Dietary carbohydrate, exercise, and human health

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Abstract

For clarity, this thesis is separated into two parts:

Part 1: Digestible carbohydrate and gastroenteropancreatic hormone release during exercise (**Chapters 1 to 5**)

Part 2: Non-digestible carbohydrate, short-chain fatty acid production and skeletal muscle metabolism (**Chapters 6 to 8**)

BACKGROUND

Carbohydrate is the principal energy-contributing macronutrient in the typical human diet. Recent evidence has however suggested high intakes of dietary carbohydrate may increase disease and mortality risk, which has been coupled with a rapid increase in popularity of low-carbohydrate diets amongst the general population. Nevertheless, health-related claims regarding carbohydrate are often too simplistic as the term 'carbohydrate' constitutes a wide spectrum of foods that likely vary in their impact on human health. The sub-division of carbohydrate into various classifications can therefore provide a greater insight into the relationship between carbohydrate-containing foods and health. Carbohydrate can be classified as digestible or non-digestible depending on its capacity to be hydrolysed by host enzymes. Despite differences in the context in which digestible and non-digestible carbohydrate may benefit human health, both carbohydrate classes can influence the release of gastroenteropancreatic hormones and provide a substrate for skeletal muscle during exercise. The overall aim of this thesis was to investigate specific contexts in which digestible and non-digestible carbohydrate may provide benefit to human health, concentrating on their interaction with skeletal muscle, exercise, and gastroenteropancreatic hormone release.

PART 1

Acute exercise is often accompanied by a temporary suppression of appetite referred to as 'exercise-induced anorexia'. This response is often attributed to changes in glucagon-like peptide 1, peptide YY and acyl-ghrelin. However, acute exercise also influences the concentrations of other hormones that are implicated in appetite-regulation (the literature surrounding this topic area is reviewed in **Chapter 1**). In **Chapter 2**, the effect of acute exercise on glucose, insulin, and glucagon concentrations in the fed and fasted state was investigated using a systematic review and meta-analysis. Acute exercise resulted in decreased concentrations of glucose and insulin in the fed state, and increased glucagon concentrations irrespective of metabolic state. As raised systemic glucagon concentrations has previously been implicated in appetite suppression, these results suggested that glucagon may play a role in exercise-induced anorexia. **Chapter 3** followed up the results of **Chapter 2** by examining the effect of acute glucagon administration on subjective appetite and energy intake in humans via a systematic review and meta-analysis, identifying an inconsistent effect on

appetite and energy intake. Therefore, the possible role of glucagon in exercise-induced anorexia could not be evaluated. **Chapter 4** explored the independent and interactive effects of digestible carbohydrate and exercise on gastroenteropancreatic hormone release, and it is implications for appetite and energy balance. Digestible carbohydrate and exercise independently and interactively modulated the hormonal milieu and plasma metabolome, resulting in the generation of distinct metabolic phenotypes. GLP-1, acetate, lactate, and succinate were also identified as putative mediators of exercise-induced suppression of appetite and energy intake. However, no strong relationship between glucagon and exercise-induced appetite suppression or energy intake was found. A general discussion of the work presented in part 1 of this thesis is provided in **Chapter 5**.

PART 2

Non-digestible carbohydrate, the majority of which constitutes dietary fibre, escapes digestion and is subsequently available for fermentation by resident colonic bacteria, resulting in the generation of metabolites such as short-chain fatty acids. Short-chain fatty acids can enter the systemic circulation where they can influence the metabolism and function of multiple organs, including skeletal muscle (the literature surrounding this topic area is reviewed in **Chapter 6**). **Chapter 7** investigated the relationship between dietary fibre intake and skeletal muscle metabolism and function in a cohort at risk of skeletal muscle atrophy using a nationally representative dataset. Increased consumption of dietary fibre was associated with increased skeletal muscle mass and strength after adjusting for key confounders. A general discussion of the work presented in part 1 of this thesis is provided in **Chapter 8**.

SUMMARY

Overall, these studies demonstrate that glucagon is unlikely to play a role in exercise-induced anorexia despite acute exercise being a potent stimulator of glucagon release. These studies also identify acetate and succinate as novel regulators of exercise-induced changes in appetite and energy intake, and show that increasing dietary fibre is associated with higher relative skeletal muscle mass and strength.

Contents

Abbreviations

AgRP - Agouti-related peptide

AMPK - AMP-activated protein kinase

Apo-A2 - Apolipoprotein A2

AUC - Area under the curve

BHB - 3-hydroxybutyrate

BMRB - Biological magnetic resonance data bank

BMI - Body mass index

CART - Cocaine-amphetamine-regulated-transcript

CAS - Composite appetite score

CI - Confidence interval

ECG - Electrocardiogram

FFAR2 - Free fatty acid receptor 2

FFAR3 - Free fatty acid receptor 3

GCGR - Glucagon receptor

GHSR1a - Growth hormone secretagogue receptor type 1a

GLP-1 - Glucagon-like peptide 1

HDACs - Histone deacetylases

HDL-C - High-density lipoprotein cholesterol

HMDB - Human metabolome data base

HOMA2-IR - Updated homeostasis model assessment

IDL-C - Intermediate-density lipoprotein cholesterol

IDL-P - Intermediate-density lipoprotein particle number

IGF-1 - Insulin-like growth factor 1

IL-6 - Interluekin-6

IVDr - *in vitro* diagnostics research

LOOCV - Leave-one-out cross-validation

MD - Mean differences

MCCV - Monte Carlo cross-validation

MHC - Myosin heavy chain

NAFLD - Non-alcoholic fatty liver disease

NEFAs - Non-esterified fatty acids

NHANES - National health and nutrition examination survey

NPY - Neuropeptide Y

p38 MAPKs - p38 mitogen-activated protein kinases

PGC1α - Peroxisome proliferator–activated receptor gamma coactivator 1α

PICO - Population, intervention, comparator, and outcome

PIR - Ratio of family income to poverty

PLS-DA - Partial least squares discriminant analysis

POMC - Proopiomelanocortin

PPARs - Peroxisome proliferator-activated receptors

PQN - Probabilistic quotient method

PYY - Peptide YY

RM - Repeated measures

RM-PLS-DA - Repeated measures partial least squares discriminant analysis

RMSEP - Root mean square error of prediction

SCFAs - Short-chain fatty acids

SNS - Sympathetic nervous system

SMDs - Standardised mean differences

STORM - Subset optimization by reference matching

USDA - The United States department of agriculture

VAS - Visual analogue scale

VIP - Variable importance in projection

$\dot{V}O_2$ max - Maximal aerobic capacity

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Declaration of contributors

The work presented in this thesis is original and primarily performed by the author (all else is appropriately referenced). All collaboration and assistance are described below:

Chapter 2:

This study was performed with assistance from Benjamin Cobbold, Mikhail Nozdrin, Htet Oo, Holly Wilson, and Professor Gary Frost.

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This study was performed with assistance from Professor Gary Frost.

Research outputs

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Frampton, J., Murphy, K. G., Frost, G., & Chambers, E. S. (2021). Higher dietary fibre intake is associated with increased skeletal muscle mass and strength in adults aged 40 years and older. *Journal of Cachexia, Sarcopenia and Muscle*, 12(6), 2134-2144. [*Chapter 7 is based on this publication]*

Frampton, J., Cobbold, B., Nozdrin, M. et al. (2021). The effect of a single bout of continuous aerobic exercise on glucose, insulin and glucagon concentrations compared to resting conditions in healthy adults: a systematic review, meta-analysis and meta-regression. *Sports Medicine*, 51(9), 1949-1966. [*Chapter 2 is based on this publication]*

Amin, A., **Frampton, J**., Liu, Z. et al. (2021). Differential effects of L-and D-phenylalanine on pancreatic and gastrointestinal hormone release in humans: a randomized crossover study. *Diabetes, Obesity and Metabolism*, 23(1), 147-157.

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Preface: Carbohydrates and metabolism

Carbohydrate is the primary energy-contributing macronutrient in the conventional human diet and accounts for 50-70% of total energy intake for the majority of human adults[1,2]. Over the past decade concerns have been raised regarding the relationship between a high carbohydrate intake and ill-health. The large-scale prospective cohort study PURE (Prospective Urban Rural Epidemiology) recently reported significant associations between a high carbohydrate intake (>60% of energy) and an increased risk of total mortality and non-cardiovascular disease mortality[3]. This rise in concern has been paralleled by an increase in popularity of low carbohydrate (<20% of energy from carbohydrate) and ketogenic diets (<5% of energy from carbohydrate), as well as the coining of terms such as 'carbotoxicity'[4]. It is however pragmatic to remember that 'carbohydrate' is an umbrella term encompassing a wide array of foods that differ in their nutritive value and their effects on postprandial physiological responses[5]. It can be argued that collectively analysing carbohydrates is too simplistic, as this groups carbohydrate based-foods which are largely considered health-promoting (e.g. whole grains[6]) with highly processed carbohydrates that are associated with ill-health (e.g. sugar-sweetened beverages[7]). Understanding the role of different types of carbohydrate in human health, and the context in which they are consumed, is therefore of importance to public health.

Carbohydrates can be classified into distinct groups on the basis of various criteria[5]. Well-established classifications include the degree of polymerization (simple vs complex), the magnitude of the rise in postprandial blood glucose concentrations (low vs high glycaemic index) and the extent to which they are metabolised by host digestive enzymes (digestible vs non-digestible). Of these classifications, the degree of carbohydrate digestibility is of particular interest due to the reported positive and negative effects following both digestible and non-digestible carbohydrate ingestion. For example, the restriction of digestible carbohydrate may improve glycaemic control in type 2 diabetics[8], but worsen endurance performance in athletes[9]. Similarly, non-digestible carbohydrate supplementation may reduce energy intake in individuals with obesity[10], but worsen symptoms in individuals with irritable bowel syndrome[11]. These divergent effects of digestible and non-digestible carbohydrate within different populations are important to understand when considering the magnitude of global carbohydrate consumption.

Despite these divergent effects, digestible and non-digestible carbohydrate can exert similar effects on human metabolism. For example, both classes of carbohydrate can modulate the release of gastroenteropancreatic hormones related to appetite and glucose homeostasis, such as glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), as well as provide a substrate for skeletal muscle during exercise (Figure 0.1). However, the underlying mechanisms by

which digestible and non-digestible carbohydrate achieve this are diverse. Digestible carbohydrate is hydrolysed by host enzymes into mono- and disaccharides and primarily absorbed in the upper gastrointestinal tract[12], resulting in the stimulation of gastroenteropancreatic hormone release within 30 minutes of ingestion[13]. Following absorption into the systemic circulation, the products of digestible carbohydrate digestion can be utilised directly by skeletal muscle as an energy source during periods of intense exercise[14]. In contrast, non-digestible carbohydrate, of which dietary fibre is the principial component, escapes digestion and is available for fermentation by colonic bacteria[15]. Fermentation of non-digestible carbohydrate can alter the composition of gut bacterial populations and the generation of metabolites derived from the metabolic activity of gut bacteria, including short-chain fatty acids (SCFAs)[16]. These SCFAs can also stimulate release of gastroenteropancreatic hormones and act as a substrate for skeletal muscle during exercise performance[17,18].

This doctoral research project will aim to investigate the contexts in which digestible and non-digestible carbohydrate (and their associated metabolites) can influence human health, with a focus on skeletal muscle, exercise, and gastroenteropancreatic hormones.

For clarity, this thesis is separated into two parts:

Part 1: *Digestible carbohydrate* and gastroenteropancreatic hormone release during exercise

Part 2: *Non-digestible carbohydrate*, short-chain fatty acid production and skeletal muscle metabolism

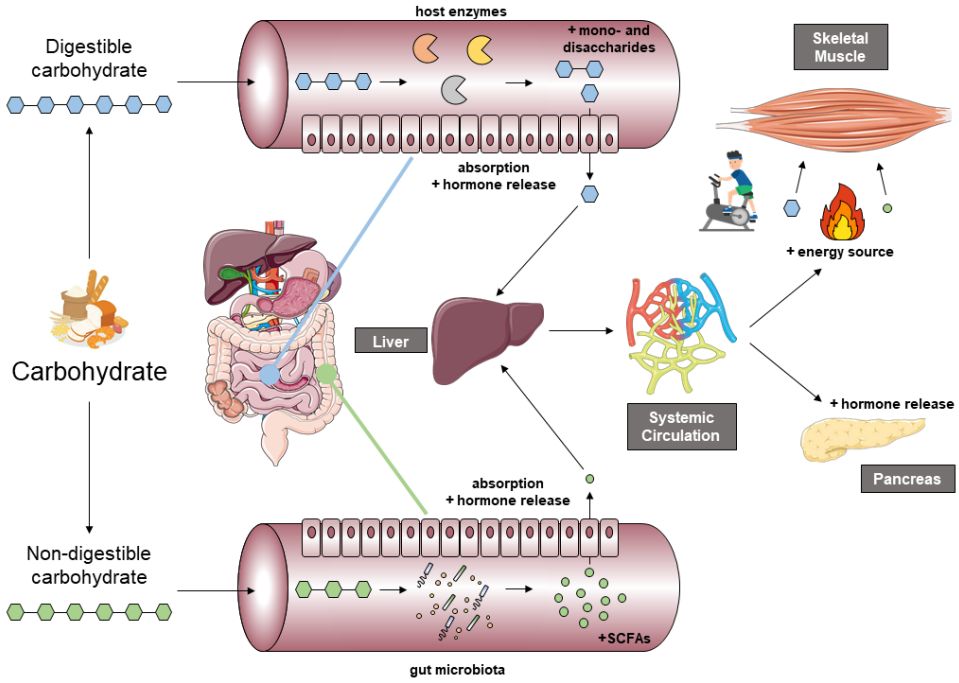


Figure 0.1: Overview of the effect of digestible and non-digestible carbohydrate on human metabolism.

