants were fasted overnight for ~10 h before starting the exercise bouts, suggesting the blood glucose increases observed after completing the HIIE bout was primarily derived from hepatic sources. This novel finding further supports the concept that glucose impairs ghrelin release only after it has made its way into circulation. Subsequently, to further discuss the changes discovered in the present study, glucose was significantly lower at 1 h and 2h post lunch during INT-PM. Suggesting, lower glucose response in the 2h after lunch might explain the reason why EI was higher 24 h after the INT-PM trial. A large cohort study (1,070 participants) revealed postprandial glucose dips 2-3 h after a meal were responsible for an increase in hunger at 2h and greater EI at 3h and a further increase in EI 24 h post-trial (Wyatt et al., 2021).

Adipose tissue lipid mobilisation increases during exercise and muscle lipid utilisation increases similarly, however the lipid oxidation rate is dependent on the relative workload which is thought to be maximised at around 65% $\dot{V}O_{2max}$ (Purdom et al., 2018). We know from work carried out within our own laboratory that fasting prior to exercise reduces carbohydrate and increases fat utilisation during exercise (McIver et al., 2018, McIver et al., 2019). These findings were further supported within Chapter 6, as exercising for 60 min at 70% $\dot{V}O_{2peak}$ caused an increase in fat

utilisation during exercise. The energy demands in the recovery period after exercise may hold the key to how individuals metabolise energy post-exercise, and critically how the body responds after consuming nutrient dense food may mitigate the increase in fat oxidation established during exercise. Within the current study, overall fat and carbohydrate oxidation shown by AUC results were not affected by mixing the modality of exercise (continuous and intermittent). This may be related to the fact fat oxidation peaked after both HIIE bouts, regardless of the fact that within INT-AM participants were fasted for ~10h and after INT-PM participants had previously consumed a relatively normal breakfast (~434 KCal) 180 min before. It is vital to consider that blood glucose levels had returned to baseline values within INT-PM (4.7 ± 0.2 vs. 4.5 ± 0.3 mmol.L) prior to starting the second exercise bout. Proposing 180 min recovery after breakfast was sufficient to increase fat oxidation during intermittent exercise, retaining the benefits of exercising fasted. Increased fat oxidation has been suggested to be beneficial for reducing fat mass (Achten and Jeukendrup, 2004), although this result should not be misinterpreted, as any increase in fat oxidation during exercise is diminished after consuming nutrient dense food.

An anomaly within the data must be discussed, as NEFA increased after the first exercise bout within INT-PM and remained elevated until post second exercise bout (285 min). This retained elevation is an unusual response for NEFA, as in previous investigations carried out within our own lab by McIver et al, (2018) and chapter 4 (Mattin et al., 2018a), found NEFA decreased shortly after consuming nutrient dense food. Nevertheless, one study using trained and untrained older adults found increased NEFA was not associated with increased fat oxidation, rather an increase in carbohydrate oxidation (Bassami et al., 2007). Consistent with the current study, carbohydrate oxidation and NEFA were significantly elevated after the first exercise bout during continuous exercise at 50% $\dot{V}O_{2peak}$ (INT-PM). Although, the metabolic reasons why NEFA was increased remains to be investigated. Subsequently, there was not an overall or between trial differences for gut hormones (PYY, PP and insulin) and metabolites (cholesterol and triglyceride).

7.4.1. Limitations

A limitation was not accounting for physiological changes in the days (24 h-48 h) following multiple exercise bouts. It must be acknowledged, the nature of measuring subjective wellbeing via a questionnaire, may make the findings within the current study variable and less reliable than using objective muscle measuring strategies. Selected literature has previously shown changes in muscle function and strength tests >60 h after strenuous exercise, with the majority using a mixture of tests to functionally understand if muscle recovery was hindered or improved (Bell et al., 2014, Bell et al., 2015, Bell et al., 2016, Clifford et al., 2016, Clifford et al., 2017, Jackman et al., 2019). Using a muscle function test may modulate if feelings of wellbeing and muscle soreness correlate. Understanding the physiological mechanisms as to why the human body responds in a certain manner post exercise may enhance how the body stores and utilises energy. Therefore, not assessing blood markers of muscle function and recovery could be considered another limitation, as changes in muscle damage markers directly post exercise, and in the days after exercise, might strengthen the concept that increased damage shown by an inflammatory response within the muscle post-exercise may lead to compensatory increases in EI. Future studies should examine the differences in post-exercise energy demands after intermittent exercise to explain why wellbeing and EI were higher within the current study. An increased energy demand 24 h post-trial, may simply be the functional response of the body to recover after an energy diminishing protocol. Nevertheless, if energy balance continues to increase over a long period of time, this might eventually lead to weight gain and health concerns.

One potential weakness with conducting any type of questionnaire-based research is the potential to introduce error or bias. Blinding participants from which exercise condition they were involved in was not impossible, and therefore the elevation in the recorded wellbeing result may have been manipulated by the fact that participants might have found the afternoon HIIE bout harder. Unfortunately, there is no documented evidence to support this statement. However, physiological HR results may show some interesting data to suggest otherwise, as participants did start the second exercise bout with a higher HR within the INT-AM trial (79 ± 9 vs. 66 ± 8 BPM: $P<0.001$). Although, the INT-PM bout had a significantly higher HR at the end

of the second exercise bout compared to INT-AM. Regardless of the fact that there was no difference in HR between trials after the first exercise bout, we know an increased HR can be a sign of increased strain or fatigue (Nelesen et al., 2008). This would suggest the modality and order of exercise is an important consideration when designing physical activity programmes which are intended for weight management strategies.

7.4.2. Conclusions

We found that a decrease in peripheral active ghrelin elicited a lower perception in hunger and an increase in circulating glucose immediately after completing HIIE in a fasted state. Despite this, no differences were observed during the 3 h monitoring period between exercise bouts, showing the timing of different exercise modes (continuous and intermittent) does not result in any compensatory effects in GER or overall substrate utilisation, regardless of the fact that active ghrelin significantly decreased during the second exercise bout post HIIE. Nevertheless, wellbeing and EI were greater 24-h post-trial after completing HIIE in the afternoon. Further work is required, to assess additional long-term responses after exercising multiple times throughout a normal working schedule, in an applied setting with a larger cohort. Additionally, it would be beneficial to correlate these subjective measurements of wellbeing with in vivo markers of muscle damage and inflammatory response. This novel approach of using blood and functional muscle assessments has been used consistently within literature which focuses on post exercise muscle recovery. Conversely, these techniques provide substantial potential to explore the possible preventive targets in the treatment of weight management, within a healthy, male, untrained population after exercise.

Chapter 8.0

GENERAL DISCUSSION

This chapter commences with a summary of the research findings (see Table 19), followed by the key overview of the main findings and their significance within the studies. Methodological considerations and limitations with respect to the recruitment of participants and the study designs are also discussed before introducing the recommendations for further investigation are suggested. Finally, an overall conclusion of this thesis is discussed. Additionally, a general public overview has been added to highlight the potential applications and implications for practice is acknowledged with a decision tree flow diagram to help guide exercise choices (see Figure 87).

The current work explored whether changing the mode, intensity or timing of exercise affected postprandial GI function, metabolic responses, appetite, and EI; by supporting the development of novel non-pharmacological interventions for weight management. This chapter will collate and consider the findings from the experimental chapters in this thesis.

A series of studies on human volunteers are presented in this thesis. The aim of this thesis is to determine whether there is a connection between GER and a consequence of, 1) adjustment in exercise intensity, 2) using an intermittent mode of exercise or 3) timing of exercise by conducting multiple exercise bouts.

Table 19: Summary of the Main Findings of the Effect of Exercise Intensity, Mode and Timing on Gastrointestinal Function, Metabolic Response and EI

Study	Participants	Design*	Measures	Main Outcomes
One (Mattin et al., 2018)	Male (n = 12); Age; 26 ± 5 yr; BMI; 25.5 ± 3.5 Kg/m^2; Body Fat; 18.9 ± 8.1 %; $\dot{V}O_{2peak}$ 42 ± 6 ml/Kg/min	60 min of CON EXE @ LOW (40% $\dot{V}O_{2Max}$), High (70% $\dot{V}O_{2Max}$) & non-exercise control. 242 KCal Semi-solid meal 30 min after EXE bout.	Pre-EI: Food record; HR & RPE: During EXE; GER: Pre-lunch; Post-lunch 15-min PP x 120min; AS VAS: Pre- & Post-EXE; Pre-lunch; 15-min PP x120mim; Metab & GH: Pre- & post-EXE; Pre-lunch; 30-min PP x 120min	GER DOB & AUC, T_{lag} (63 ± 13 min, 56 ± 10 min, 60 ± 16 min) and T_{half} (89 ± 13 min, 82 ± 8 min 94 ± 31 min) CON > LOW > HIGH, N.S; N.S. for AS VAS; Fullness-AUC; LOW > HIGH (3150 ± 2091 vs 2555 ± 1828 mm 225 min^{-1}; P = 0.023). Nausea-AUC; CON > HIGH (1939 ± 2359 vs 1106 ± 1575 mm 225 min^{-1}; P = 0.020); N.S. for Metab; NEFA-AUC; LOW > HIGH (68.5 ± 22.9 vs 43 ± 11.5 mmol.L 225 min^{-1}; P = 0.050); N.S. for GH; Ghrelin-AUC; LOW > HIGH (3150 ± 2091 vs 2555 ± 1828 pg.mL^{-1}. 225 min^{-1}; P = 0.006).
Two (Mattin et al., 2020)	Male (n= 12); Age; 30 ± 6 yr; BMI; 24.6 ± 2.0 Kg/m^2; Body Fat; 17.8 ± 3.9 %; $\dot{V}O_{2peak}$ 38 ± 6 ml/Kg/min	60 min of CON EXE & INT EXE [20x (1 min cycle / 2 min rest)] @ LOW (40% $\dot{V}O_{2Max}$) & MOD (60% $\dot{V}O_{2Max}$). 376 KCal Semi-solid meal 30 min after EXE bout.	Pre- & Post EI: Food record; HR & RPE: During EXE; GER: Pre-lunch; Post-lunch 15-min PP x 120min; AS VAS: Pre- & Post-EXE; Pre-lunch; Immediately post-lunch; 15-min PP x 120 mim; GLU: Pre- mid & Post-EXE; Pre-lunch; 30-min PP x 120min; SU; Pre-EXE; 30-min PP x 120 min	Post EI (3500 ± 1419 vs. 2777 ± 1042 kCal: $p < 0.001$) MOD-INT > MOD-CON. N.S. for LOW-INT > LOW-CON; GER DOB faster @ 30-120-min, $p = 0.050$; DOB-AUC higher, $p = 0.002$ MOD-CON > MOD-INT; T_{lag} (58 ± 15 min, 62 ± 15 min, 60 ± 17 min, 59 ± 13 min); T_{half} (110 ± 31 min, 106 ± 29 min 95 ± 20 min, 107 ± 13 min) LOW-INT, LOW-CON, MOD-INT and MOD-CON, N.S; AS VAS: PFC was lower $p = 0.011$ Post-EXE and nausea higher $p = 0.011$ for MOD-INT; Hunger-AUC (10011 ± 96 vs. 3103 ± 93 mm . 225 min: $p = 0.002$) MOD-INT > MOD-CON; N.S. for LOW-INT > LOW-CON; GLU higher Mid-EXE ≠ BL (4.9 ± 0.4 mmol.L, $p = 0.016$) MOD-INT; Post-lunch,MOD-INT > MOD-CON (7.5 ± 0.9 vs. 6.5 ± 0.9 mmol.L, $p < 0.001$) & LOW-CON > LOW-INT (7.6 ± 1.0 vs. 7.0 ± 1.0 mmol.L, $p<0.001$); N.S. SU, LOW-INT, LOW-CON, MOD-INT and MOD-CON

Three	Male (n = 14); Age; 27 ± 6 yr; BMI; 24.9 ± 2.0 Kg/m^2; Body Fat; 18.5 ± 4.1 %; $\dot{V}O_{2peak}$ 42 ± 9 ml/Kg/min	60 min of CON (SINGLE) & 2 x 30 min EXE (SPLIT) @ High (70% $\dot{V}O_{2Max}$). Post-EXE breakfast 30% ETEE; ~172 KCal semi-skimmed milk; TMC 500 mL Post-2EXE lunch 70% of ETEE; ~400 KCal Semi-solid meal 30 min after EXE bout; TMC 1000 mL	Pre- & Post EI: Food record; HR & RPE: During EXE and EXE-rest; GER: Pre-lunch; Post-lunch 15-min PP x 120min; AS VAS: Pre-EXE & Pre-BK; 30-min PP x 120 mim; Pre-Lunch; 30-min PP x 120 min; Metab & GH: Pre-EXE & Pre-BK; 60-min PP x 120 min; Pre-lunch; 60-min PP x 120min; SU; Pre-EXE; 15-min mid-EXE x 60 min; Post-BK, 30-min PP x 120 min; Second EXE bout, 15-min mid-EXE x 30 min; Post-lunch, 30-min PP x 120 min; I-FABP$_2$: Pre-EXE, Post-EXE & Post-2EXE	Post EI (3240 ± 1171 vs. 2556 ± 1049 kCal: p = 0.007) SPLIT > SINGLE; GER DOB faster @ 45-60-min, p <0.001 SPLIT > SINGLE; T_{lag} (73 ± 15 min vs 89 ± 24 min, p = 0.007); T_{half} (118 ± 29 min vs 149 ± 49 min, p = 0.027) SPLIT > SINGLE; AS VAS: Pre-BK hunger increase (70 ± 14 vs. 53 ± 22 mm: p = 0.025) SPLIT > SINGLE. Remaining AS VAS & AUC N.S; Metab: GLU & CHOL, p < 0.005 increased @ 405 min SINGLE > SPLIT; NEFA & TRIG, p < 0.005 increased @ 405 min SPLIT > SINGLE; Higher GLU & NEFA-AUC, p < 0.005 SINGLE > SPLIT; GH: Ghrelin decreased Pre-BK (138 ± 49 vs. 166 ± 67 pg.mL, p = 0.024) SINGLE > SPLIT; Increased @ 405 min (223 ± 82 vs. 151 ± 87 pg.mL, p =0.017) SPLIT > SINGLE. Insulin increased @ 405 min (1056 ± 514 vs. 547± 356 pg.mL: p =0.011) SINGLE > SPLIT; SU, FAT-AUC (51 ± 18 vs 42 ± 12 g/min^{-1} 405 min^{-1}, P =0.036) SINGLE > SPLIT; N.S. I-FABP$_2$, SINGLE = SPLIT.
Four	Male (n = 12); Age; 26 ± 6 yr; BMI; 24.6 ± 4.0 Kg/m^2; Body Fat; 20.4 ± 6.7 %; $\dot{V}O_{2peak}$ 48 ± 10 ml/Kg/min	30 min of CON EXE @ MOD (50% $\dot{V}O_{2Max}$) & INT EXE [10x (1 min cycle / 2 min rest)] @ Very High (100% $\dot{V}O_{2Max}$) PPO.	Pre- & Post EI: Food record; HR & RPE: During EXE; GER: Pre-lunch; Post-lunch 15-min PP x 120min; AS VAS, Metab & GH: Pre- & Post EXE;	Post EI (3511 ± 879 vs 2829 ± 614 Kcal: P =0.007) INT-PM > INT-AM; HR: Pre-2EXE (79 ± 9 vs. 66 ± 8 BPM: P <0.001) INT-AM > INT-PM; GER-DOB & AUC, T_{lag} (62 ± 11 min vs 57 ± 8 min) & T_{half} (151 ± 111 min vs 99 ± 24 min) INT-AM > INT-PM , N.S; AS VAS; hunger increased Post-EXE (72 ± 20 vs. 39 ± 22 mm: p = 0.002) & Pre-BK (79 ± 25 vs. 51 ± 26 mm: p = 0.016) INT-PM > INT-AM; Nausea increased Post-EXE (19 ± 16 vs. 6 ± 6 mm: p = 0.003) INT-AM > INT-PM;

Four (*Continued*)	Post-EXE breakfast 434 KCal mixed solid and liquid food. Post-2EXE lunch, 376 KCal Semi-solid meal 30 min after EXE bout.	Pre-BK; 60-min PP x 180 mim; Post-2EXE; Pre-Lunch; 60-min PP x 120 min; SU: Pre- & Post-EXE; 60-min PP x 180 min;Post-2EXE; 60-min PP x 120min; WB; Pre- & Post-EXE; Pre- & Post-2EXE; End-TR; Post-24H	Metab: GLU increased Post-EXE (5.07 ± 0.43 vs. 4.70 ± 0.25 mmol.L: $p = 0.017$), 1h Post-lunch (6.27 ± 0.91 vs. 5.22 ± 0.69 mmol.L: $p = 0.001$) & End-TR (5.09 ± 0.5 vs. 4.51 ± 0.36 mmol.L: $p = 0.004$) INT-AM > INT-PM; Higher NEFA-AUC, p = 0.022 INT-PM > INT-AM; Ghrelin increased Pre-BK (435 ± 236 vs. 267 ± 211 pg.mL: $p = 0.001$) & decreased Post-2EXE (267 ± 167 vs 585 ± 419 pg.mL: $p = 0.003$) & Pre-lunch (361 ± 223 vs 519 ± 357 pg.mL: $p = 0.022$) INT-PM > INT-AM; Remaining GH & AUC N.S; SU, Fat increased Post-EXE (0.119 ± 0.048 vs 0.040 ± 0.037 g/min: $P < 0.001$) INT-AM > INT-PM & Post-2EXE (0.119 ± 0.048 vs 0.073 ± 0.042 g/min: $P = 0.023$) INT-PM > INT-AM; N.S. Overall Fat & Carb AUC; WB: Increased Post-EXE (14 ± 2 vs. 12 ± 2 total: $P = 0.018$) INT-AM > INT-PM & Post-2EXE (14 ± 2 vs. 12 ± 3 total: $P = 0.013$) and Post-24H (13 ± 3 vs. 11 ± 2 total: $P = 0.043$) INT-PM > INT-AM.