References

1. Mokhtar, N. *et al.* Diet Culture and Obesity in Northern Africa. *J. Nutr.* **131**, 887S-892S (2001).
2. Austin, G. L., Ogden, L. G. & Hill, J. O. Trends in carbohydrate, fat, and protein intakes and association with energy intake in normal-weight, overweight, and obese individuals: 1971–2006. *Am. J. Clin. Nutr.* **93**, 836–843 (2011).
3. Dehghan, M. *et al.* Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study. *Lancet* **390**, 2050–2062 (2017).
4. Kroemer, G., López-Otín, C., Madeo, F. & de Cabo, R. Carbotoxicity—Noxious Effects of Carbohydrates. *Cell* **175**, 605–614 (2018).
5. Lunn, J. & Buttriss, J. L. Carbohydrates and dietary fibre. *Nutr. Bull.* **32**, 21–64 (2007).
6. Reynolds, A. *et al.* Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet* **393**, 434–445 (2019).
7. Malik, V. S. *et al.* Sugar-Sweetened Beverages and Risk of Metabolic Syndrome and Type 2 Diabetes: A meta-analysis. *Diabetes Care* **33**, 2477–2483 (2010).
8. Samkani, A. *et al.* A carbohydrate-reduced high-protein diet acutely decreases postprandial and diurnal glucose excursions in type 2 diabetes patients. *Br. J. Nutr.* **119**, 910–917 (2018).
9. Burke, L. M. *et al.* Low carbohydrate, high fat diet impairs exercise economy and negates the performance benefit from intensified training in elite race walkers. *J. Physiol.* **595**, 2785–2807 (2017).
10. Pasman, W. J., Saris, W. H., Wauters, M. A. & Westerterp-Plantenga, M. S. Effect of one week of fibre supplementation on hunger and satiety ratings and energy intake. *Appetite* **29**, 77–87 (1997).
11. Ong, D. K. *et al.* Manipulation of dietary short chain carbohydrates alters the pattern of gas production and genesis of symptoms in irritable bowel syndrome. *J. Gastroenterol. Hepatol.* **25**, 1366–1373 (2010).
12. Gray, G. M. Carbohydrate Digestion and Absorption. *N. Engl. J. Med.* **292**, 1225–1230 (1975).
13. Færch, K. *et al.* GLP-1 Response to Oral Glucose Is Reduced in Prediabetes, Screen-Detected Type 2 Diabetes, and Obesity and Influenced by Sex: The ADDITION-PRO Study. *Diabetes* **64**, 2513–2525 (2015).
14. Egan, B. & Zierath, J. R. Exercise Metabolism and the Molecular Regulation of Skeletal Muscle Adaptation. *Cell Metab.* **17**, 162–184 (2013).
15. Wong, J. M. W., de Souza, R., Kendall, C. W. C., Emam, A. & Jenkins, D. J. A. Colonic Health: Fermentation and Short Chain Fatty Acids. *J. Clin. Gastroenterol.* **40**, 235–243 (2006).
16. Fan, Y. & Pedersen, O. Gut microbiota in human metabolic health and disease. *Nat. Rev. Microbiol.* (2020).
17. Frampton, J., Murphy, K. G., Frost, G. & Chambers, E. S. Short-chain fatty acids as potential regulators of skeletal muscle metabolism and function. *Nat. Metab.* **2**, 840–848 (2020).

18. den Besten, G. *et al.* The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J. Lipid Res.* **54**, 2325–2340 (2013).

Chapter 1: Digestible carbohydrate and gastroenteropancreatic hormone release during exercise

1.1 The obesity pandemic

The continued rise in global obesity prevalence is widely considered a public health crisis. As of 2016, the number of adults worldwide who were classified as overweight (body mass index [BMI] ≥ 25.0 kg/m^2) exceeded 1.9 billion, of which 650 million were obese (BMI ≥ 30.0 kg/m^2)[1]. If current trends remain unaltered, it is predicted that approximately 40% of the UK adult population will be obese by 2030[2]. This is disconcerting given that obesity is associated with numerous comorbidities, including type 2 diabetes[3], cardiovascular disease[4] and several forms of cancer[5]. Furthermore, obesity places a large economic burden on society, with recent analyses estimating a global economic cost of $2 trillion[6]. Despite bariatric surgery and pharmaceutical interventions both offering highly effective treatment options for obesity[7,8], they are not universally available or acceptable for all individuals. Strategies that effectively prevent and reverse obesity at the population level are therefore increasingly needed.

Obesity is the direct result of a chronic imbalance between energy intake and energy expenditure, in which intake exceeds expenditure[9]. Interventions that aim to reduce bodyweight consequently target a reduction in energy intake, an increase in energy expenditure, or both simultaneously[10]. A reduction in energy intake is conventionally achieved through a hypocaloric diet, whereas an increase in energy expenditure is typically achieved via exercise. Acutely, an energy deficit created by meal omission produces a subsequent increase in *ad libitum* energy intake[11]. In contrast, an identical energy deficit created by a single bout of exercise has no effect on subsequent *ad libitum* energy intake[11]. If this response persists over time, exercise should theoretically be a more useful intervention for weight reduction relative to dietary restriction when energy deficits are matched. Contradicting this hypothesis, data from meta-analyses show that exercise results in significantly less weight-loss that dietary restriction over the long-term[12,13]. Indeed, weight-loss following long-term exercise interventions is approximately 30% of expected weight loss given the energy expended during exercise training[14]. The reduced effectiveness of exercise on long-term weight-loss is primarily attributed to compensatory increases in dietary energy intake rather than changes in physical activity energy expenditure[15,16]. Understanding the mechanisms by which exercise induces changes in acute energy balance and the influence of dietary strategies on this response may therefore help to improve the effectiveness of exercise as a long-term weight loss tool.

1.2 The effect of a single bout of exercise on post-exercise subjective appetite

Subjective appetite is typically measured using visual analogue scales (VAS). VAS are a psychometric tool which consist of questions and corresponding 100-150 mm lines anchored by two contrasting statements. These questions (also referred to as scales) can be used to assess various dimensions of subjective appetite, including hunger, fullness, satiety, desire to eat and prospective food consumption[17]. These scales are often combined to form a composite score that is considered a general estimate of subjective appetite[18]. VAS scores are considered reliable and reproducible[19] but may not accurately predict subsequent energy intake[20].

1.2.1 The effect of a single bout of aerobic exercise on subjective appetite

A single bout of aerobic exercise has been repeatedly shown to suppress subjective hunger[21,22] and composite appetite scores[23,24] - a phenomenon commonly referred to as exercise-induced anorexia[25]. This suppression occurs within 10 minutes of exercise onset[26] and persists for 30 to 90 minutes following exercise completion[21,23,24,27–29]. Nevertheless, this response may not be present in all populations. Studies using individuals with obesity/overweightness (BMI ≥25 kg/m^2) commonly report no significant changes in subjective appetite following exercise completion[30–32] unless exercise intensity is high (>70% $\dot{V}O_2$ max)[29,33,34]. Indeed, high intensity interval exercise appears to produce a greater suppression of subjective appetite relative to moderate intensity continuous exercise (~65% $\dot{V}O_2$ max)[34,35]. Children with obesity also do not exhibit significant changes in post-exercise subjective appetite[36,37]. However, it is unclear whether this is attributable to age or obesity. Males and females both experience a decrease in subjective appetite with no differences in the magnitude of suppression between sexes[23,24].

1.2.2 The effect of a single bout of resistance exercise on subjective appetite

In contrast to aerobic exercise, few studies have investigated the effects of a single bout of resistance exercise on post-exercise subjective appetite. Of these studies, only one has demonstrated a significant decrease in hunger during and 30 mins post-exercise[21]. Most studies have reported no effect of resistance exercise on subjective appetite during or post-exercise[32,38–40]. The reason for this discrepancy between aerobic and resistance exercise is currently unknown. It may however relate to exercise intensity, as energy expenditure during resistance-based exercise is typically lower than that achieved during aerobic exercise[21].

1.2.3 Medium to long-term effects of exercise on subjective appetite

Daily exercise for seven[41,42] or 14 days[43] does not alter any dimension of subjective appetite (hunger, fullness, satiety, desire and prospective food consumption) in males or females. Long-term exercise interventions (≥4 weeks) have however produced mixed results, with

studies reporting an increase[44–46], decrease[47] or no change[48–50] in fasting measures of subjective appetite. Despite these mixed findings, long-term exercise may increase the sensitivity of the appetite control system[51]. For example, participants who undertook a long-term exercise programme (12 weeks) reported increased fullness following a 75g carbohydrate bolus relative to sedentary controls[47]. However, this finding is not consistent across all studies[50].

1.3 The effect of a single bout of exercise on post-exercise *ad libitum* energy intake

Post-exercise *ad libitum* energy intake is often measured 0-2 hours following exercise completion using either a single (limited food variety) or buffet (high food variety) style meal[17,52]. The amount of food consumed at this meal and/or the time taken to request this meal following exercise completion (latency to eat) are commonly assessed.

1.3.1 The effect of a single bout of aerobic exercise on post-exercise *ad libitum* energy intake

The majority of studies demonstrate that aerobic exercise does not affect[53–57] or decreases[30,58,59] post-exercise *ad libitum* energy intake. Aerobic exercise therefore decreases 'relative energy intake' (energy intake considering energy expended via exercise) and appears to be an effective method for creating a short-term energy deficit. In addition to this, latency to eat is also increased following aerobic exercise[60,61] suggesting that exercise may modulate both inter- and intra-meal satiety. The absence of a compensatory response in energy intake is observed in males[25,35] and females[27,62], as well as in children[36,53] and individuals with obesity[23]. The magnitude of the compensatory response in energy intake following acute aerobic exercise appears to be similar between men and women (despite higher absolute energy intakes seen in men)[63,64] and between lean and overweight individuals[23].

1.3.2 The effect of a single bout of resistance exercise on post-exercise *ad libitum* energy intake

Similar to aerobic exercise, post-exercise energy intake in response to a single bout of resistance exercise is unchanged[40,65] or decreased[66]; therefore creating a reduction in relative energy intake. The effect of sex, age and BMI on this response is unknown due to the comparatively little research performed using this exercise modality.

1.3.3 Medium to long-term effect of exercise on *ad libitum* energy intake

The lack of compensatory changes in energy intake following exercise interventions likely persist beyond the initial hours following exercise completion. No changes in self-reported energy intake were observed in males following seven days of medium- (~350 kcal/day) and high-level (~750 kcal/day) exercise[41]. However, females did exhibit compensatory increases in self-reported energy intake following seven days of high-level (~800 kcal/day) but not medium-level exercise (~450 kcal/day), although this compensation was only partial

(approximately 33% of energy expended via exercise)[42]. When the exercise intervention was extended to 14 days, Whybrow et al.[43] reported that approximately 30% of energy expended through exercise was compensated for, suggesting that total compensation could take several weeks. They also noted that the degree of compensation as highly variable amongst participants. Long-term exercise interventions (>60 weeks) show compensatory responses in energy intake approaching 100% of the energy expended through exercise[67], suggesting that challenges to energy balance via energy expenditure are initially ignored but compensated for over time.

1.4 Mechanisms underlying the effect of acute exercise on subjective appetite and energy intake

Acute moderate- (40-70% $\dot{V}O_2$ max) and high-intensity exercise (>70% $\dot{V}O_2$ max) modulates the release of gastrointestinal hormones related to appetite, namely acyl-ghrelin, glucagon-like peptide 1 (GLP-1) and peptide tyrosine tyrosine (PYY)[68]. These changes have been proposed as a central mechanism underlying the suppression of subjective appetite and relative energy intake following a single bout of exercise[69].

1.4.1 Acyl-ghrelin

Ghrelin is a 28 amino acid peptide that is primarily synthesised and released into the systemic circulation by X/A-like endocrine cells located in the stomach[70,71]. It is an endogenous ligand for the G protein-coupled receptor 'growth hormone secretagogue receptor type 1a' (GHSR1a) and is produced from the post-translational processing of the 117 amino acid peptide preproghrelin[72].

Ghrelin exists in two major forms: acyl- and desacyl-ghrelin. Acyl-ghrelin represents approximately 10% of total circulating ghrelin concentration and has undergone acylation (attachment of a fatty acid side chain to its serine 3 residue) by the enzyme ghrelin O-acyltransferase[72]. Depending on the fatty acid attached, acyl-ghrelin can be further subdivided into octanoyl (C8:0), decanoyl (C10:0) and possibly decenoyl (C10:1) ghrelin[73].