* All were randomised, repeated-measure counter-balanced designs. Means ± SD. CON, continuous; EI, energy intake; HR, heart rate; RPE, rate of perceived exertion; EXE, exercise; GER, gastric emptying rate; min-PP, minutes postprandial; AS, appetite score; VAS, visual analogue scale; Metab, (glucose, triglycerides, cholesterol, and NEFA); GH, (ghrelin, insulin, GLP-1, PYY, and PP); N.S, non-significant; INT, intermittent; MOD, moderate; GLU, blood glucose; Mid-EXE, during exercise SU, substrate utilisation; ETEE, estimated trial energy expenditure; TMC, total meal consumed; Pre-BK, pre-breakfast; ≠, compared; PPO, peak power output; BL, baseline; Post-2EXE, post second exercise bout; End-TR, final sample; Carb, carbohydrate; WB, wellbeing;

8.1. Overview of Key Findings

The effect of exercise on GER in response to a subsequent meal post-exercise is an important consideration in the pathophysiology of obesity and metabolic syndrome. GER is considered an extremely critical step in the food digestion process as the emptying rate of chyme into the small intestines, determines the amount of nutrients that are absorbed (Liu et al., 2021).

The results from **Chapter 4**, report no difference in GER between a low (40% $\dot{V}O_{2peak}$), or high (70% $\dot{V}O_{2peak}$) intensity continuous exercise bout when a standardised semi-solid meal was consumed 30 min after completing an exercise bout ($T_{1/2}$ = low; 82 ± 8 min, and high; 94 ± 31 min). A previous investigation compared post-exercise GER using a 5% glucose solution, revealed exercise intensity does not affect GER- $T_{1/2}$ = low; 22 ± 9 min, and high; 22 ± 7 min (Evans et al., 2016). It is important to consider the time difference indicated by $T_{1/2}$ which demonstrates how food composition has a central and prolific effect on GER. Further investigations found a carbohydrate solution emptied faster than a protein solution (Evans et al., 2018a). Glucose empties faster than protein, although, both glucose and protein empty faster than lipids (Mackie et al., 2013, Lundin et al., 2008). The presence of different macronutrients within the digestive system stimulate the secretion of a unique mixture of satiety peptides released from enteroendocrine cells in the wall of the gut, which signal through the vagus nerve to stimulate the brain (Woods, 2004). Therefore, the characteristics of food or drink consumed after exercise is potentially more important for the delivery of nutrients to the small intestines than exercise intensity, when performed in a continuous manner. Literature has confirmed characteristics of food such as food volume, calories, and viscosity all affect digestibility and therefore GER (Mazzawi et al., 2019, Mackie et al., 2013, Zhu et al., 2013, Kwiatek et al., 2009), although minimal research has focused on different food characteristics after exercise. Within this thesis, conclusions cannot be made on individual macronutrient responses, as a mixed macronutrient standardised semi-solid meal was consumed. Supplementary work is required to explore the possible links between food characteristics and GER.

The experimental procedure in **Chapter 5** indicates GER was maintained at a similar rate between continuous and intermittent exercise performed at 40 and 60%

$\dot{V}O_{2peak}$. This discovery is fundamentally important as GER is already known to be impaired during continuous exercise >75 $\dot{V}O_{2max}$ (Neufer et al., 1989) and during intermittent sprint exercise (Leiper et al., 2001a). **Chapter 5** shows GER does not seem to be affected when a semi-solid meal is consumed 30 min after exercising in a continuous or intermittent manner. Mechanistically during exercise GER is regulated by the CNS and splanchnic blood flow. Therefore, a 30 min break after exercise is sufficient for the GI system to recover after a low to high continuous exercise bout.

The results from **Chapter 6** add novel insight into gastric emptying research, since exercising twice-a-day accelerated GER compared to a single continuous exercise bout matched at a high intensity (70% $\dot{V}O_{2peak}$). The mechanism behind the increase in GER after exercising twice-a-day cannot be fully determined from the result within this thesis. Although, the fact GER responded at a similar rate after mixing mode of exercise when exercising twice-a-day (**Chapter 7**). This Suggests, the stomach responded to a change in energy demand from exercise or an interactive sensing mechanism relating to energy nutrient sensors in the GI tract, which has a role in transport and absorption in the recovery period after exercise. It must be considered, liquid food empties significantly faster than solid food (Goyal et al., 2019); within all four studies of this thesis a soup meal containing a high liquid content was provided. The lunch meal within **Chapter 4**, was considered to be a relatively small in volume and energy content and might have been one of the potential reasons why GER was unaffected between the two exercise conditions. Within **Chapters 5 & 7**, the meal volume and energy content were doubled. Whereas, in **Chapter 6** the meal was standardised relevant to the participants estimated energy requirements. The meals were matched for total volume, suggesting the viscosity of the meals might have been vastly different. In defence, Zhu et al (2013), found increasing food viscosity of a semi-solid meal at equal-calories were shown to prolong satiety and slow GER. The difference in GER highlighted in **Chapter 6,** after twice-a-day exercise may be relevant to meal viscosity, or the fact participants exercised in a non-fasted state consuming a milk-drink 2 h prior to starting the second exercise bout.

In all investigations within this thesis food diaries were recorded to ensure standardisation of food 24 h before each trial. The fasting period before each trial were consistent across all subjects. In turn, GER has been reported to empty at a

similar rate between fasted and fed exercise in the morning (McIver et al., 2018, McIver et al., 2019), although in the afternoon GER has been reported to be typically slower (Grammaticos et al., 2015, Goo et al., 1987). Emerging evidence suggests the circadian clocks regulate the digestive process, causing a delay in GER in the evening (Hoogerwerf, 2010). The mechanisms why faster GER was reported within **Chapter 6,** warrants further investigation as slower GER may be favourable for reducing food intake and prolonging satiety (Zhu et al., 2013, Jones et al., 1997). It is not conclusive from the data within this thesis, if having a faster or a slower GER when consuming a meal after exercise will affect metabolic responses in the postprandial period.

Gastric emptying is understood to be regulated by a complex interaction of neuronal and hormonal input through the CNS (Horner et al., 2015). The results from **Chapter 4,** reported continuous exercise at a low intensity (40% $\dot{V}O_{2peak}$) caused an increase in circulating ghrelin prior to consuming an energy dens meal. Overall AUC was higher during low intensity when compared to high intensity (70% $\dot{V}O_{2peak}$) continuous exercise bouts. This increase in ghrelin before consuming a meal was also shown in **Chapter 7**, after moderate continuous exercise (50% $\dot{V}O_{2peak}$). Alternatively, **high** intensity continuous exercise (70% $\dot{V}O_{2peak}$) decreased ghrelin immediately after exercise in **Chapter 6**. A decrease was also reported during **Chapter 7**, after a very high intense intermittent exercise bout (100% $\dot{V}O_{2peak}$). Ghrelin fluctuations after exercise diminish after consuming a nutrient dens meal, as ghrelin decreased below fasted values in all Chapters 4-7, this response in not unusual (Mani et al., 2019).

Over a 2 h recovery phase ghrelin concentrations begin to slowly rise, although this increase was not shown to be significant, apart from when multiple exercise bouts were performed during **Chapter 6**, as ghrelin values were elevated compared to a one-off exercise bout 2 h after food. It can be concluded that exercise intensity does stimulate ghrelin differently when exercising in a fasted state, as very high intensity intermittent exercise seems to follow a similar pattern to high intensity continuous exercise, as ghrelin decreases post-exercise regardless of the mode of exercise. The results from this thesis do correspond with previous literature suggesting elevated ghrelin correlates with accelerated GER, this finding was only discovered in **Chapter 6**, therefore this information should be used cautiously.

As circulating levels of ghrelin increase priming meal initiation, GLP-1 signalling is usually shown to be lower (Decarie-Spain and Kanoski, 2021). Although, there were no further difference in appetite hormones (GLP-1, PP and PYY) across the experimental chapters within this thesis, except insulin concentrations were higher 120 min after consuming a standardised semi-solid meal after the SINGLE exercise trial reported in **Chapter 6**. Elevated insulin levels have been shown to regulate appetite by mobilising metabolism (Qaid and Abdelrahman, 2016), by secreting insulin following a meal in response to increased glucose within the bloodstream (Röder et al., 2016b). The increased insulin concentration within the postprandial period could be in response to the sedentary nature of the SINGLE trial. This can be explained by an increased sensitivity of muscle glucose metabolism to insulin during and post-exercise (Richter et al., 1985). The benefit of exercise on insulin sensitivity may have been exposed within **Chapter 6,** since insulin values were lower across the full 120 min recovery period, consuming a meal 30 min after completing a continuous exercise bout at 70% $\dot{V}O_{2peak}$. Insulin concentrations were only reported to be different within one chapter of this thesis. Further work is required to understand if completing multiple exercise bouts within the same day are beneficial to control insulin sensitivity, after consuming a nutrient dens meal post exercise. Nevertheless, changing the mode, intensity and timing of exercise wasn't significant enough to provoke any further hormonal responses, regardless of the fact there were metabolic modifications.

The studies presented in this thesis measured several different metabolites (glucose, triglyceride, cholesterol and NEFA). Although, no further key differences in triglycerides nor cholesterol were discovered across the experimental chapters. Glucose concentration changed depending on the exercise intensity to a greater extent than mode during and post-exercise. Performing intermittent exercise >60% $\dot{V}O_{2peak}$ appears to increase blood glucose during exercise (**Chapter 5)**, or immediately after an exercise bout (**Chapter 7)**. Although, this increase is not replicated within the hours after consuming calorific nutrients post intermittent exercise. Glucose causes a rapid and profound suppression of ghrelin in humans and rodents when administered by either parental or oral routes (Briatore et al., 2003, McCowen et al., 2002, Nakagawa et al., 2002, Shiiya et al., 2002). As briefly mentioned earlier, ghrelin concentrations were decreased immediately after exercise within **Chapters 4, 6 & 7** at an intensity

>70% $\dot{V}O_{2peak}$, yet an increase in glucose was only revealed within **Chapter 7**. It is likely that glucose only impacts ghrelin release once it has made its way into circulation (Mani et al., 2019), as inserting a pyloric cuff within rat models failed to suppress plasma ghrelin after intragastric administration (Williams et al., 2003). It is likely that a direct effect of glucose on ghrelin cells figures extensively in the inhibitory effect of glucose on ghrelin secretion. Sakata et al, (2012) proposes ghrelin cells express machinery associated with glucose sensing and metabolism such as glucose transporters and ATP-sensitive potassium (K_{ATP}) channels. Some research believes glucose must enter and be metabolised by ghrelin cells before ghrelin secretion is suppressed (Mani et al., 2019, Mani et al., 2017, Sakata et al., 2012, Chuang et al., 2011). The exact metabolic pathways modulating ghrelin release, are unclear but align with macronutrient ingestion, in particular glucose intake.

To further support this point glucose increased in all four investigations within this thesis (**Chapters 4-7**) 30 min after consuming a mixed macronutrient meal. Although, conflicting evidence revealed continuous exercise causes a larger spike in blood glucose, compared to intermittent exercise (**Chapter 5**), causing blood glucose to remain elevated during a 2 h recovery period within **Chapter 6 & 7**. The metabolic response after consuming energy dense material post-exercise is strongly related to GI hormone responses (insulin, glucagon, GLP-1 and PYY) (Mani et al., 2019), the data within this thesis is not conclusive and further clarity is needed to strengthen this theory.

It is important to consider the changes in NEFA concentrations reported in **Chapters 6 & 7**. NEFA were higher after continuous exercise in a fasted state and overall NEFA AUC was higher after continuous exercise at 70% $\dot{V}O_{2peak}$ (**Chapter 4)**, indicating greater fat mobilisation for metabolism, consistent with the knowledge that fat oxidation is increased after fasting (Achten and Jeukendrup, 2004). This increase in NEFA was only seen after continuous exercise and not after intermittent. Mulla et al, (2000) reports muscle lipid utilisation increases during exercise, but the lipid oxidation rate is maximised at 65% $\dot{V}O_{2Max}$ making this metabolic pathway workload sensitive. The results from **Chapter 6**, report that NEFA increased substantially more after exercising for 60 min compared to exercising for 30 min at the same intensity (70% $\dot{V}O_{2peak}$). Although, during endurance exercise the

contribution of lipid to the total oxidative metabolism increases with time duration of exercise (Paul, 1975). The increase in NEFA suggests continuous exercise might rely on different metabolic pathways during continuous exercise than intermittent exercise, especially when male participants exercise in a fasted state. This is further supported as walking in a fasted state for 45 min caused a significantly large increase in NEFA than when participants consumed breakfast (McIver et al., 2018). Nevertheless, postprandial NEFA concentrations were similar within all chapters (4-7), after consuming a calorific nutrient meal showing a decrease in NEFA mobilisation regardless of intensity, mode, or timing of exercise after food. This data aligns with previous literature as NEFA concentrations are regularly shown to decline after the ingestion of food containing carbohydrates, due to its stimulatory effect on insulin release (Martins et al., 2007a, Clayton et al., 2016, Yau et al., 2017a).

Minimal investigations have measured lipid oxidation rates in prolonged periods post exercise or after consuming food (Mulla et al., 2000). Within this thesis substrate utilisation was measured. Interestingly, substrate utilisation shifts to predominantly fat oxidation after continuous and intermittent exercise at various intensities between 40- 60% $\dot{V}O_{2peak}$ (**Chapter 5**), and after intermittent exercise at PPO (100% $\dot{V}O_{2peak}$) **Chapter 7**. Therefore, fat oxidation probably remains elevated ~30-60 min after consuming food, in response to a lag phase in delivering digested chyme to the small intestines and absorbing nutrients into circulation (Horner et al., 2015). Further reinforcing changes in GER may affect metabolic processes and appetite control. Fasting prior to exercise has regularly been shown to reduce carbohydrate utilisation during exercise (McIver et al., 2018, McIver et al., 2019). In **Chapter 6**, fat oxidation were higher in both trials compared to baseline, although immediately after exercising for 60 min, caused a larger increase in fat oxidation compared to exercising for 30 min at the same intensity (70% $\dot{V}O_{2peak}$). Overall fat oxidation-AUC were higher within the SINGLE one-off exercise bout, possibly related to the increased time exercising within a fasted state. While increased fat oxidation post-exercise within this thesis suggests an increase in lipolysis after continuous and intermittent exercise, this increase is unfortunately short lived after nutrient dense foods are consumed, yet within **Chapter 6** substrate utilisation does begin to shift towards prioritising fat oxidation 2h after consuming food. Further

research is needed to understand the relationship between consuming food after exercise and the changes in substrate utilisation leading to overall changes in lipolysis.

The evidence within this thesis suggests appetite is affected by intensity and the mode of exercise as variations in hunger-AUC were significantly suppressed after exercising intermittently but not after exercising continually despite the fact, exercise bouts were matched for power output (60% $\dot{V}O_{2peak}$) (**Chapter 5**). The results reported in **Chapter 7** further support the notion, mode of exercise effects appetite differently as exercising intermittently for 30 min at PPO suppressed hunger. Although exercising continually for 30 min at 50% $\dot{V}O_{2peak}$ increased the perception of hunger. This potentially suggests intermittent modes of exercise might acutely suppress appetite to a greater extent than continuous exercise. While another point to consider is the differences in hunger within **Chapter 6**, as 60 min of continuous exercise at 70% $\dot{V}O_{2peak}$ suppresses appetite, while 30 min at the same intensity resulted in an increase in hunger. This may suggest duration of exercise might also be an important consideration when designing exercise programs. Nevertheless, a subsequent meal following both intermittent and continuous exercise seems to abolish any compensatory effects in subjective feelings of hunger within the first 2 h post-meal, as a small breakfast drink ~ 334 ml of semi-skimmed milk were sufficient to counterbalance the exercise effects on appetite (**Chapter 6**). Showing subjective appetite can be offset by relatively small nutrient dense material after exercise, independent of the changes observed after exercise in a fasted state. It must be acknowledged that all suppressions in appetite within this thesis were all above the >60% $\dot{V}O_{2max}$ threshold that had already been previously shown within the literature (Martins et al., 2007a, Becker et al., 2012, King et al., 2013b).

What we do know is that the feeling of appetite is a good indicator of a person's metabolic state but a poor marker of EI (Stubbs et al., 2000). For this reason, EI were assessed using a 24 h weighed food intake log which revealed moderate intensity intermittent exercise (**Chapter 5**) and multiple bouts of continuous exercise (**Chapter 6**), caused a transient increase in EI within the first 24h. These two investigations were not consistent with the literature, as the majority of weight management research focusing on appetite have not found changes in EI in the hours and days after exercise (McIver et al., 2020, McIver et al., 2019, King et al., 2013a, Hanlon et al., 2012, King

et al., 2010a, Pomerleau et al., 2004, King et al., 1997). Therefore, a wellbeing assessment was added to **Chapter 7**, to assess one potential mechanism why EI has shown to be increased within previous chapters of this thesis. It has been suggested the recovery process after energy depleting exercise bouts might affect EI and appetite response with wellbeing and EI being reported to be increased within **Chapter 7**. The key findings that have been discussed within this section can be found in Figure 86, an illustrated key findings diagram.