Acylation of ghrelin is necessary for binding to the GHSR1a as well as its metabolic, hormonal and orexigenic effects[74]. Acyl-ghrelin is a potent secretagogue of growth hormone[70] and a key stimulator of appetite and energy intake[75]. The orexigenic effects of acyl-ghrelin are achieved by both endocrine (activation of GHSR1a in the hypothalamus) and neural (activation of GHSR1a on vagal afferents) mechanisms[76]. Beyond these roles, acyl-ghrelin is implicated in cardiac function[77], insulin sensitivity[78], glucose[79] and lipid metabolism[80].

In contrast, desacyl-ghrelin accounts for ~90% of total ghrelin in circulation but lacks this acyl modification; and therefore does not activate the GHSR1a[72]. The exact biological role of

desacyl-ghrelin remains controversial, but evidence suggests it may act as an antagonist to acyl-ghrelin at a yet unidentified receptor[81].

1.4.2 Acyl-ghrelin secretion

Acyl-ghrelin is largely regulated by energy intake, with circulating concentrations increasing pre-prandially and decreasing post-prandially[74]. The rate of decrease is proportional to the energy content of the meal[82] and dependent on the macronutrient content of the meal, with carbohydrate arguably producing the greatest suppression[83].

Despite the mechanisms regulating acyl-ghrelin secretion not being fully understood, it is believed to be primarily under the control of neural mechanisms[84]. For example, cephalic phase reflexes (i.e. the sight, smell, taste, or thought of food) have been found to stimulate acyl-ghrelin section in humans[85,86]. Postprandial suppression of acyl-ghrelin levels may be also be due to cephalic phase reflexes, conditioned reflexes, and/or hormones such as insulin, GLP-1, and PYY[87–91]. Interestingly, acyl-ghrelin secreting cells do express nutrient receptors (e.g. the calcium-sensing receptor) even though they are not in direct contact with the contents of the gastrointestinal tract[84]. These nutrient receptors may be stimulated by metabolites from the systemic circulation diffusing into the lamina propria or possibly by gastric contents[84].

1.4.3 The effect of a single bout of exercise on acyl-ghrelin concentrations

Acute exercise has also been shown to temporarily suppress acyl-ghrelin concentrations. Aerobic exercise decreases acyl-ghrelin concentrations during exercise and throughout the post-exercise period[29,92]. A reduction in acyl-ghrelin levels can be detected 30 minutes after exercise commencement[26,93] and persists for up to 120 minutes following exercise completion[29]. This response is seen in various exercise modalities, including high-intensity interval aerobic exercise[35] and resistance exercise[21,38,65]. The magnitude of acyl-ghrelin suppression may, however, relate to exercise intensity, with higher intensities (notably high-intensity interval training) generating a greater suppression relative to moderate intensities (~65% $\dot{V}O_2$ max)[35,93]. Acyl-ghrelin suppression is reported in both males[35] and females[94], as well as in individuals with overweightness/obesity[32]. There also appears to be no difference in the magnitude of exercise-induced acyl-ghrelin release between males and females and between lean and overweight individuals[95].

1.4.4 Relationship between exercise-induced changes in acyl-ghrelin concentrations, subjective appetite and energy intake

The relationship between acyl-ghrelin correlations and subjective appetite are unclear, with studies reporting significant correlations with subjective appetite scales[31,35,96,97] and others reporting no correlation[21–23,62,63]. In contrast, most studies report no correlation between acyl-ghrelin concentrations and subsequent intake at an *ad libitum* meal[23,62,63,92,97].

1.4.5 Long-term effects of exercise on acyl-ghrelin concentrations

Results from exercise training studies (≥12 weeks) show mixed results regarding the effect of chronic exercise on fasting acyl-ghrelin concentrations. Most studies report no effect on fasting acyl-ghrelin concentrations following exercise training[47,98–100]. However, studies which measure total ghrelin (acyl- and deacyl ghrelin) report increased concentrations following exercise training[44,101,102]. The increase in total ghrelin (and perhaps acyl-ghrelin) could be a homeostatic response to exercise-induced weight loss in which the body increases energy intake to return body weight to pre-exercise training levels. Another possibility is that long-term exercise may modify desacyl-ghrelin concentrations (i.e. acyl-ghrelin concentrations may not change but total ghrelin may) but this is yet to be directly investigated. This uncertainty is also reflected in the relationships between changes in acyl-ghrelin concentrations and body weight during exercise training, for which negative[100] and no correlations[44,102] have been reported. Furthermore, exercise training does not appear to influence postprandial acyl-ghrelin concentrations following a standardized meal[44,47,100].

1.4.6 GLP-1

GLP-1 is an incretin hormone secreted from enteroendocrine L-cells of the gastrointestinal tract[103]. Its effects are mediated through the ubiquitously expressed GLP-1 receptor, which is found in the kidney, pancreas, adipose tissue, and central nervous system amongst other tissues[104]. GLP-1 is produced via the post-translational cleavage of proglucagon by prohormone convertase 1/3 (also known as proprotein convertase subtilisin-kexin type 1)[105].

GLP-1 is released from L-cells in two equipotent biologically active forms: GLP-$1_{7\text{-}36}$ and GLP-$1_{7\text{-}37}$[103]. GLP-$1_{7\text{-}36}$ is the amidated form of GLP-1 and accounts for approximately 80% of circulating levels[106], whereas GLP-$1_{7\text{-}37}$ is the glycine-extended form of GLP-1 and accounts for around 20% of circulating levels[106]. However, only 10-15% of secreted GLP-1 enters the systemic circulation due to degradation by endothelial and hepatic dipeptidyl peptidase-4, forming the metabolites GLP-$1_{9\text{-}36}$ and GLP-$1_{9\text{-}37}$[103]. Initially thought to be biologically redundant cleavage products, evidence suggest that GLP-$1_{9\text{-}36}$ and GLP-$1_{9\text{-}37}$ may influence glucoregulation (albeit weakly)[107] as well as exert cardio-and neuroprotective actions[108]. GLP-$1_{9\text{-}36}$ and GLP-$1_{9\text{-}37}$ can be further cleaved by neprilysin into the metabolites GLP-$1_{28\text{-}36}$, GLP-$1_{32\text{-}36}$, GLP-$1_{28\text{-}37}$ and GLP-$1_{32\text{-}37}$[108]. These peptides also have putative roles in regulating glucose homeostasis and energy expenditure[108].

Due to the rapid metabolism of GLP-1 following its release, GLP-1 secretion is best estimated by the sum of active and inactive GLP-1 in the systemic circulation[84]. Hence, this thesis will generally refer to ‘GLP-1’ instead of a specific GLP-1 form.

GLP-1 possesses pleiotropic effects on the human body. Of relevance, GLP-1 increases satiety[109], reduces food intake[110] and is likely involved in the activation of the ileal brake (the principal inhibitory feedback mechanism that regulates the transit of ingested food through the gastrointestinal tract to increase nutrient digestion and absorption)[103]. GLP-1 likely exerts its anorectic effect via multiple routes, including activation of sensory afferent neurons within the nodose ganglion which then relays impulses to the hypothalamus, and binding to central GLP-1 receptors in the hypothalamus[103]. GLP-1 also has important roles in liver[111] and skeletal muscle metabolism[112], as well as glucose homeostasis (GLP-1 is a potent insulin secretagogue)[113].

1.4.7 GLP-1 secretion

Circulating GLP-1 concentrations are primarily determined by meal intake, with low levels found during periods of fasting and rapid increases observed following meal ingestion[105]. Protein, carbohydrate and lipids all elicit GLP-1 responses, with protein arguably the most potent of the three macronutrients[83].

GLP-1 secretion can exhibit a monophasic (a single peak in concentrations) or biphasic (two peaks in concentrations) postprandial pattern[114]. The mechanisms responsible for determining the GLP-1 response pattern are however not fully understood but are thought to be related to differences in gastric emptying rate and meal digestibility[84]. GLP-1 is typically secreted within 5-15 minutes of meal ingestion, with concentrations peaking around 30 minutes post-meal ingestion and returning to baseline concentrations 3-4 hours post-meal ingestion[84]. GLP-1-secreting cells (L-cells) express a variety of nutrient receptors (including the calcium-sensing receptor, free fatty acid receptors and glucose transporters) that are stimulated by a variety of digestive products (e.g. amino acids, mono- and di-saccharides, fatty acids); this nutrient sensing by the L cell is believed to be the primary regulator of GLP- release[84]. GLP-1 release may also be controlled by nutrient-induced bile secretion[115] and vagal signalling[116].

1.4.8 The effect of a single bout of exercise on GLP-1 concentrations

Acute exercise is also a potent stimulator of GLP-1. Aerobic exercise increases GLP-1 concentrations both during and post-exercise, with a rise observed 15 minutes following exercise onset[117] and GLP-1 levels remaining elevated for 30-90 minutes following exercise cessation[23,24,28,30,69]. Elevated GLP-1 levels are reported following both continuous[97] and high-intensity interval aerobic exercise[28]. However, the effect of resistance exercise on GLP-1 levels is less clear, with the single study to measure GLP-1 concentrations reporting no significant effect[32]. This increase in GLP-1 concentrations can be nonetheless observed in various populations following aerobic exercise, including individuals with overweightness/obesity[33], as well as both males[117] and females[28]. Indeed, post-exercise GLP-

1 responses are comparable in magnitude amongst men and women, and between lean individuals and individuals with overweightness/obesity[95]. Exercise-induced GLP-1 release also exhibits no differences between moderate-intensity (~65% $\dot{V}O_2$ max) and high-intensity interval exercise[24,28,33].

1.4.9 Relationship between exercise-induced changes in GLP-1 concentrations, subjective appetite and energy intake

Relative to acyl-ghrelin concentrations, few studies have investigated the relationship between GLP-1 concentrations, subjective appetite and energy intake during and post-exercise. Exercise-induced changes in GLP-1 concentrations do not seem to correlate with changes in subjective appetite measures[23,62,97] but may predict subsequent *ad libitum* energy intake[97,117].

1.4.10 Long-term effects of exercise on GLP-1 concentrations

Studies investigating exercise training interventions (≥12 weeks) report no changes in fasting or postprandial GLP-1 concentrations[44,99,100,102]. Furthermore, individual changes in GLP-1 concentrations have been shown to both correlate (a larger increase in GLP-1 was associated with lower body weight)[102] and not correlate[44] with changes in bodyweight due to exercise training. Exercise training may however increase post-exercise GLP-1 concentrations following a single bout of aerobic exercise[102].

1.4.11 PYY

PYY is a 36 amino acid peptide synthesised and released, like GLP-1, from L-cells of the gastrointestinal tract. It is part of the 'pancreatic polypeptide-fold family', which comprises pancreatic polypeptide, neuropeptide Y and PYY[118]. These hormones share a hair-pin-fold motif structure and act via the G-protein coupled receptors Y1, Y2, Y4, Y5 and Y6 (with the Y6 present in rabbits and mice, but not humans)[119].

PYY circulates in two forms: PYY_{1-36} and PYY_{3-36}. PYY_{1-36} is prevalent in the fasted state and activates all five Y receptors[120]. In contrast, PYY_{3-36} is the predominant form postprandially and is produced via the cleavage of PYY_{1-36} by dipeptidyl peptidase-4[120]. This modification also alters its selectivity, resulting in PYY_{3-36} possessing a high affinity for the Y2 receptor and a moderate affinity for Y1 and Y5 receptors[118].

PYY reduces subjective appetite and energy intake[121], and along with GLP-1 is implicated in activation of the ileal brake[122]. Like acyl-ghrelin and GLP-1, PYY likely exerts these effect via multiple routes, including binding to Y2 receptors in the hypothalamus and binding to Y2 receptors on vagal afferent fibres[120]. PYY also inhibits gastric acid secretion, pancreatic enzymes and gall bladder contractions, and may increase both energy expenditure and fat oxidation[121,123].

Owing to the difficulties in assaying PYY_{3-36}[124–126], this thesis will refer to a non-specific 'PYY' instead of either PYY_{1-36} or PYY_{3-36}.

1.4.12 PYY secretion

PYY concentrations are low in the fasted state and increase within 15 minutes of meal ingestion[118]. This rise in PYY concentrations is proportional to the energy content of the meal[127]. All three primary macronutrients (carbohydrate, protein and fat) promote the release of PYY, with protein reported to be the most potent stimulator of PYY[83].

PYY is secreted within 15-30 minutes of meal ingestion, peaking 60-90 minutes postprandially and then returning to baseline levels within 3-4 hours[128–131]. PYY release from L-cells appears to be stimulated by nutrient detection via membrane receptors (such as the calcium-sensing receptor and taste receptors) and neurohumoral reflexes[84]. For example, human studies demonstrate that infusion of cholecystokinin increases circulating PYY[132] concentrations, whereas GLP-1 infusions decrease systemic concentrations[133]. Bile acid secretion may also be an important regulator of PYY release[134] and animal models suggest a putative role for the vagus nerve[135,136].