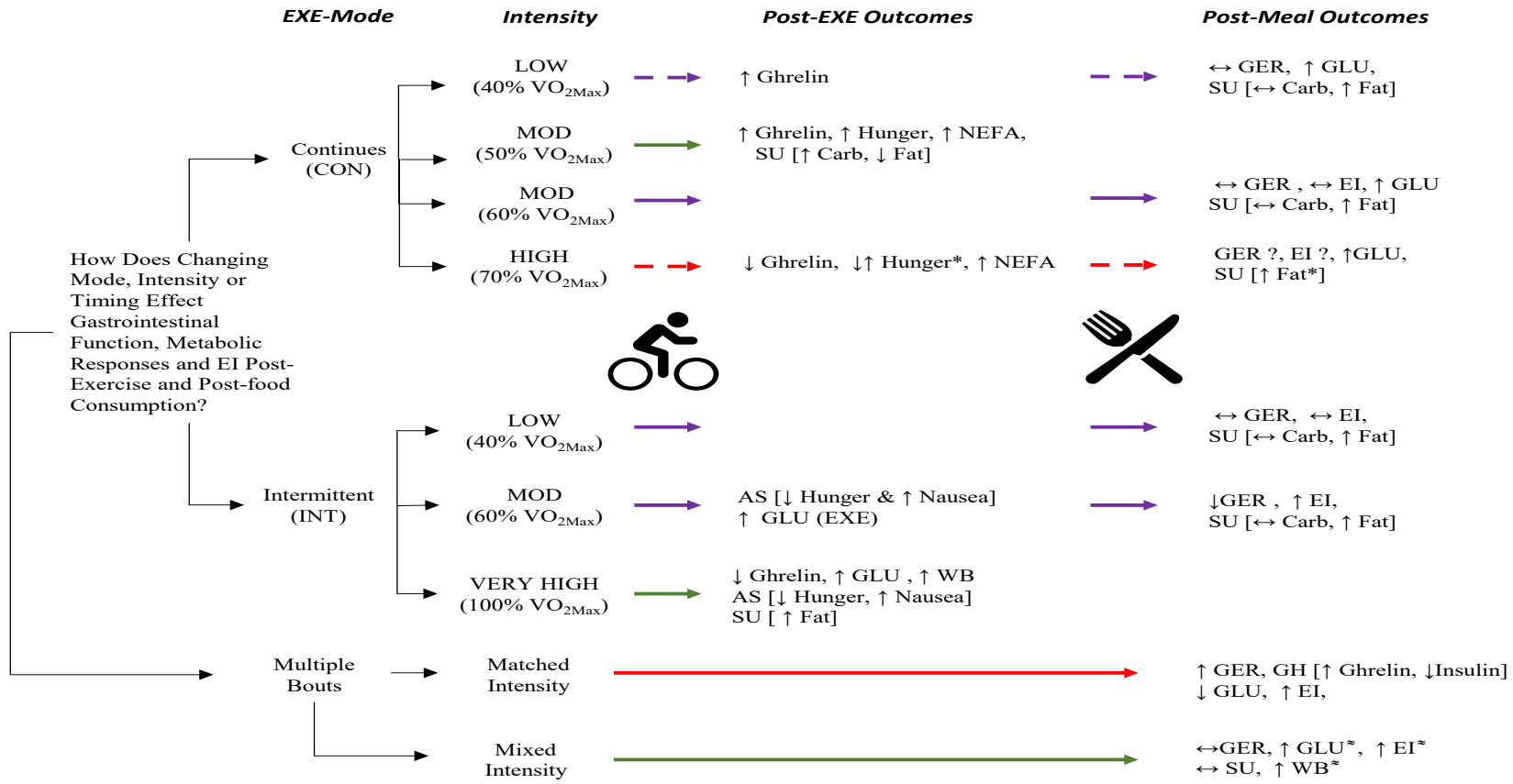

Figure 86: Flow diagram illustrating the key finding of this thesis. The influences of exercising on a bike at different modes, intensity or timing on Post-EXE and Post-meal responses to: GER, AS, GH, Metab, SU, and EI. Dashed lines represent, data from multiple study's; Dashed purple & red lines findings from Chapter 4; Purple arrows findings from Chapter 5. Red arrows, findings from Chapter 6. Green arrows, findings from Chapter 7. *Effect of exercise duration, 30 min at 70% VO2Max increased hunger and 60 min decreased hunger and increased fat oxidation.≈ Int undertaken in the second EXE bout . EXE, exercise; Metab, metabolites; GLU, glucose; SU, substrate utilisation; AS, appetite score; WB, wellbeing score. ↑, increase or faster; ↓, decrease or slower; ↔ no change or similar result; ?, Inconclusive.

8.2. Limitations

Each study included in this thesis discusses individual limitations to the clarification of the data collection in turn. However, it is relevant to discuss some general limitations to the body of work as a whole. The financial and equipment constraints to data collection must be discussed. The deviations in the number of analyte to analyte measured are because of limitations in the lack of access to equipment over the testing period and the ability to collect blood samples at single time points, in some cases whole trials. The capacity of the ELISA kits used to run every sample in duplicate were limited, although any metabolite or hormone disputes have been acknowledged within each research chapter. It is important to add the measurement of triglyceride used during this thesis did not correct for free glycerol. In all four studies presented within this thesis, participants performed an exercise bout with the expression of the control trial within **Chapter 4**. For this reason, an increased level of free glycerol may have been present in response to whole body lipolysis. Using the assumption that all glycerol released in the process of lipolysis, whether in adipose tissue or skeletal muscle, appears in plasma, and that glycerol cannot be produced in the body other than from lipolysis (van Hall et al., 2002). This might suggest the triglyceride results presented might not be accurate and in future investigations correcting for free glycerol level would make the triglyceride result more reliable. It is important to add, there were no significant differences observed for triglyceride values within this thesis and therefore, no major outcomes have been placed of these results.

The omission of gut hormones from **Chapter 5** were for participant safety, due to the high intensity intermittent nature from some of the trials. Equipment was standardised across all research chapters within this thesis apart from changing the cycle ergometer used within **Chapter 4-5**. The new cycle ergometer used in **Chapters 6-7** allowed a direct connection to a Cortex metalyzer permitting continuous monitoring of cycling cadence, HR and metabolic output. Additionally, breath-by-breath data collection was not measured during **Chapter 4**, because there was a lack of access to equipment over the testing periods as testing took place over a 6- 8month period, simultaneously while other investigations were conducted within the same time period.

This thesis was standardised to a single gender design using male participants only. Conducting research involving female participants is a very important consideration and the decision to solely recruit males was not made lightly. Although, there is compelling rationale for focusing on a single gender when investigating appetite regulatory effects. Fundamentally Camilleri et al (2012), found differences in GER and Monrroy et al, (2019) established female participants had different sensory experiences after consuming a meal which may influence subsequent nutrient ingestion. In addition, studies have reported a change in appetite response following ingestion of Mediterranean diets (Bédard et al., 2015) and differences in brain response to hunger and subsequent food ingestion between genders (Del Parigi et al., 2002b, Del Parigi et al., 2002a). Given that the subjective feelings after food ingestion was a key objective such gender differences may have influenced the results obtained. Similarly, additional studies have demonstrated a difference in appetite hormones (PYY and GLP-1) after exercise (Hazell et al., 2017b) and Atkinson et al, (2020) revealed differences in acute metabolic response to food ingestion after different fasting periods between males and females. Therefore, the current literature suggests gender differences in gut hormone response to exercise as well as post-food ingestion processes could influence metabolic response and the desire to eat. It is important that these differences are investigated further to establish how important they are for weight management and appetite control.

Equally, exploring whether different populations such as age, body composition (adipose tissue) and training states may be external factors which determine how the GI system responds to food ingestion post-exercise. Within the current thesis the age range was controlled between 18-40 years, as the ageing process has been shown to drastically effect the function of the digestive system (Nigam and Knight, 2017). Particularly GI hormones, with the secretion of ghrelin decreasing (Di Francesco et al., 2008) and PYY levels increasing (Hickson et al., 2016) supporting the control in age range. Nevertheless, other age-related changes in food consumption such as loss of taste and smell and targeted feeding (Doty and Kamath, 2014, Wright et al., 2008) was not measured. These sensory responses can also crossover into young, healthy individuals. Understanding the sensory elements of consuming food may further expand how appetite regulation fluctuates between individuals.

Participants within the current thesis were controlled by BMI not adiposity values. Using a more reliable measurement of body fat such as a DEXA scan is possibly a stronger determinant if someone is lean, overweight or obese, rather than using BMI and body fat determined from a bioimpedance analyser. This potential limitation in this thesis may be an important consideration. The amount of body fat the human body retains has been suggested to affect the rate of which food is emptied from the stomach (Lavigne et al., 1978), it has also been suggested being overweight/obese may result in different sized osmolar receptors in comparison to lean individuals (Wright et al., 1983). Nevertheless, the literature is divided whether GER is effected by body fat, as some investigations have found a rapid emptying rate (Wright et al., 1983, Zahorska-Markiewicz et al., 1986, Tosetti et al., 1996) and others slower (Di Ciaula et al., 2012, Buchholz et al., 2013). The main consideration is the fact previous studies have used different meals and food components to measure GER, which could account for the inconsistencies in the results.

Additionally, it is not possible to establish whether the participants involved in the thesis were pre-disposed to certain exercise training responses which may be described as a potential limitation. All participants were asked to control their exercise on the approach to taking part and were entrusted to standardise exercise 48h prior to each experimental trial. Although, this could be easily monitored and controlled by asking participants to wear an accelerometer (Ward et al., 2005). Furthermore, each study within this thesis included an aerobic exercise phase and **Chapter 5 & 7** a moderate to high intermittent exercise element. Skeletal muscle adapts to exercise-induced muscle damage in such ways that it protects the working muscle from subsequent damaging stimuli, through several mechanisms such as neural adaption, extracellular matrix remodelling and biochemical signalling (Hyldahl et al., 2017). Therefore, whether the participants were subjected to the repeated bout effect (RBE) must be considered, regardless to the fact each trial was separated by a minimum of seven days and were an acute exercise bout (Sugimoto et al., 2020). To continue this point, wellbeing was assessed using a VAS questionnaire, this method is an indirect method of muscle soreness and recovery. Using more robust techniques such as muscle function tests to measure inflammation markers within the blood may further

support the qualitative data found within this thesis with additional quantitative physiological data.

Gastric emptying was assessed in this thesis using the ^{13}C breath test, which is an indirect, non-invasive technique to assess GER, which has been strongly validated with gastric aspiration technique (Ghoos et al., 1993, Braden et al., 1995). One of the benefits of using the ^{13}C breath test over the gastric aspiration is the ability to measure solid foods (Maes et al., 1994), regrettably only semi-solid liquid meals were measured during all four investigations within this thesis. Future studies should expand the research on GER after exercise by using solid foods, as emptying rates are usually significantly delayed compared to liquids meals (Hellström et al., 2006). Although, solid food may remain a more realistic meal category post-exercise for the general-public. $C0_2$ production was estimated using the participants height and weight. It is important to consider that this estimated value could be seen as a potential weakness in the analysis of the GER measurement as baseline expired air data was available and exact $C0_2$ values could have been used. In future investigations using the ^{13}C technique to measure GER, exact measurements will be used.

Moreover, the research within this thesis was collected within a controlled laboratory setup, additional investigations need to comprehend how appetite and EI may be controlled by a number of different factors within the external environment. This may influence signals from the GI tract to the vagus nerve, which in turn stimulates the nucleus tractus solitarius in the brain stem, signalling to the arcuate nucleus in the hypothalamus which mediates metabolic requirements (Näslund and Hellström, 2007). It has been well documented that longer observation periods are needed in the hours and day after exercise (Blundell et al., 2003, Blundell and King, 2000). For this reason, it is vitally important applied research is conducted which acknowledges external stimuli that possibly contributes to the regulation of EI.

A final methodological limitation is the studies described in **Chapters 6 & 7** did not involve a non-exercise control trial which, therefore, could be consisted of a limitation. A true determination of power within any data set should consider the information provided by a placebo or control (Jakobsen et al., 2017, Wetterslev et al., 2017), therefore allowing the true effect-size to be defined. This is usually more important when calculating data used within a meta-analysis. However, by using a

repeated measures design within **Chapter 6 &7** meant participants acted as their own control.

8.3. Direction for Future research

In this thesis, a more comprehensive understanding has been established regarding how the GI system is affected when exercising on a bike is manipulated for intensity, mode, and timing. The research that has been conducted during this thesis has emphasised that although changes are present, they vary across different levels of the system or systems and highlight why it is not possible to predict whether a faster or a slower GER is more beneficial for improving health and wellbeing after physical activity. While the aims of this thesis have been met, the continual expansion of knowledge in regard to the physiological response to the GI system after exercise have provoked more questions that warrant investigation. Future understanding of how the fundamental process of GER might be key for the prevention of metabolic syndrome are now apparent, recommendations for future work are:

a) Whether body composition (adiposity values) determine the volume or amount GI peptide hormones are secreted or the response of the designated receptor developing resistance as seen within obese populations in regard to insulin (Kashyap and Defronzo, 2007), might be fundamental to controlling weight management. Future investigations should focus on clinically obese populations and how metabolic responses can be disrupted by the neural drive from the vagus nerve to the appetite regulatory regions of the brain.

b) Further investigations should be driven by a specific hypothesis which include female and male participates in parallel arms, in order for comparisons to be made between genders and within group gender-to-gender comparisons.

c) Implementing a calculated calorie replacement method during meal periods reflected individual absolute trial energy loss more accurately. Proposing a more controlled method to ensure participants completed each trial in a state of energy balance. Although, this method could also help to better understand

if and how GER is affected by macronutrient content of meals consumed after exercise. Theories have suggested GER is determined by meal volume and calorie content (Moran and Dailey, 2011). However, if these factors are standardised and the macronutrient content (carbohydrate, protein, and fat) are altered stimulating the secretion of different peptides from the enteroendocrine cells in the wall of the gut, this could help grasp why GER and therefore intestinal absorption might be different when one macronutrient is more dominant within a feeding-period. Identifying, if the stomach can adapt to different nutrients may promote the idea that the stomach is influenced and trainable.

d) One area of interest is the relationship between glucose fluctuation and the period after exercise using continuous glucose monitoring (CGM). This technology is a novel and effective method to improve glycaemic control within clinical populations (Mariani et al., 2017). Ishihara et al, (2020) has used CGM during ultramarathon races. Combining these techniques will help to link whether changes in blood glucose levels in the hours and days after exercise might begin the shift in appetite and EI post-exercise.

e) It was not possible from the results within this thesis to determine if the GI system adapts to prolonged changes in exercise. Horner et al, (2015) advises the relationship of GER after long-term and acute exercise remains largely unexplored. Therefore, longitudinal training investigations are needed to comprehend whether the stomach adapts to exercise if performed regularly, and whether this leads to changes in the GI tract is possibly vital to determine if GER is a catalyst to metabolic changes and recovery.

Further avenues which would be interesting to explore, although not directly related to the study of GER, would be to investigate if muscle recovery post exercise is directly responsible for the increase in EI in the hours after exercise. Evidence in epidemiological literature suggest that increasing polyphenols within the diet have beneficial effects on health improvements such as, heart disease (Tung et al., 2020), coronary artery disease (Huxley and Neil, 2003) and increased cholesterol metabolism (Chambers et al., 2019). Nevertheless, limited research has focused on whether

consuming a polyphenol supplement post-exercise can minimise muscle inflammation leading to a suppression in appetite and therefore, EI by blunting the inflammation response.

8.4 Conclusions

The experimental studies discussed within this thesis have expanded the understanding and knowledge whether manipulating mode, intensity, and timing of a bout or bouts of cycling exercise would affect GER, gut hormones, metabolic responses, appetite, substrate utilisation, and EI following exercise and a subsequent meal. Disassembling an exercise bout has presented viable and effective strategies to maintain and improve health benefits in the light of increasing incidence of obesity, cardiovascular diseases, and metabolic syndrome. The evidence presented in this thesis suggests an untrained "normal weight" male population responded somewhat differently to the stimulus of exercise when the mode, intensity, or timing is changed. Although unfortunately, not all situations resulted in positive health outcomes. The mechanisms of these physiological adaptations are closely characterised by deviations in the physical and environmental setting, which are most likely the result of external influences on different metabolic and endocrinal pathways within the recovery process. This thesis adds novel insights into the manner in which manipulating an exercise bout can disrupt GER effecting postprandial metabolic and hormonal responses, while further enquiries are needed to comprehend the role gastric emptying performs in the regulation of appetite and EI. The main conclusions that can be drawn from this work are:

1) Multiple bouts of continuous exercise initiate the volume of a semi-solid meal to empty from the stomach faster when compared to a one-off exercise bout matched at a high intensity 70% $\dot{V}O_{2peak}$. Weather it is more beneficial for health to have a faster or slower GER is not conclusive from the data presented within this thesis.
2) Subjective feelings of hunger are reduced immediately post-exercise when exercising intermittently or continuously >60% $\dot{V}O_{2peak}$. Nevertheless, a

subsequent meal following both intermittent and continuous exercise seems to abolish any compensatory effects in subjective feelings of hunger.

3) The equilibrium of substrate utilisation shifts to predominantly fat oxidation after continuous and intermittent exercise at various intensities between 40- 70% $\dot{V}O_{2peak}$. Unfortunately, this increase is short lived after a relatively small calorific semi-solid (~242 KCal) or liquid meal (~169 KCal) is consumed.

4) Acylated ghrelin decreases immediately after continuous exercise > 70% $\dot{V}O_{2peak}$ and intermittent exercise at PPO (100% $\dot{V}O_{2peak}$). These results strengthen the concept that the appetite regulatory hormone ghrelin is responsive to changes in exercise intensity, promoting its orexigenic properties. To further support this notion acylated ghrelin is also shown to be increased in the postprandial period, whereas insulin returns to bassline values faster after multiple exercise bouts. The mechanisms for this increase in acylated ghrelin requires further investigation but may be due to subtle differences in blood glucose in the postprandial period.

5) Continuous exercise triggers a spike in blood glucose after a calorific meal, causing blood glucose to remain elevated during recovery periods. The metabolic response after consuming energy dense material post-exercise is strongly related to GI hormone responses, although the data within this thesis is not conclusive and further clarity is needed to strengthen this theory.

6) Continuous exercise regardless of intensity and intermittent exercise performed at a low intensity induces an energy deficit without subsequent compensatory response in 24 h EI. In contrast, moderate intensity intermittent exercise and multiple bouts of continuous exercise may potentially cause a transient increase in EI within the first 24h, which could in the long-term cause weight gain. The mephanisms of adaptation requires further investigation but may be due to the wellbeing or recovery process after completing an energy depleting exercise bout.