1.4.13 The effect of a single bout of exercise on PYY concentrations

Acute exercise also stimulates PYY release. Aerobic exercise increases PYY concentrations during (within 15 minutes of commencement)[117] and following exercise completion (elevated for 30 minutes following cessation)[21,117,137]. Individuals with overweightness/obesity[32], as well as males[137] and females[24] all exhibit this response. However, resistance exercise does not appear to influence PYY concentrations[21,32,65]. There appears to be no differences between males and females in the magnitude of PYY release[95], with higher intensities possibly producing a greater PYY release[24,35,138]. Furthermore, evidence suggests exercise-induced PYY release may be greater in lean individuals relative to individuals with overweightness/obesity[23], but this is not a consistent finding across studies[30].

1.4.14 Relationship between exercise-induced changes in PYY concentrations, subjective appetite and energy intake

Like GLP-1, few studies have undertaken correlational analyses between PYY, subjective appetite and energy intake. The findings from those studies that have are mixed, with studies reporting significant correlations[31,35,97,139] and no correlations[21,23,63,117] between PYY, subjective appetite and *ad libitum* energy intake.

1.4.15 Long-term effects of exercise on PYY concentrations

Most chronic exercise interventions lasting ≥12 weeks show no effect on fasting or postprandial PYY concentrations[44,47,99,100,140]. Only Jones et al.[98] reported an increase in fasting PYY concentrations following a 32-week exercise training programme. Exercise

training may however increase the post-exercise PYY response following an acute bout of exercise[102]. Nevertheless, there appears to be no relationship between changes in PYY concentrations and changes in bodyweight during exercise training [44,101,102].

1.5 Mechanisms underlying the acute effect of exercise on changes in gastrointestinal hormones

Several mechanisms have been proposed for the changes in acyl-ghrelin, GLP-1 and PYY concentrations during and following acute exercise[141]. These include increased sympathetic nervous system (SNS) activity, decreased splanchnic blood flow, decreased gastric emptying rate, increased interleukin-6 production, increased non-esterified fatty acid concentrations, and increased lactate concentrations.

1.5.1 Increased sympathetic nervous system activity

At the onset of acute exercise, central command (feedforward increase in SNS activity that accompanies skeletal muscle contraction) and exercise pressor reflex (skeletal muscle afferents that respond to mechanical and chemical stimuli) mechanisms operate to increase SNS activity and catecholamine release[142,143]. Acute exercise increases SNS activity and circulating catecholamine concentrations (adrenaline and noradrenaline) in an intensity-dependent manner[144–146].

With regards to the effect of the SNS on gastrointestinal hormone release, treating isolated perfused rat ileum with adrenaline increases PYY and GLP-1 secretion via activation of adrenergic receptors on L-cells[147]. Similarly, infusion of beta-adrenergic agonists into perfused rat colon stimulates PYY secretion[148]. Despite acylated ghrelin concentrations negatively correlating with noradrenaline and adrenaline concentrations during and post-exercise[149], *in vitro* and *in vivo* data suggest that increased SNS activity and catecholamine release promote ghrelin secretion. Adrenaline and noradrenaline stimulate ghrelin release from rodent-derived stomach ghrelinoma cell lines[150,151]. Moreover, electrical stimulation of sympathetic nerves in Wister rats increases circulating ghrelin concentrations, but interestingly adrenaline administration does not[152].

The increase in SNS activity observed with exercise also results in vasoconstriction of splanchnic blood vessels (and consequent decrease in splanchnic blood flow), decreased gastric emptying rate and increased non-esterified fatty acid concentrations[141]. These downstream effects may be - at least in part - responsible for the modulation of gut hormone concentrations induced by acute exercise and are consequently discussed below.

1.5.2 Decreased splanchnic blood flow

The splanchnic circulation comprises the hepatic, celiac, superior mesenteric and inferior mesenteric arteries which supplies the splanchnic organs (stomach, small intestine, large

intestine, pancreas, spleen and liver). Acute exercise decreases splanchnic blood flow in proportion to exercise intensity, allowing a greater proportion of cardiac output to be redistributed to working skeletal muscle[153–155]. This decrease in splanchnic blood flow occurs within 10 minutes of exercise onset and returns to resting values within 10 minutes of exercise cessation[153,156].

Splanchnic hypoperfusion can induce gastric ischemia and consequently increase local inflammation, intestinal damage and permeability[157]. Rodent models have also demonstrated that gastric ischemia decreases acylated ghrelin concentrations and food intake[158,159]. The reduction in splanchnic blood flow may inhibit ghrelin release into the systemic circulation[141], or the mucosal injuries caused by gastric ischemia may decrease ghrelin production in the enteroendocrine cells found in the mucosal layer[159]. These mechanisms are however speculative and do not account for the concurrent increase in PYY and GLP-1 concentrations observed during and post-exercise[68]. This suggests that decreased gastrointestinal blood flow is not primarily responsible for the changes in gut hormone concentrations with exercise, or that the increase in GLP-1 and PYY is driven by a mechanism distinct to that regulating ghrelin release.

1.5.3 Decreased gastric emptying rate

Relative to resting conditions, high-intensity exercise (>70% $\dot{V}O_2$ max) decreases gastric emptying rate (the speed at which constituents leave the stomach) whereas low-intensity exercise (<40% $\dot{V}O_2$ max) accelerates gastric emptying rate[160]. A decrease in the rate of gastric emptying would prolong gastrointestinal tract exposure to nutrients, possibly augmenting the release of GLP-1/PYY and suppression of acyl-ghrelin[161]. This may explain why changes to gut hormone concentrations are most likely to be seen with higher-intensity exercise protocols. Nevertheless, it is possible that exercise-induced decreases in gastric emptying rate are a consequence of gut hormone modulation rather than a cause, owing to GLP-1 and PYY's role in the ileal brake[103,122]. Indeed, the release of GLP-1 and PYY in response to acute exercise is observed in the overnight fasted state, and therefore the prolongation of nutrient exposure due to a decrease in gastric emptying is unlikely to be the primary mechanism underlying exercise-induced modulation of gastrointestinal hormones[21,23,137].

1.5.4 Increased interleukin-6 production

Interluekin-6 (IL-6) is a cytokine that is secreted by various cell types and involved in a diverse set of physiological functions[162]. IL-6 concentrations increase during and following acute exercise in proportion to intensity and duration, with the rise in circulating IL-6 being primarily driven by production in skeletal muscle[163]. Human data suggests that changes in IL-6 may

explain changes in gastrointestinal hormones following an acute bout of exercise. GLP-1, acylated ghrelin, subjective appetite and energy intake have all been demonstrated to correlate with circulating IL-6 concentrations during the post-exercise period[164–166]. This is supported by rodent data demonstrating that IL-6 is required for exercise-induced anorexia and can stimulate GLP-1 release from L-cells[167,168]. IL-6 may also act centrally on key neural circuits implicated in energy homeostasis[169,170]. Indeed, muscle-specific deletion of IL-6 influences bodyweight in mice, possibly via the modulation of the appetite-regulating neuropeptides agouti-related peptide (AgRP), proopiomelanocortin (POMC), and neuropeptide Y (NPY)[171].

1.5.5 Increased non-esterified fatty acid concentrations

Circulating levels of non-esterified fatty acids (NEFAs; also known as free fatty acids) increases two- to four-fold with acute moderate-intensity exercise (45% $\dot{V}O_2$ max)[172] and can remain elevated for up to two hours following exercise termination[173]. This increase in NEFA availability is thought to be primarily driven by the suppression of insulin and increase in catecholamine concentrations that also accompany exercise, which consequently increases lipolysis and NEFA release from adipose tissue[172]. NEFAs released from adipose tissue vary in their carbon length and degree of saturation, with NEFAs with a higher degree of unsaturation and smaller chain length predominating following exercise[174,175]. These fatty acids (which include short-, medium- and long-chain fatty acids) are a major energy source during both rest and exercise, as well as being putative signalling molecules in the regulation of energy balance[176–178].

Human studies using pancreatic-pituitary and hyperinsulinaemic glucose clamps (to eliminate interference from insulin and growth hormone) demonstrate that intravenous NEFA infusion suppresses circulating ghrelin levels in a dose-dependent manner[179,180]. Intravenous NEFA infusions in humans not using pancreatic-pituitary and hyperinsulinaemic glucose clamps also significantly suppressed ghrelin, but showed no effect on PYY or GLP-1 concentrations relative to saline[181,182]. Nevertheless, it has been shown that intravenous lipid infusion increases acylated ghrelin concentrations in rodents[183] and that NEFA concentrations positively correlate with both ghrelin and hunger in humans and animal models respectively[184–186]. The reason for these discrepant findings is unclear, but may relate to the context in which high NEFA concentrations are present (e.g. postprandial, fasting, post-exercise) and the accompanying changes in other physiological factors (e.g. hormones) that are present in these situations. Alternatively, differences in the composition of the plasma NEFA pool (i.e. fatty acid chain length and saturation) may also play a role, considering NEFAs exert divergent physiological effects depending on these characteristics[187].

It is important to note that NEFA mobilisation from adipose tissue decreases with increasing exercise intensity, such that high-intensity exercise (85% $\dot{V}O_2$ max) results in a significantly lower rate of NEFA appearance compared to lower exercise intensities (25% and 50%)[188,189]. This is incongruent with the notion that exercise-induced changes in gastrointestinal hormone concentrations are intensity-dependent. However, post-exercise circulating NEFA levels show a transient spike that is also related to intensity, with higher intensities (60 vs 40% $\dot{V}O_2$ max) resulting in a greater peak NEFA response[190]. NEFA concentrations may therefore play a greater role in post-exercise appetite suppression (compared to appetite suppression during exercise) when high-intensity exercise is performed.

1.5.6 Increased lactate concentrations

Circulating lactate concentrations increase during exercise as a result of skeletal muscle lactate production exceeding removal, with levels remaining elevated for 30-60 minutes post-exercise[191–193]. Importantly, plasma lactate concentrations remain similar to resting levels during moderate-intensity (40% $\dot{V}O_2$ max) exercise and only rise with high-intensity exercise (>70% $\dot{V}O_2$ max)[193] .

Treatment of primary gastric mucosal cells with physiological concentrations of lactate (1-10 mM) inhibits the secretion of acyl-ghrelin via the lactate receptor GPR81 (which is highly expressed in gastric ghrelin cells)[194]. Furthermore, post-exercise changes in lactate are associated with changes in acyl-ghrelin and GLP-1 concentrations[166], while central and intravenous administration of lactate suppresses energy intake in animals[195] and humans[196], respectively. However, neither the relationship between lactate and PYY during the post-exercise period nor the effect of lactate administration on PYY release has been investigated.

1.6 The role of pancreatic hormones in exercise-induced changes in subjective appetite and energy intake

Most research investigating the relationship between acute exercise and changes in subjective appetite and energy intake has focused on the release of the gastrointestinal hormones acyl-ghrelin, GLP-1 and PYY. This is likely due to their well-established and prominent roles in appetite regulation[74,103,118]. However, a single bout of exercise also modulates the systematic concentration of several other hormones, including insulin and glucagon[30]. These hormones are commonly associated with glucoregulatory functions but are also implicated in appetite regulation[197,198].

1.6.1 Insulin and appetite regulation

Insulin is a 51 amino acid peptide that is produced and released into the systemic circulation by pancreatic beta cells, its release is predominantly stimulated by elevated circulating levels of amino acids and glucose[199]. Following its release, insulin binds to the insulin receptor (which

exists in two isoforms, A and B) in order to exert various metabolic effects, including glycogenesis, fatty acid uptake, and glucose uptake[199,200]. Despite the insulin receptor being found in essentially all human cells, it primarily exerts its metabolic effects on skeletal muscle, adipose tissue, the liver, and neurons[201].

Insulin is capable of crossing the blood-brain barrier via a saturable transport system, allowing it to exert central effects[202]. The insulin receptor is widely expressed in the brain, most notably in the hypothalamus, a region of the brain that is central to appetite regulation[203]. Insulin has been to show to stimulate anorexigenic neurons (e.g. POMC and cocaine-amphetamine-regulated-transcript [CART] neurons) and inhibit orexigenic neurons (e.g. NPY and AgRP) present in the arcuate nucleus of the hypothalamus[204–206]. Clinical data also supports a role of insulin in appetite regulation, with insulin administration shown to reduce energy intake and induce satiety in individuals with and without obesity[207].

1.6.2 Glucagon and appetite regulation

Glucagon is a 29 amino acid formed from the precursor molecule proglucagon and secreted into the systemic circulation by the alpha cells of the pancreas[208]. Glucagon is traditionally recognised as an anti-hypoglycaemic hormone that is released in response to low blood glucose concentrations[209]. However, glucagon is also released in circumstances of psychological and metabolic stress[210]. Glucagon exerts its effects by binding to the glucagon receptor, which is widely expressed throughout the body in organs such as the liver, kidney, adipose tissue, pancreas, heart, and brain[211]. Once bound, glucagon can induce a variety of physiological effects including hepatic gluconeogenesis and lipolysis, as well as increasing heart rate and energy expenditure[212].

Rodent models have also shown that glucagon can suppress energy intake[213]. While the mechanism underlying this effect is not fully understood, it is believed to be mediated by the liver-vagus-hypothalamus axis[212]. This hypothesis is based on studies demonstrating that hepatic portal glucagon infusions suppress energy intake (whereas glucagon infusion into the vena cava at the same dose produces no effect), the infusion of glucagon antibodies results in an increase in energy intake, and that the anorectic effect of glucagon is abolished following hepatic vagotomy[213,214]. Nevertheless, clinical data investigating the effect of glucagon administration on energy intake is less consistent, with studies showing both a suppression of energy intake[215] and no effect[216].

1.7 Identification of novel mediators of exercise-induced changes in subjective appetite and energy intake via metabolomics

Recent advancements in technology have permitted the quantification of a wide array of non-traditional metabolites in biological samples; commonly referred to as metabolomics[217]. This

analytical technology has now become widespread in medical science, and has subsequently been applied to the field of exercise physiology[218]. Studies employing metabolomic analyses have repeatedly demonstrated that acute exercise bouts cause large changes in metabolite concentrations, generally characterised by an increase in lactate, pyruvate, fatty acids, acylcarnitines, nucleotides, and ketone bodies, as well as a decrease in bile acids (effects on amino acids are mixed)[219]. Nevertheless, many of these studies use pre-post designs[220–223], inappropriate control groups[224,225], or comparisons between exercise modalities only[226]. The inferences that can be drawn from such data are therefore often limited. Additionally, no study has investigated changes in the metabolome in response to a combination of diet and exercise interventions, and how these changes are associated with exercise-induced changes in subjective appetite, energy intake, and gastrointestinal hormone release. The identification of novel metabolic mediators of exercise-induced suppression of appetite and energy intake could facilitate the development of future therapeutics aimed at body weight reduction.

1.8 The role of diet in exercise-induced changes in subjective appetite, energy intake and gastrointestinal hormone release

Chronic exercise results in a compensatory increase in energy intake characterised by both an increased drive to eat but also an enhanced satiating efficiency of a fixed meal[46,227]. The decisions that individuals make with respect to food choice and timing to meet this increased drive may therefore explain the discrepancy between the acute and chronic effects of exercise on energy balance. Indeed, acute laboratory studies investigating exercise and energy balance often control when and what is eaten[35,138]. This method contrasts that of exercise training interventions, in which participants are studied in free-living situations and consequently have full autonomy on food-related decisions[47,99,102]. Identifying dietary patterns or choices that modify the appetite response to exercise may facilitate the development of strategies to increase the potency of exercise as a long-term weight loss tool.

1.9 Fed versus fasted exercise

An important decision to be made by all individuals is whether to consume a meal prior to exercise commencement. Performing exercise in the fasted stated (>6 hours since last meal) has recently grown in popularity with individuals looking to decrease bodyweight because of research demonstrating increased fat oxidation during fasted relative to fed exercise[228]. Regularly performing exercise in the fasted state is consequently believed to augment fat and bodyweight loss. Despite its popularity, increased fat oxidation does not necessarily translate to a decrease in bodyweight; only a long-term change in energy balance (energy balance = energy intake – energy expenditure) will result in a decrease in bodyweight[10].

While performing exercise in the fed or fasted state undoubtedly increases energy expenditure, the impact of any diet-exercise strategy on appetite and energy intake is likely to

be of more importance due to the relatively small energy deficit created by a single exercise bout[229].

1.9.1 The acute effect of fed versus fasted exercise on subjective appetite

It has been conclusively demonstrated that acute exercise in the fed state significantly reduces subjective appetite relative to exercise in the fasted state throughout the experimental period[230–237]. Interestingly, fasted exercise significantly decreases subjective appetite during the exercise period i.e. a decrease between pre- and post-exercise measurements[232,234]; an effect that is not present with fed exercise.

1.9.2 The acute effect of fed versus fasted exercise on post-exercise *ad libitum* energy intake

Despite the greater decrease in subjective appetite observed with fed exercise, there appears to be no difference in energy intake at an *ad libitum* meal served post-exercise[230,232,236–239]. When considering the total intake consumed during the experimental period (including the energy content of the pre-exercise meal), fasted exercise consequently results in a significantly lower total energy intake[232,237]. Indeed, some studies have reported a lower 24-hour energy intake following fasted exercise relative to fed exercise[236,239] but this is not always found[230,235]. It is also important to note that there appears to be no difference in energy expenditure - at least when measured in the laboratory environment - between fasted and fed exercise[234,237–239]. This suggests that the possible energy deficit created by fasted exercise is not acutely compensated for by a change in energy expenditure.

1.9.3 Medium to long-term effect of fed versus fast exercise on *ad libitum* energy intake

Despite the differences in energy balance between fed and fasted exercise observed acutely, this does not appear to translate to any meaningful effect when performed chronically. Long-term training interventions (≥4 weeks) report no significant differences in body mass between fed and fasted exercise groups, regardless of training status (i.e. trained vs untrained) and sex[240]. However, these studies only employed aerobic exercise as an intervention and therefore the effect of chronic resistance exercise performed in the fasted relative to fed state is unknown.

1.9.4 The acute effect of fed versus fasted exercise on gastrointestinal hormone release

Very few studies have investigated the acute effect of fed and fasted exercise on acyl-ghrelin, GLP-1 and PYY release. Fasted exercise increases acyl-ghrelin concentrations during exercise[231,233] and possibly in the postprandial period relative to fed exercise[237]. The effect of fed and fasted exercise on GLP-1 and PYY concentrations is difficult to interpret due to an insufficient number of studies. There appears to be no difference in GLP-1 concentrations between fed and fasted exercise[232], and possibly an increase in PYY concentrations following fed exercise[231].

1.9.5 Timing of pre-exercise meal

Most studies comparing the acute effect of fed versus fasted exercise on subjective appetite and energy intake provide a pre-exercise meal 1 - 2.5 hours prior to exercise commencement[230–237,239]. Therefore, little is known regarding the effects of a pre-exercise meal consumed less than 1 hour or more than 2.5 hours prior to exercise commencement on subjective appetite and energy intake. Interestingly, the only study to use a time interval outside this period reported that consuming a meal 30 mins before exercise results in no difference between fed and fasted exercise in subjective appetite during the course of the study visit[238]. This suggest that timing of the pre-exercise meal may influence the subsequent subjective appetite response to exercise, ultimately having implications for energy balance.

Timing of the pre-exercise meal may also influence the prevalence and severity of gastrointestinal symptoms experienced during exercise. Kondo et al.[241] found that immediately performing high-intensity exercise (70-80% heart rate reserve) after meal consumption increased the prevalence and severity of subjective nausea.

1.9.6 Composition of pre-exercise meal

The pre-exercise meal selected by studies comparing fed and fasted exercise is often high in carbohydrate (>55% carbohydrate by energy content) and provides ~300-700 kcal of energy[230,232–237]. Moreover, this meal is typically grain-based (e.g. oats) and consumed with a liquid energy source (e.g. milk or orange juice). This type of meal is likely selected as it is representative of the foods consumed prior to exercise[242]. Individuals are recommended to consume a high-carbohydrate meal prior to exercise to maximise performance during the exercise period[243]. This has however resulted in a limited understanding of the influence of a pre-exercise meal high in protein or fat on subjective appetite and energy intake following an acute exercise bout. Despite its ergogenic benefits, high carbohydrate intake may increase the risk of gastrointestinal complaints. High carbohydrate intakes are correlated with an increased prevalence of nausea during endurance events[244]. This is believed to be due to an increase in water retention caused by the incomplete absorption of carbohydrate[245].

1.9.7 Carbohydrate metabolism during acute exercise and appetite regulation

Endogenous carbohydrate stores in the form of skeletal muscle glycogen and liver glycogen are a major energy source during acute exercise[246]. Relative to endogenous lipid stores (>100,000 kcal), carbohydrate is severely restricted in its storage capacity (<1200 kcal)[193]. The status of endogenous carbohydrate stores has consequently been proposed as a potential regulator of energy balance and mediator of energy compensation following an exercise-induced energy deficit[247].

Despite reports of whole-body carbohydrate utilization during exercise being positively associated with post-exercise energy compensation[248], the relationship between endogenous glycogen stores and energy balance is believed to be tissue specific[247]. Higher rates of hepatic-derived glucose utilization during exercise are associated with an increase in post-exercise energy compensation, whereas no such effect was reported for skeletal muscle glycogen utilization[239]. This is believed to be due to the liver's gluconeogenic capacity and consequent provision of a systemic glucose source[247]. Skeletal muscle cannot provide systemic glucose due to the absence of a key enzyme involved in the metabolism of glycogen to free glucose - glucose 6-phosphatase[249]. The mechanisms by which hepatic glycogen may regulate energy balance are not yet fully understood, but evidence from animal models implicate neural signalling via the vagus nerve and/or endocrine signalling via Fibroblast growth factor 21[247].

Nutrient intake influences fuel utilization during exercise[243]. The consumption of exogenous carbohydrate before or during exercise may therefore decrease hepatic glycogen utilization and compensatory responses to exercise-induced energy deficits[247]. However, this may be modulated by the type of carbohydrate (e.g. glucose vs fructose), timing of carbohydrate ingestion (e.g. immediately before or 2 hours before), as well as exercise intensity, duration and modality.

1.9.8 Carbohydrate intake and gastrointestinal hormone release

Ingestion of carbohydrate suppresses the release of acyl ghrelin and stimulates the release of GLP-1 and PYY[83]. This hormonal response mirrors that produced during and following an acute bout of exercise[68]. However, there is currently no information into the additive or possibly synergetic effects of combining carbohydrate ingestion with acute exercise on acyl-ghrelin, GLP-1 and PYY concentrations, and its subsequent influence on subjective appetite and energy intake.

1.10 Synopsis and general aims

A single bout of exercise results in a decrease in subjective appetite and relative energy intake. If this response persists over time, chronic exercise interventions should result in substantial weight loss. Contradicting this prediction, regularly performing exercise for >12 weeks produces modest weight loss, especially when compared to dietary energy restriction. The reason for this discrepancy is largely attributed to compensatory eating behaviours following exercise energy expenditure, rather than a compensatory decrease in energy expenditure. Understanding the mechanisms by which acute exercise influences appetite and the impact of diet on this response may facilitate the development of strategies that improve the effectiveness of chronic exercise as a weight loss tool.

The decrease in subjective appetite and relative energy intake following acute exercise is thought to be due to modulation of gastrointestinal hormones related to appetite, primarily acyl-ghrelin, GLP-1 and PYY. However, other hormones such as insulin and glucagon may also be involved. Furthermore, advancements in technology such as metabolomics now enable the identification of metabolites that may also play a causal role in exercise induced changes in appetite and energy intake.

The metabolic response during and following exercise is likely influenced by provision, type and timing of pre-exercise nutrient ingestion. Dietary carbohydrate is widely recommended for consumption prior to exercise due to its ergogenic properties, and is also capable of influencing gastrointestinal hormone release. Despite its widespread use, there is no research investigating the effects of carbohydrate consumption less than 30 minutes prior to exercise commencement (a dietary strategy employed by many recreational athletes) on gastrointestinal hormone release, subjective appetite, and energy intake.

1.10.1 General aims:

Chapter 2:

- To determine the effect of acute exercise on insulin and glucagon concentrations
- To investigate the impact of pre-exercise nutritional state on insulin and glucagon responses to acute exercise

Chapter 3:

- To better understand the role of glucagon in energy intake and subjective appetite in humans

Chapter 4:

- To examine the influence of carbohydrate ingestion in the 30-minute period prior to exercise on gastrointestinal hormone release, subjective appetite, and energy intake.
- To explore the potential mechanisms by which acute exercise modulates subjective appetite and energy intake.
- To identify novel metabolic mediators of exercise-induced changes in subjective appetite and energy intake

1.11 References

1. WHO. Obesity and overweight factsheet. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (2018).

2. Wang, Y. C., McPherson, K., Marsh, T., Gortmaker, S. L. & Brown, M. Health and economic burden of the projected obesity trends in the USA and the UK. *Lancet* **378**, 815–825 (2011).

3. Schnurr, T. M. *et al.* Obesity, unfavourable lifestyle and genetic risk of type 2 diabetes: a case-cohort study. *Diabetologia* **63**, 1324–1332 (2020).

4. Rashid, M. N., Fuentes, F., Touchon, R. C. & Wehner, P. S. Obesity and the Risk for Cardiovascular Disease. *Prev. Cardiol.* **6**, 42–47 (2003).

5. Gallagher, E. J. & LeRoith, D. Obesity and Diabetes: The Increased Risk of Cancer and Cancer-Related Mortality. *Physiol. Rev.* **95**, 727–748 (2015).

6. McKinsey Global Institute. Overcoming obesity: An initial economic analysis. (2014).

7. O'Neil, P. M. *et al.* Efficacy and safety of semaglutide compared with liraglutide and placebo for weight loss in patients with obesity: a randomised, double-blind, placebo and active controlled, dose-ranging, phase 2 trial. *Lancet* **392**, 637–649 (2018).

8. O'Brien, P. E. *et al.* Long-Term Outcomes After Bariatric Surgery: a Systematic Review and Meta-analysis of Weight Loss at 10 or More Years for All Bariatric Procedures and a Single-Centre Review of 20-Year Outcomes After Adjustable Gastric Banding. *Obes. Surg.* **29**, 3–14 (2019).