Chapter 9.0

Practical Applications of Gastric Emptying Rate and Appetite after Manipulating Exercise on a Bike for the General Public.

9.1. Overview

The gastrointestinal system is one of the most diverse systems within the human body. The main role of the digestive system is to break down food mechanically and chemically into smaller pieces, that can be absorbed and utilised as energy by the human body (Nigam and Knight, 2017). The stomach is the first organ within the gastrointestinal system. Gastric emptying or gastric emptying rate (GER) refers to the process in which the stomach discharges its contents into the small intestine, where further digesting and the majority of food (nutrients once within the digestive system) are absorbed (Liu et al., 2021). Therefore, consuming large quantities of food and drink without appropriate amounts of exercise will eventually lead to weight gain, which is probably one of the largest precursors to obesity, diabetes, and cardiovascular disease. Fundamentally, research has focused on enhancing understanding related to the way gastrointestinal gut hormones such as insulin, ghrelin and GLP-1 interact to control body weight and weight management. Minimal research has been carried out to further understand the process of how gastric emptying might regulate appetite and satiety; a feeling or condition of being full after eating food.

This thesis was designed to focus on manipulating exercise by changing intensity from a low (still able to talk to someone next to you during exercise), through to a high (not able to talk to someone as your breathing rate would increase becoming heavy with other physical signs such as of sweating). Secondly, manipulating the mode of exercise is performed in, either continuously (non-stop for the duration of the exercise) or intermittently (stop-start exercise to rest for the time duration). Finally, using a mixture of the two conditions. Intensity and mode were used to assess whether completing more than one exercise bout within the same day, such as using a form of exercise to commute to work and then home, would have similar responses to a one-off exercise bout such as going the gym as your daily activity.

Understanding the mechanisms of the stomach by examining the process of GER will help to formulate if there is a relationship between food ingestion after exercise. Therefore, this research is of paramount importance for the development of weight management programmes, to reduce the UK's ever-increasing overweight

problem, in order to prevent an obesity epidemic, by avoiding filling belly's with high-streets temptation snacks after well earnt physical activity.

9.2. Findings -What is the Take Home Message for Someone Aspiring to Change their Physique

The research was designed to raise awareness of the human body in relation to the consumption of semi-solid food after different exercise conditions on a bike. All findings within this thesis were short-term laboratory-based investigations using healthy, untrained, adult males, who endured several experimental investigations to draw upon the conclusions documented.

1) At this moment it is not conclusive whether the speed at which a standardised semi-solid meal empties from the stomach after exercising on a bike has a positive or a negative effect on health.

2) Low intensity continuous exercise increases the feeling of hunger. Although, after consuming food hunger is maintained, leading to a controlled energy intake, helping to achieve negative energy balance.

3) Moderate intensity intermittent exercise reduces the feeling of hunger after exercise, which results in compensation in energy intake in the first 24h. Suggesting, appetite suppression does not guarantee a reduction in energy intake or to achieve negative energy balance.

4) Consuming food or drink containing calories changes the sensitive balance between fat and carbohydrate energy metabolism. Proposing a delay in calorie intake in the hour/hours after exercise might increase the formulation of increased fat metabolism.

5) The gut hormone ghrelin increases and decreases reflecting the feeling of hunger after exercise in a fasted state. Ghrelin might be the first physiological sign notifying the brain to initiate energy intake.

6) Consuming food after continuous exercise causes blood glucose to increase and remain elevated more so than intermittent exercise. Any increase in blood glucose over long-periods of time are potentially detrimental to health.

The recommendations and suggestions documented, are related to findings from this thesis alone, and therefore directly related to a male population. Whether these findings are related to other populations are currently unclear.

9.3. Traffic Light Decision Tree for Quick Tips for Exercise

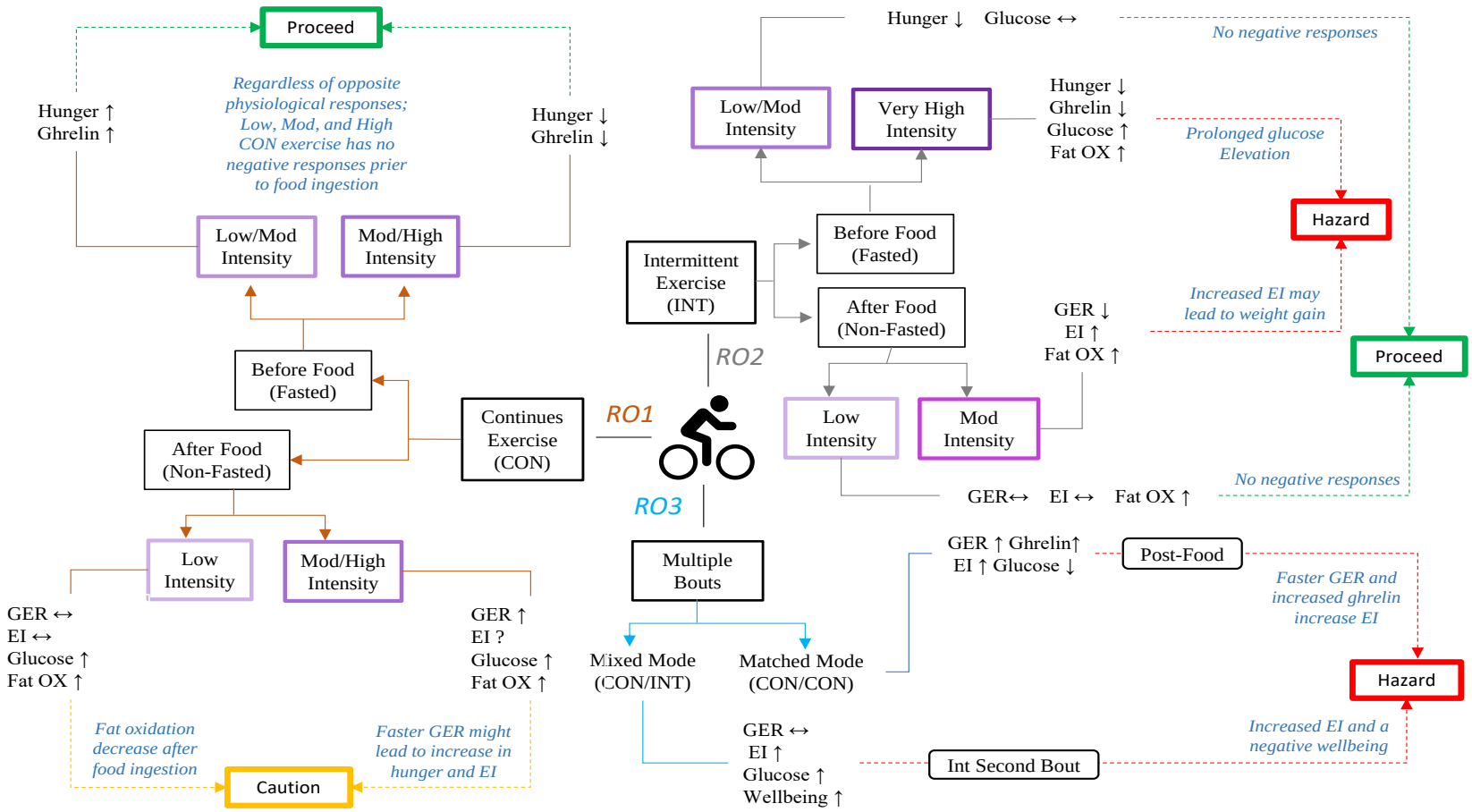

Figure 87:Decision tree illustrating the potential outcome when deciding how to manipulate exercise. With an easy-to-use traffic light conclusion. RO1, Research objective 1. Adjustments in exercise intensity; RO2, Research objective 2. Using an intermittent mode of exercise; RO3, Research objective 3; Organisation of exercise by conducting multiple exercise bouts; Dashed lines represent, recommendations using data only from this thesis; Dashed green lines (proceed), continue with ease; Dashed orange lines (caution), think before undertaking this exercise method; Dashed red lines (hazard), may cause negative physiological effect during or post-exercise; Blue italicize writing, key considerations. Change in the colour purple, indicates the increase in exercise intensity. CON, continuous exercise; INT, intermittent exercise; GER, gastric emptying rate; EI, energy intake; Fat OX, Fat oxidation rate; ↑, increase or faster; ↓, decrease or slower; ↔ no change or similar result; ?, Inconclusive result.