9. Hill, J. O. Understanding and Addressing the Epidemic of Obesity: An Energy Balance Perspective. *Endocr. Rev.* **27**, 750–761 (2006).

10. Hall, K. D. *et al.* Quantification of the effect of energy imbalance on bodyweight. *Lancet* **378**, 826–837 (2011).

11. Hubert, P., King, N. A. & Blundell, J. E. Uncoupling the effects of energy expenditure and energy intake: appetite response to short-term energy deficit induced by meal omission and physical activity. *Appetite* **31**, 9–19 (1998).

12. Miller, W., Koceja, D. & Hamilton, E. A meta-analysis of the past 25 years of weight loss research using diet, exercise or diet plus exercise intervention. *Int. J. Obes.* **21**, 941–947 (1997).

13. Verheggen, R. J. H. M. *et al.* A systematic review and meta-analysis on the effects of exercise training versus hypocaloric diet: distinct effects on body weight and visceral

adipose tissue. *Obes. Rev.* **17**, 664–690 (2016).

14. Ross, R. & Janssen, I. Physical activity, total and regional obesity: dose-response considerations. *Med. Sci. Sports Exerc.* **33**, S521-7 (2001).

15. Thomas, D. M. *et al.* Why do individuals not lose more weight from an exercise intervention at a defined dose? An energy balance analysis. *Obes. Rev.* **13**, 835–47 (2012).

16. Fedewa, M. V., Hathaway, E. D., Williams, T. D. & Schmidt, M. D. Effect of Exercise Training on Non-Exercise Physical Activity: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Sport. Med.* **47**, 1171–1182 (2017).

17. Blundell, J. *et al.* Appetite control: methodological aspects of the evaluation of foods. *Obes. Rev.* **11**, 251–270 (2010).

18. Stubbs, R. J. *et al.* The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *Br. J. Nutr.* **84**, 405–415 (2000).

19. Flint, A., Raben, A., Blundell, J. E. & Astrup, A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int. J. Obes.* **24**, 38–48 (2000).

20. Holt, G. M. *et al.* Systematic literature review shows that appetite rating does not predict energy intake. *Crit. Rev. Food Sci. Nutr.* **57**, 3577–3582 (2017).

21. Broom, D. R., Batterham, R. L., King, J. A. & Stensel, D. J. Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. *Am. J. Physiol. Integr. Comp. Physiol.* **296**, R29–R35 (2009).

22. Broom, D. R. *et al.* Acute effect of exercise intensity and duration on acylated ghrelin and hunger in men. *J. Endocrinol.* **232**, 411–422 (2017).

23. Douglas, J. A. *et al.* Acute effects of exercise on appetite, ad libitum energy intake and appetite-regulatory hormones in lean and overweight/obese men and women. *Int. J. Obes.* **41**, 1737–1744 (2017).

24. Hazell, T. J., Townsend, L. K., Hallworth, J. R., Doan, J. & Copeland, J. L. Sex differences in the response of total PYY and GLP-1 to moderate-intensity continuous and sprint interval cycling exercise. *Eur. J. Appl. Physiol.* **117**, 431–440 (2017).

25. King, N. A., Burley, V. J. & Blundell, J. E. Exercise-induced suppression of appetite:

effects on food intake and implications for energy balance. *Eur. J. Clin. Nutr.* **48**, 715–24 (1994).

26. Kawano, H. *et al.* Effects of different modes of exercise on appetite and appetite-regulating hormones. *Appetite* **66**, 26–33 (2013).

27. Tsofliou, F., Pitsiladis, Y. P., Malkova, D., Wallace, A. M. & Lean, M. E. J. Moderate physical activity permits acute coupling between serum leptin and appetite–satiety measures in obese women. *Int. J. Obes.* **27**, 1332–1339 (2003).

28. Hallworth, J. R., Copeland, J. L., Doan, J. & Hazell, T. J. The Effect of Exercise Intensity on Total PYY and GLP-1 in Healthy Females: A Pilot Study. *J. Nutr. Metab.* **2017**, (2017).

29. Holliday, A. & Blannin, A. Very Low Volume Sprint Interval Exercise Suppresses Subjective Appetite, Lowers Acylated Ghrelin, and Elevates GLP-1 in Overweight Individuals: A Pilot Study. *Nutrients* **9**, 362 (2017).

30. Ueda, S. *et al.* Changes in gut hormone levels and negative energy balance during aerobic exercise in obese young males. *J. Endocrinol.* **201**, 151–159 (2009).

31. Holmstrup, M. E., Fairchild, T. J., Keslacy, S., Weinstock, R. S. & Kanaley, J. A. Satiety, but not total PYY, Is increased with continuous and intermittent exercise. *Obesity* **21**, 2014–2020 (2013).

32. Larsen, P. S. *et al.* Effects of Aerobic, Strength or Combined Exercise on Perceived Appetite and Appetite-Related Hormones in Inactive Middle-Aged Men. *Int. J. Sport Nutr. Exerc. Metab.* **27**, 389–398 (2017).

33. Martins, C. *et al.* Effect of moderate- and high-intensity acute exercise on appetite in obese individuals. *Med. Sci. Sports Exerc.* **47**, 40–8 (2015).

34. Matos, V. *et al.* Acute Effects of High-Intensity Interval and Moderate-Intensity Continuous Exercise on GLP-1, Appetite and Energy Intake in Obese Men: A Crossover Trial. *Nutrients* **10**, 889 (2018).

35. Deighton, K., Barry, R., Connon, C. E. & Stensel, D. J. Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise. *Eur. J. Appl. Physiol.* **113**, 1147–1156 (2013).

36. Masurier, J. *et al.* Effect of Exercise Duration on Subsequent Appetite and Energy Intake in Obese Adolescent Girls. *Int. J. Sport Nutr. Exerc. Metab.* **28**, 593–601 (2018).

37. Miguet, M. *et al.* Appetite, energy intake and food reward responses to an acute High Intensity Interval Exercise in adolescents with obesity. *Physiol. Behav.* **195**, 90–97 (2018).

38. Ballard, T. P. *et al.* Effect of resistance exercise, with or without carbohydrate supplementation, on plasma ghrelin concentrations and postexercise hunger and food intake. *Metabolism* **58**, 1191–1199 (2009).

39. Laan, D. J., Leidy, H. J., Lim, E. & Campbell, W. W. Effects and reproducibility of aerobic and resistance exercise on appetite and energy intake in young, physically active adults. *Appl. Physiol. Nutr. Metab.* **35**, 842–847 (2010).

40. Cadieux, S., McNeil, J., Lapierre, M. P., Riou, M.-È. & Doucet, É. Resistance and aerobic exercises do not affect post-exercise energy compensation in normal weight men and women. *Physiol. Behav.* **130**, 113–119 (2014).

41. Stubbs, R. *et al.* The effect of graded levels of exercise on energy intake and balance in free-living men, consuming their normal diet. *Eur. J. Clin. Nutr.* **56**, 129–140 (2002).

42. Stubbs, R. *et al.* The effect of graded levels of exercise on energy intake and balance in free-living women. *Int. J. Obes.* **26**, 866–869 (2002).

43. Whybrow, S. *et al.* The effect of an incremental increase in exercise on appetite, eating behaviour and energy balance in lean men and women feeding ad libitum. *Br. J. Nutr.* **100**, 1109–1115 (2008).

44. Martins, C., Kulseng, B., King, N. A., Holst, J. J. & Blundell, J. E. The Effects of Exercise-Induced Weight Loss on Appetite-Related Peptides and Motivation to Eat. *J. Clin. Endocrinol. Metab.* **95**, 1609–1616 (2010).

45. Caudwell, P. *et al.* No sex difference in body fat in response to supervised and measured exercise. *Med. Sci. Sports Exerc.* **45**, 351–8 (2013).

46. King, N. A. *et al.* Dual-process action of exercise on appetite control: increase in orexigenic drive but improvement in meal-induced satiety. *Am. J. Clin. Nutr.* **90**, 921–927 (2009).

47. Guelfi, K. J., Donges, C. E. & Duffield, R. Beneficial effects of 12 weeks of aerobic compared with resistance exercise training on perceived appetite in previously sedentary overweight and obese men. *Metabolism* **62**, 235–243 (2013).

48. Martins, C., Kulseng, B., Rehfeld, J. F., King, N. A. & Blundell, J. E. Effect of chronic

exercise on appetite control in overweight and obese individuals. *Med. Sci. Sports Exerc.* **45**, 805–12 (2013).

49. Morishima, T., Kurihara, T., Hamaoka, T. & Goto, K. Whole body, regional fat accumulation, and appetite-related hormonal response after hypoxic training. *Clin. Physiol. Funct. Imaging* **34**, 90–97 (2014).

50. Martins, C., Truby, H. & Morgan, L. M. Short-term appetite control in response to a 6-week exercise programme in sedentary volunteers. *Br. J. Nutr.* **98**, 834–842 (2007).

51. Beaulieu, K., Hopkins, M., Blundell, J. & Finlayson, G. Does Habitual Physical Activity Increase the Sensitivity of the Appetite Control System? A Systematic Review. *Sport. Med.* **46**, 1897–1919 (2016).

52. Almiron-Roig, E. *et al.* Factors that determine energy compensation: a systematic review of preload studies. *Nutr. Rev.* **71**, 458–473 (2013).

53. Fillon, A. *et al.* Effect of exercise-meal timing on energy intake, appetite and food reward in adolescents with obesity: The TIMEX study. *Appetite* **146**, 104506 (2020).

54. Rocha, J., Paxman, J., Dalton, C., Winter, E. & Broom, D. Effects of an acute bout of aerobic exercise on immediate and subsequent three-day food intake and energy expenditure in active and inactive men. *Appetite* **71**, 369–378 (2013).

55. Rocha, J., Paxman, J. R., Dalton, C. F., Hopkins, M. & Broom, D. R. An acute bout of cycling does not induce compensatory responses in pre-menopausal women not using hormonal contraceptives. *Appetite* **128**, 87–94 (2018).

56. Rocha, J., Paxman, J., Dalton, C., Winter, E. & Broom, D. Effects of an acute bout of aerobic exercise on immediate and subsequent three-day food intake and energy expenditure in active and inactive pre-menopausal women taking oral contraceptives. *Appetite* **89**, 183–191 (2015).

57. Charlot, K. & Chapelot, D. Energy compensation after an aerobic exercise session in high-fat/low-fit and low-fat/high-fit young male subjects. *Br. J. Nutr.* **110**, 1133–1142 (2013).

58. Ueda, S., Yoshikawa, T., Katsura, Y., Usui, T. & Fujimoto, S. Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. *J. Endocrinol.* **203**, 357–364 (2009).

59. Kojima, C. *et al.* Appetite Regulations After Sprint Exercise Under Hypoxic Condition in Female Athletes. *J. Strength Cond. Res.* **33**, 1773–1780 (2019).

60. King, J. A., Wasse, L. K. & Stensel, D. J. Acute exercise increases feeding latency in healthy normal weight young males but does not alter energy intake. *Appetite* **61**, 45–51 (2013).

61. Charlot, K. & Chapelot, D. Comparison of energy-matched high-intensity interval and moderate-intensity continuous exercise sessions on latency to eat, energy intake, and appetite. *Appl. Physiol. Nutr. Metab.* **44**, 665–673 (2019).

62. Unick, J. L. *et al.* Acute effect of walking on energy intake in overweight/obese women. *Appetite* **55**, 413–419 (2010).

63. Hagobian, T. A. *et al.* Effects of acute exercise on appetite hormones and ad libitum energy intake in men and women. *Appl. Physiol. Nutr. Metab.* **38**, 66–72 (2013).

64. Alajmi, N. *et al.* Appetite and Energy Intake Responses to Acute Energy Deficits in Females versus Males. *Med. Sci. Sports Exerc.* **48**, 412–420 (2016).

65. Balaguera-Cortes, L., Wallman, K. E., Fairchild, T. J. & Guelfi, K. J. Energy intake and appetite-related hormones following acute aerobic and resistance exercise. *Appl. Physiol. Nutr. Metab.* **36**, 958–966 (2011).

66. Nemet, D., Arieli, R., Meckel, Y. & Eliakim, A. Immediate post-exercise energy intake and macronutrient preferences in normal weight and overweight pre-pubertal children. *Int. J. Pediatr. Obes.* **5**, 221–229 (2010).

67. Doucet, É., McInis, K. & Mahmoodianfard, S. Compensation in response to energy deficits induced by exercise or diet. *Obes. Rev.* **19**, 36–46 (2018).

68. Schubert, M. M., Sabapathy, S., Leveritt, M. & Desbrow, B. Acute Exercise and Hormones Related to Appetite Regulation: A Meta-Analysis. *Sport. Med.* **44**, 387–403 (2014).

69. Martins, C., Morgan, L. M., Bloom, S. R. & Robertson, M. D. Effects of exercise on gut peptides, energy intake and appetite. *J. Endocrinol.* **193**, 251–258 (2007).

70. van der Lely, A. J., Tschöp, M., Heiman, M. L. & Ghigo, E. Biological, Physiological, Pathophysiological, and Pharmacological Aspects of Ghrelin. *Endocr. Rev.* **25**, 426–457 (2004).

71. Sato, T. *et al.* Structure, regulation and function of ghrelin. *J. Biochem.* **151**, 119–128 (2012).

72. Delporte, C. Structure and Physiological Actions of Ghrelin. *Scientifica (Cairo).* **2013**, 1–25 (2013).

73. Hosoda, H., Kojima, M., Mizushima, T., Shimizu, S. & Kangawa, K. Structural Divergence of Human Ghrelin. *J. Biol. Chem.* **278**, 64–70 (2003).

74. Müller, T. D. *et al.* Ghrelin. *Mol. Metab.* **4**, 437–460 (2015).

75. Chen, C.-Y., Asakawa, A., Fujimiya, M., Lee, S.-D. & Inui, A. Ghrelin Gene Products and the Regulation of Food Intake and Gut Motility. *Pharmacol. Rev.* **61**, 430–481 (2009).

76. Howick, K., Griffin, B., Cryan, J. & Schellekens, H. From Belly to Brain: Targeting the Ghrelin Receptor in Appetite and Food Intake Regulation. *Int. J. Mol. Sci.* **18**, 273 (2017).

77. Kishimoto, I., Tokudome, T., Hosoda, H., Miyazato, M. & Kangawa, K. Ghrelin and cardiovascular diseases. *J. Cardiol.* **59**, 8–13 (2012).

78. Amini, P. *et al.* Serum Acylated Ghrelin Is Negatively Correlated with the Insulin Resistance In the CODING study. *PLoS One* **7**, e45657 (2012).

79. Gray, S. M., Page, L. C. & Tong, J. Ghrelin regulation of glucose metabolism. *J. Neuroendocrinol.* **31**, 0–3 (2019).

80. Vestergaard, E. T. *et al.* Acute Effects of Ghrelin Administration on Glucose and Lipid Metabolism. *J. Clin. Endocrinol. Metab.* **93**, 438–444 (2008).

81. Fernandez, G. *et al.* Des-Acyl Ghrelin Directly Targets the Arcuate Nucleus in a Ghrelin-Receptor Independent Manner and Impairs the Orexigenic Effect of Ghrelin. *J. Neuroendocrinol.* **28**, (2016).

82. Callahan, H. S. *et al.* Postprandial Suppression of Plasma Ghrelin Level Is Proportional to Ingested Caloric Load but Does Not Predict Intermeal Interval in Humans. *J. Clin. Endocrinol. Metab.* **89**, 1319–1324 (2004).

83. Karhunen, L. J., Juvonen, K. R., Huotari, A., Purhonen, A. K. & Herzig, K. H. Effect of protein, fat, carbohydrate and fibre on gastrointestinal peptide release in humans. *Regul. Pept.* **149**, 70–78 (2008).

84. Steinert, R. E. *et al.* Ghrelin, CCK, GLP-1, and PYY(3–36): Secretory Controls and Physiological Roles in Eating and Glycemia in Health, Obesity, and After RYGB. *Physiol. Rev.* **97**, 411–463 (2017).

85. Monteleone, P., Serritella, C., Scognamiglio, P. & Maj, M. Enhanced ghrelin secretion in the cephalic phase of food ingestion in women with bulimia nervosa. *Psychoneuroendocrinology* **35**, 284–288 (2010).

86. Simonian, h. p., Kresge, k. m., Boden, g. h. & Parkman, h. p. Differential effects of sham feeding and meal ingestion on ghrelin and pancreatic polypeptide levels: evidence for vagal efferent stimulation mediating ghrelin release. *Neurogastroenterol. Motil.* **17**, 348–54 (2005).

87. Crum, A. J., Corbin, W. R., Brownell, K. D. & Salovey, P. Mind over milkshakes: Mindsets, not just nutrients, determine ghrelin response. *Heal. Psychol.* **30**, 424–429 (2011).

88. Heath, R., Jones, R., Frayn, K. & Robertson, M. Vagal stimulation exaggerates the inhibitory ghrelin response to oral fat in humans. *J. Endocrinol.* **180**, 273–281 (2004).

89. Flanagan, D. E. *et al.* The influence of insulin on circulating ghrelin. *Am. J. Physiol. Metab.* **284**, E313–E316 (2003).

90. Batterham, R. L. *et al.* Inhibition of Food Intake in Obese Subjects by Peptide YY 3–36. *N. Engl. J. Med.* **349**, 941–948 (2003).

91. Hagemann, D. *et al.* Glucagon-like peptide 1 (GLP-1) suppresses ghrelin levels in humans via increased insulin secretion. *Regul. Pept.* **143**, 64–68 (2007).

92. King, J. A., Miyashita, M., Wasse, L. K. & Stensel, D. J. Influence of prolonged treadmill running on appetite, energy intake and circulating concentrations of acylated ghrelin. *Appetite* **54**, 492–498 (2010).

93. Sim, A. Y., Wallman, K. E., Fairchild, T. J. & Guelfi, K. J. High-intensity intermittent exercise attenuates ad-libitum energy intake. *Int. J. Obes.* **38**, 417–422 (2014).

94. Tiryaki-Sonmez, G. *et al.* Effect of exercise on appetite-regulating hormones in overweight women. *Biol. Sport* **30**, 75–80 (2013).

95. Dorling, J. *et al.* Acute and Chronic Effects of Exercise on Appetite, Energy Intake, and Appetite-Related Hormones: The Modulating Effect of Adiposity, Sex, and Habitual Physical Activity. *Nutrients* **10**, 1140 (2018).

96. King, J. A., Wasse, L. K., Broom, D. R. & Stensel, D. J. Influence of brisk walking on appetite, energy intake, and plasma acylated ghrelin. *Med. Sci. Sports Exerc.* **42**, 485–92 (2010).

97. Holliday, A. & Blannin, A. Appetite, food intake and gut hormone responses to intense aerobic exercise of different duration. *J. Endocrinol.* **235**, 193–205 (2017).

98. Jones, T. E., Basilio, J. L., Brophy, P. M., McCammon, M. R. & Hickner, R. C. Long-term Exercise Training in Overweight Adolescents Improves Plasma Peptide YY and

Resistin. *Obesity* **17**, 1189–1195 (2009).

99. Gibbons, C. *et al.* The Role of Episodic Postprandial Peptides in Exercise-Induced Compensatory Eating. *J. Clin. Endocrinol. Metab.* **102**, 4051–4059 (2017).

100. Martins, C. *et al.* High-Intensity Interval Training, Appetite, and Reward Value of Food in the Obese. *Med. Sci. Sports Exerc.* **49**, 1851–1858 (2017).

101. Gueugnon, C. *et al.* Ghrelin and PYY levels in adolescents with severe obesity: effects of weight loss induced by long-term exercise training and modified food habits. *Eur. J. Appl. Physiol.* **112**, 1797–1805 (2012).

102. Ueda, S. *et al.* Effects of exercise training on gut hormone levels after a single bout of exercise in middle-aged Japanese women. *Springerplus* **2**, 83 (2013).

103. Holst, J. J. The Physiology of Glucagon-like Peptide 1. *Physiol. Rev.* **87**, 1409–1439 (2007).

104. Andersen, A., Lund, A., Knop, F. K. & Vilsbøll, T. Glucagon-like peptide 1 in health and disease. *Nat. Rev. Endocrinol.* **14**, 390–403 (2018).

105. Müller, T. D. *et al.* Glucagon-like peptide 1 (GLP-1). *Mol. Metab.* **30**, 72–130 (2019).

106. Orskov, C., Rabenhoj, L., Wettergren, A., Kofod, H. & Holst, J. J. Tissue and Plasma Concentrations of Amidated and Glycine-Extended Glucagon-Like Peptide I in Humans. *Diabetes* **43**, 535–539 (1994).

107. Elahi, D. *et al.* GLP-1 (9-36) Amide, Cleavage Product of GLP-1 (7-36) Amide, Is a Glucoregulatory Peptide. *Obesity* **16**, 1501–1509 (2008).

108. Guglielmi, V. & Sbraccia, P. GLP-1 receptor independent pathways: emerging beneficial effects of GLP-1 breakdown products. *Eat. Weight Disord.* **22**, 231–240 (2017).

109. Flint, A., Raben, A., Astrup, A. & Holst, J. J. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J. Clin. Invest.* **101**, 515–520 (1998).

110. Verdich, C. *et al.* A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans. *J. Clin. Endocrinol. Metab.* **86**, 4382–9 (2001).

111. Jin, T. & Weng, J. Hepatic functions of GLP-1 and its based drugs: current disputes and perspectives. *Am. J. Physiol. Metab.* **311**, E620–E627 (2016).

112. Chai, W. *et al.* Glucagon-like peptide 1 recruits microvasculature and increases

glucose use in muscle via a nitric oxide-dependent mechanism. *Diabetes* **61**, 888–896 (2012).

113. Nadkarni, P., Chepurny, O. G. & Holz, G. G. Regulation of Glucose Homeostasis by GLP-1. in *Progress in Molecular Biology and Translational Science* vol. 121 23–65 (2014).

114. Carr, R. D. *et al.* Secretion and Dipeptidyl Peptidase-4-Mediated Metabolism of Incretin Hormones after a Mixed Meal or Glucose Ingestion in Obese Compared to Lean, Nondiabetic Men. *J. Clin. Endocrinol. Metab.* **95**, 872–878 (2010).

115. Brighton, C. A. *et al.* Bile Acids Trigger GLP-1 Release Predominantly by Accessing Basolaterally Located G Protein–Coupled Bile Acid Receptors. *Endocrinology* **156**, 3961–3970 (2015).

116. Brubaker, P. L. & Anini, Y. Direct and indirect mechanisms regulating secretion of glucagon-like peptide-1 and glucagon-like peptide-2. *Can. J. Physiol. Pharmacol.* **81**, 1005–1012 (2003).

117. Ueda, S., Yoshikawa, T., Katsura, Y., Usui, T. & Fujimoto, S. Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. *J. Endocrinol.* **203**, 357–364 (2009).

118. Karra, E., Chandarana, K. & Batterham, R. L. The role of peptide YY in appetite regulation and obesity. *J. Physiol.* **587**, 19–25 (2009).

119. Pedragosa-Badia, X., Stichel, J. & Beck-Sickinger, A. G. Neuropeptide Y receptors: how to get subtype selectivity. *Front. Endocrinol. (Lausanne).* **4**, 1–13 (2013).

120. Silva, A. De & Bloom, S. R. Gut Hormones and Appetite Control: A Focus on PYY and GLP-1 as Therapeutic Targets in Obesity. *Gut Liver* **6**, 10–20 (2012).

121. Sloth, B., Holst, J. J., Flint, A., Gregersen, N. T. & Astrup, A. Effects of PYY 1–36 and PYY 3–36 on appetite, energy intake, energy expenditure, glucose and fat metabolism in obese and lean subjects. *Am. J. Physiol. Metab.* **292**, E1062–E1068 (2007).

122. Pironi, L. *et al.* Fat-induced heal brake in humans: A dose-dependent phenomenon correlated to the plasma levels of peptide YY. *Gastroenterology* **105**, 733–739 (1993).

123. Cooper, J. A. Factors affecting circulating levels of peptide YY in humans: a comprehensive review. *Nutr. Res. Rev.* **27**, 186–197 (2014).

124. Manning, S. & Batterham, R. L. The Role of Gut Hormone Peptide YY in Energy and Glucose Homeostasis: Twelve Years On. *Annu. Rev. Physiol.* **76**, 585–608 (2014).

125. Toräng, S. *et al.* In vivo and in vitro degradation of peptide YY 3–36 to inactive peptide YY 3–34 in humans. *Am. J. Physiol. Integr. Comp. Physiol.* **310**, R866–R874 (2016).

126. Toräng, S., Veedfald, S., Rosenkilde, M. M., Hartmann, B. & Holst, J. J. The anorexic hormone Peptide YY 3-36 is rapidly metabolized to inactive Peptide YY 3-34 in vivo. *Physiol. Rep.* **3**, e12455 (2015).

127. Adrian, T. E. *et al.* Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology* **89**, 1070–1077 (1985).

128. Essah, P. A., Levy, J. R., Sistrun, S. N., Kelly, S. M. & Nestler, J. E. Effect of Macronutrient Composition on Postprandial Peptide YY Levels. *J. Clin. Endocrinol. Metab.* **92**, 4052–4055 (2007).

129. Hill, B. R., Souza, M. J. De, Wagstaff, D. A., Sato, R. & Williams, N. I. 24-Hour profiles of circulating ghrelin and peptide YY are inversely associated in normal weight premenopausal women. *Peptides* **38**, 159–162 (2012).

130. Lomenick, J. P., Clasey, J. L. & Anderson, J. W. Meal-related Changes in Ghrelin, Peptide YY, and Appetite in Normal Weight and Overweight Children. *Obesity* **16**, 547–552 (2008).

131. Batterham, R. L. *et al.* Critical role for peptide YY in protein-mediated satiation and body-weight regulation. *Cell Metab.* **4**, 223–233 (2006).

132. Brennan, I. M. *et al.* Dose-dependent effects of cholecystokinin-8 on antropyloroduodenal motility, gastrointestinal hormones, appetite, and energy intake in healthy men. *Am. J. Physiol. Metab.* **295**, E1487–E1494 (2008).