10.0. References

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11.0 Appendices

A) *Ethical approval letter Study 1 (Chapter 4)*

FACULTY OF SCIENCE AND ENGINEERING

Manchester Metropolitan University

MEMORANDUM

TO Lewis Mattin

FROM Karen Hartley

DATE 28th September 2016

SUBJECT Application for Ethical Approval **(SE151667)**

On the 28th September 2016 the Head of Ethics for Science & Engineering considered your application for Ethical Approval (SE151667) entitled "*Changes in appetite and gut hormones after exercising at different intensities in a thermo-neutral environment*". 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 (Dr Nick Costen) 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

B) *Ethical approval letter, Study 2 (Chapter 5)*

FACULTY OF SCIENCE AND ENGINEERING

Manchester Metropolitan University

MEMORANDUM

TO	Lewis Mattin
FROM	Karen Hartley
DATE	27th September 2017
DATE OF EXPIRY:	27th June 2018
SUBJECT	Application for Ethical Approval **(SE1617159)**

On the 27th September 2017 the Head of Ethics for Science & Engineering considered your application for Ethical Approval (**SE1617159**) entitled "The effect of high and low intensity, intermittent and continuous exercise on gastric emptying, appetite and 24-h post energy intake". 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

C) *Ethical approval letter, Study 3 (Chapter 6)*

16/11/2018

Project Title: Split exercise effects gastrointestinal health

EthOS Reference Number: 1089

Ethical Opinion

Dear Lewis Mattin,

The above application was reviewed by the Science and Engineering Research Ethics and Governance Committee and, on the 16/11/2018, was given a favourable ethical opinion. The approval is in place until 26/08/2020 .

Conditions of favourable ethical opinion

Application Documents

Document Type	File Name	Date	Version
Additional Documentation	100-mm VAS_Study3	10/08/2018	1
Additional Documentation	Dietary_Record_Post-trial3	10/08/2018	1
Additional Documentation	Dietary_Record_Pre-trial(3)	10/08/2018	1
Additional Documentation	Medical_screening_questionnaire_study3	10/08/2018	1
Additional Documentation	Consent_form_Study3	10/08/2018	1
Project Proposal	Study3	10/08/2018	1
Recruitment Media	Advertising_poster_Study3	10/08/2018	1
Consent Form	Consent_form_Study3	10/08/2018	1
Information Sheet	Participant_information_Study3	10/08/2018	1

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.

We wish you every success with your project.

Science and Engineering Research Ethics and Governance Committee

D) *Ethical approval letter, Study 4 (Chapter 7)*

Manchester Metropolitan University

15/10/2019
Project Title: The effects of multiple exercise bouts (HIIE and continuous) over the same day on appetite and metabolic response to food ingestion

EthOS Reference Number: 11619

Ethical Opinion

Dear Lewis Mattin,

The above application was reviewed by the Science and Engineering Research Ethics and Governance Committee and, on the 15/10/2019, was given a favourable ethical opinion. The approval is in place until 14/09/2020 .

Conditions of favourable ethical opinion

Application Documents

Document Type	File Name	Date	Version
Information Sheet	100-mm VAS_Study4	23/07/2019	1
Information Sheet	Wellbeing_2_Study4	23/07/2019	1
Information Sheet	Dietary_Record_Post-trial(4)	23/07/2019	1
Information Sheet	Dietary_Record_Pre-trial(4)	23/07/2019	1
Information Sheet	Medical_screening_questionnaire_study4	23/07/2019	1
Information Sheet	Participant_information_Study4	23/07/2019	1
Information Sheet	Participant_order_Study4	23/07/2019	1
Project Protocol	Project Proposal-ETHOS	06/09/2019	2
Recruitment Media	Poster(4)	06/09/2019	2
Consent Form	Consent_form_Study4	06/09/2019	2
Additional Documentation	RiskAssessment-labcup-study4	06/09/2019	1
Additional Documentation	RiskAssessment-labcup-2.1.html	30/09/2019	1

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.

We wish you every success with your project.

Science and Engineering Research Ethics and Governance Committee

E) *Medical screening questionnaire*

Does workload matched exercise split over two sessions effect gastrointestinal health

Participant number:

Medical Screening Questionnaire

It is important that the investigators are aware of any health conditions before participation in this research study. This is to ensure that the study protocol will not exacerbate any existing conditions of the participant. Please answer the following questions as accurately as possible.

Are you currently taking any prescribed medication? YES/NO

Are you currently attending your GP? YES/NO

Have you ever suffered from a cardiovascular problem?

i.e. high blood pressure, anaemia, heart attack etc YES/NO

Have you ever suffered from a neurological disorder?

i.e. epilepsy, convulsions etc YES/NO

Have you ever suffered from an endocrine disorder?

i.e. diabetes etc YES/NO

Have you ever suffered from a chronic gastrointestinal disorder?

i.e. Crohn's disease, irritable bowel syndrome etc YES/NO

Have you ever suffered from a skin disorder?

i.e. eczema etc YES/NO

Have you ever had a blood borne virus infection?

i.e. HIV, Hepatitis B and hepatitis C etc YES/NO

Do you suffer from any allergies?

i.e. any medications, foods etc YES/NO

Are you aware of any other medical condition that may prevent you from participating in this investigation?

i.e. immunological disorders, numbness in extremities, asthma etc YES/NO

Are you currently taking any supplements?

i.e. creatine YES/NO

If you have answered "yes" to any of these questions, please provide details below:_____

_____

F) *Manufacturers details for breakfast meal*

Breakfast details*	
Semi-Skimmed Milk Per 100 mL	
Energy (KJ/ KCal)	208/50
Carbohydrate (g)	4.8
Protein (g)	3.6
Fat (g)	1.8
Fibre (g)	0
Kellogg's Special K original Cereal Per 100 g	
Energy (KJ/KCal)	1588/375
Carbohydrate (g)	79
Protein (g)	9
Fat (g)	1.5
Fibre (g)	4.5
Sainsbury's Pure Orange Juice Per 100mL	
Energy(KJ/KCal)	176/42
Carbohydrate (g)	8.6
Protein (g)	0.6
Fat (g)	<0.5
Fibre (g)	<0.5
Sainsbury's Butter Croissant Per 100g	
Energy(KJ/KCal)	1826/438
Carbohydrate (g)	41.5
Protein (g)	8.5
Fat (g)	25.8
Fibre (g)	2.7

* Breakfast was provide during Chapter 6 & Chapter 7, refer to methods section for details

G) Manufacturers details for lunch meal

Lunch details	
Heinz Chicken Noodle Soup [1] Per 100 g	
Energy (KJ/ KCal)	138/32
Carbohydrate (g)	6.1
Protein (g)	1.3
Fat (g)	0.3
Fibre (g)	0.2
Heinz Vegetable Soup [2] Per 100 g	
Energy (KJ/ KCal)	198/47
Carbohydrate (g)	8.3
Protein (g)	1.1
Fat (g)	0.8
Fibre (g)	0.9

[1] Semi-solid meal for Chapter 4. **[2]** Semi-solid meal for Chapters 5, 6 and 7

H) *Pre-trial dietary and physical activity diary*

Trial: Subject Number: Pre-Trial

SCHOOL OF HEALTHCARE SCIENCE

PRE-TRIAL DIETARY & PHYSICAL ACTIVITY DIARIE

The effect of high and low intensity, intermittent and continuous exercise on gastric emptying, appetite and 24-h post energy intake

- It is important that conditions are standardised before each trial and therefore we would like you to follow the same patterns of diet and exercise before each of the trial days.
- For the 24 hours before the start of the first trial, we would ask you to weigh and record all the food and drink that you consume and any activity that you undertake in the space provided on the following pages.
- You should then keep these sheets as a record of your diet and activity to allow you to follow the same pattern before the second and third trial.
- We would like to remind you that you should not consume any alcohol or caffeine and also undertake any strenuous activity in the 24 hours prior to each test.
- Furthermore, please do not eat anything after 2200h on the evening before a trial except the consumption of water.
- If you have any problems don't hesitate to call me (Lewis Mattin) on [07775430533] or email [l.mattin@mmu.ac.uk] at any time.

Name: .....

Date: .....

Trial: Subject Number: Pre-Trial

Dietary record Sheet

- Please record all food and drink consumed in the 24 hours prior to the start of the first trial.
- Start in the morning when you first get out of bed, and record the food you eat until the evening when you stop eating at 2200h.
- Use a separate line for each item of food.

Example Dietary

Time	Item of Food	Weight	Description/Brand
e.g. 13 :00	Baked Beans	200g	Heinz, low sugar
13 : 00	Toasted bread	143g	Wholemeal, thick cut
13 : 00	Orange soft drink	1 500 ml bottle	Diet Fanta
24h Prior to trial dietary record			

Trial: Subject Number: Pre-Trial

Activity Record Sheet

- Please Record all activity undertaken for the 24 hour period prior to the start of the first trial
- Start in the morning when you first get up until bed time in the evening.

Example Dietary

Time	Activity	Duration
e.g.17:00	Cycled home from college	30 minutes
20:00	Walked the dog	1hour
24h Prior to trial Activity record		

I) *Appetite VAS*

Subject number _____ Trial _____ Time point _____

Satiety Scorecard

1. How hungry do you feel ?

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

2. How full do you feel ?

Not at all full _____ *Totally full*

3. How much do you think you can eat ?

Nothing at all _____ *A lot*

4. How Satisfied do you feel ?

I am completely empty _____ *I can't eat another bite*

---

5. How nauseous do you feel ?

Not at all nauseous _____ *Very nauseous*

6. How bloated do you feel ?

Not at all bloated _____ *Very bloated*

J) *Wellbeing Scale*

Subject number _____ Trial _____ Time point _____

Wellbeing Questionnaire

1. How sore do your muscles feel?

2. How fatigued do you feel ?

3. How is your mood ?

4. How stressed do you feel ?

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5. How sleepy do you feel ?