133. Brennan, I. M. *et al.* Intravenous CCK-8, but not GLP-1, suppresses ghrelin and stimulates PYY release in healthy men. *Peptides* **28**, 607–611 (2007).

134. Adrian, T. E. *et al.* Deoxycholate is an important releaser of peptide YY and enteroglucagon from the human colon. *Gut* **34**, 1219–1224 (1993).

135. Fu-Cheng, X. *et al.* Mechanisms of peptide YY release induced by an intraduodenal meal in rats: neural regulation by proximal gut. *Pflugers Arch. Eur. J. Physiol.* **433**, 571–579 (1997).

136. Sheikh, S. P., Holst, J. J., Ørskov, C., Ekman, R. & Schwartz, T. W. Release of PYY

from pig intestinal mucosa; luminal and neural regulation. *Regul. Pept.* **26**, 253–266 (1989).

137. King, J. A. *et al.* Differential Acylated Ghrelin, Peptide YY3–36, Appetite, and Food Intake Responses to Equivalent Energy Deficits Created by Exercise and Food Restriction. *J. Clin. Endocrinol. Metab.* **96**, 1114–1121 (2011).

138. Deighton, K., Karra, E., Batterham, R. L. & Stensel, D. J. Appetite, energy intake, and PYY 3–36 responses to energy-matched continuous exercise and submaximal high-intensity exercise. *Appl. Physiol. Nutr. Metab.* **38**, 947–952 (2013).

139. Deighton, K., Batterham, R. L. & Stensel, D. J. Appetite and gut peptide responses to exercise and calorie restriction. The effect of modest energy deficits. *Appetite* **81**, 52–59 (2014).

140. Kelly, K. R. *et al.* The glucose-dependent insulinotropic polypeptide and glucose-stimulated insulin response to exercise training and diet in obesity. *Am. J. Physiol. Metab.* **296**, E1269–E1274 (2009).

141. Hazell, T. J., Islam, H., Townsend, L. K., Schmale, M. S. & Copeland, J. L. Effects of exercise intensity on plasma concentrations of appetite-regulating hormones: Potential mechanisms. *Appetite* **98**, 80–88 (2016).

142. Boulton, D., Taylor, C. E., Macefield, V. G. & Green, S. Contributions of Central Command and Muscle Feedback to Sympathetic Nerve Activity in Contracting Human Skeletal Muscle. *Front. Physiol.* **7**, 1–9 (2016).

143. Barman, S. M. & Yates, B. J. Deciphering the Neural Control of Sympathetic Nerve Activity: Status Report and Directions for Future Research. *Front. Neurosci.* **11**, (2017).

144. Christensen, N. J. & Galbo, H. Sympathetic Nervous Activity During Exercise. *Annu. Rev. Physiol.* **45**, 139–153 (1983).

145. Leuenberger, U. *et al.* Effects of exercise intensity and duration on norepinephrine spillover and clearance in humans. *J. Appl. Physiol.* **75**, 668–674 (1993).

146. Pritzlaff, C. J. *et al.* Catecholamine release, growth hormone secretion, and energy expenditure during exercise vs. recovery in men. *J. Appl. Physiol.* **89**, 937–946 (2000).

147. Claustre, J., Brechet, S., Plaisancie, P., Chayvialle, J. & Cuber, J. Stimulatory effect of beta-adrenergic agonists on ileal L cell secretion and modulation by alpha-

adrenergic activation. *J. Endocrinol.* **162**, 271–278 (1999).

148. Brechet, S. *et al.* Involvement of β1- and β2- but not β3-adrenoceptor activation in adrenergic PYY secretion from the isolated colon. *J. Endocrinol.* **168**, 177–183 (2001).

149. Shiiya, T. *et al.* Significant lowering of plasma ghrelin but not des-acyl ghrelin in response to acute exercise in men. *Endocr. J.* **58**, 335–342 (2011).

150. Zhao, T.-J. *et al.* Ghrelin secretion stimulated by 1-adrenergic receptors in cultured ghrelinoma cells and in fasted mice. *Proc. Natl. Acad. Sci.* **107**, 15868–15873 (2010).

151. Iwakura, H. *et al.* Oxytocin and Dopamine Stimulate Ghrelin Secretion by the Ghrelin-Producing Cell Line, MGN3-1 in Vitro. *Endocrinology* **152**, 2619–2625 (2011).

152. Mundinger, T. O., Cummings, D. E. & Taborsky, G. J. Direct Stimulation of Ghrelin Secretion by Sympathetic Nerves. *Endocrinology* **147**, 2893–2901 (2006).

153. Qamar, M. I. & Read, A. E. Effects of exercise on mesenteric blood flow in man. *Gut* **28**, 583–587 (1987).

154. Perko, M. J. *et al.* Mesenteric, coeliac and splanchnic blood flow in humans during exercise. *J. Physiol.* **513**, 907–913 (1998).

155. Otte, J. a, Oostveen, E., Geelkerken, R. H., Groeneveld, A. B. J. & Kolkman, J. J. Exercise induces gastric ischemia in healthy volunteers: a tonometry study. *J. Appl. Physiol.* **91**, 866–871 (2001).

156. van Wijck, K. *et al.* Exercise-Induced Splanchnic Hypoperfusion Results in Gut Dysfunction in Healthy Men. *PLoS One* **6**, e22366 (2011).

157. Wijck, K. Van *et al.* Physiology and pathophysiology of splanchnic hypoperfusion and intestinal injury during exercise : strategies for evaluation and prevention. *Am. J. Physiol. - Gastrointest. Liver Physiol.* **303**, G155–G168 (2012).

158. Wu, R. *et al.* Orexigenic Hormone Ghrelin Attenuates Local and Remote Organ Injury after Intestinal Ischemia-Reperfusion. *PLoS One* **3**, e2026 (2008).

159. Mogami, S. *et al.* Reduced ghrelin production induced anorexia after rat gastric ischemia and reperfusion. *Am. J. Physiol. Liver Physiol.* **302**, G359–G364 (2012).

160. Horner, K. M., Schubert, M. M., Desbrow, B., Byrne, N. M. & King, N. A. Acute exercise and gastric emptying: a meta-analysis and implications for appetite control. *Sport. Med.* **45**, 659–78 (2015).

161. Janssen, P. *et al.* Review article: the role of gastric motility in the control of food intake. *Aliment. Pharmacol. Ther.* **33**, 880–894 (2011).

162. Garbers, C., Heink, S., Korn, T. & Rose-John, S. Interleukin-6: designing specific therapeutics for a complex cytokine. *Nat. Rev. Drug Discov.* **17**, 395–412 (2018).

163. Pedersen, B. K., Steensberg, A. & Schjerling, P. Muscle-derived interleukin-6: possible biological effects. *J. Physiol.* **536**, 329–337 (2001).

164. Almada, C. *et al.* Plasma levels of interleukin-6 and interleukin-18 after an acute physical exercise: relation with post-exercise energy intake in twins. *J. Physiol. Biochem.* **69**, 85–95 (2013).

165. Hunschede, S., Kubant, R., Akilen, R., Thomas, S. & Anderson, G. H. Decreased Appetite after High-Intensity Exercise Correlates with Increased Plasma Interleukin-6 in Normal-Weight and Overweight/Obese Boys. *Curr. Dev. Nutr.* **1**, e000398 (2017).

166. Islam, H. *et al.* Potential involvement of lactate and interleukin-6 in the appetite-regulatory hormonal response to an acute exercise bout. *J. Appl. Physiol.* **123**, 614–623 (2017).

167. Ropelle, E. R. *et al.* IL-6 and IL-10 Anti-Inflammatory Activity Links Exercise to Hypothalamic Insulin and Leptin Sensitivity through IKKβ and ER Stress Inhibition. *PLoS Biol.* **8**, e1000465 (2010).

168. Ellingsgaard, H. *et al.* Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. *Nat. Med.* **17**, 1481–1489 (2011).

169. Pazos, P., Lima, L., Casanueva, F. F., Diéguez, C. & García, M. C. Interleukin 6 Deficiency Modulates the Hypothalamic Expression of Energy Balance Regulating Peptides during Pregnancy in Mice. *PLoS One* **8**, e72339 (2013).

170. Schéle, E. *et al.* Inter-relation between Interleukin (IL)-1, IL-6 and Body Fat Regulating Circuits of the Hypothalamic Arcuate Nucleus. *J. Neuroendocrinol.* **25**, 580–589 (2013).

171. Ferrer, B. *et al.* Muscle-specific interleukin-6 deletion influences body weight and body fat in a sex-dependent manner. *Brain. Behav. Immun.* **40**, 121–130 (2014).

172. Jensen, M. D. Fate of fatty acids at rest and during exercise: regulatory mechanisms. *Acta Physiol. Scand.* **178**, 385–390 (2003).

173. Peake, J. M. *et al.* Metabolic and hormonal responses to isoenergetic high-intensity interval exercise and continuous moderate-intensity exercise. *Am. J. Physiol. - Endocrinol. Metab.* **307**, E539–E552 (2014).

174. Raclot, T., Mioskowski, E., Bach, A. C. & Groscolas, R. Selectivity of fatty acid mobilization: a general metabolic feature of adipose tissue. *Am. J. Physiol. Integr. Comp. Physiol.* **269**, R1060–R1067 (1995).

175. Raclot, T., Langin, D., Lafontan, M. & Groscolas, R. Selective release of human adipocyte fatty acids according to molecular structure. *Biochem. J.* **324 (Pt 3**, 911–5 (1997).

176. Le Foll, C. Hypothalamic Fatty Acids and Ketone Bodies Sensing and Role of FAT/CD36 in the Regulation of Food Intake. *Front. Physiol.* **10**, (2019).

177. Obici, S. *et al.* Central Administration of Oleic Acid Inhibits Glucose Production and Food Intake. *Diabetes* **51**, 271–275 (2002).

178. Alvarez-Curto, E. & Milligan, G. Metabolism meets immunity: The role of free fatty acid receptors in the immune system. *Biochem. Pharmacol.* **114**, 3–13 (2016).

179. Gormsen, L. C. *et al.* Free fatty acids decrease circulating ghrelin concentrations in humans. *Eur. J. Endocrinol.* **154**, 667–673 (2006).

180. Gormsen, L. C. *et al.* Effects of free fatty acids, growth hormone and growth hormone receptor blockade on serum ghrelin levels in humans. *Clin. Endocrinol. (Oxf).* **66**, 641–645 (2007).

181. Lindgren, O. *et al.* Incretin Hormone and Insulin Responses to Oral Versus Intravenous Lipid Administration in Humans. *J. Clin. Endocrinol. Metab.* **96**, 2519–2524 (2011).

182. Vamvini, M. T. *et al.* Differential Effects of Oral and Intravenous Lipid Administration on Key Molecules Related to Energy Homeostasis. *J. Clin. Endocrinol. Metab.* **101**, 1989–1997 (2016).

183. Barazzoni, R. *et al.* Intravenous lipid infusion and total plasma fatty acids positively modulate plasma acylated ghrelin in vivo. *Clin. Nutr.* **36**, 775–781 (2017).

184. Fox, M. T., Gerrelli, D., Pitt, S. R. & Jacobs, D. E. The relationship between appetite and plasma non-esterified fatty acid levels in housed calves. *Vet. Res. Commun.* **15**, 127–133 (1991).

185. Cummings, D. E., Frayo, R. S., Marmonier, C., Aubert, R. & Chapelot, D. Plasma

ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. *Am. J. Physiol. Metab.* **287**, E297–E304 (2004).

186. Ren, P. *et al.* Plasma acyl ghrelin and nonesterified fatty acids are the best predictors for hunger status in pregnant gilts1. *J. Anim. Sci.* **95**, 5485–5496 (2017).

187. Nunez, E. A. Biological complexity is under the 'strange attraction' of non-esterified fatty acids. *Prostaglandins, Leukot. Essent. Fat. Acids* **57**, 107–110 (1997).

188. Frayn, K. N. Fat as a fuel: emerging understanding of the adipose tissue-skeletal muscle axis. *Acta Physiol.* **199**, 509–518 (2010).

189. Horowitz, J. F. Fatty acid mobilization from adipose tissue during exercise. *Trends Endocrinol. Metab.* **14**, 386–392 (2003).

190. Mulla, N., Simonsen, L. & Bülow, J. Post-exercise adipose tissue and skeletal muscle lipid metabolism in humans: the effects of exercise intensity. *J. Physiol.* **524**, 919–928 (2000).

191. Gleeson, T. T. Post-Exercise Lactate Metabolism: A Comparative Review of Sites, Pathways, and Regulation. *Annu. Rev. Physiol.* **58**, 565–581 (1996).

192. Menzies, P. *et al.* Blood lactate clearance during active recovery after an intense running bout depends on the intensity of the active recovery. *J. Sports Sci.* **28**, 975–982 (2010).

193. Gonzalez, J. T., Fuchs, C. J., Betts, J. A. & van Loon, L. J. C. Liver glycogen metabolism during and after prolonged endurance-type exercise. *Am. J. Physiol. Metab.* **311**, E543–E553 (2016).

194. Engelstoft, M. S. *et al.* Seven transmembrane G protein-coupled receptor repertoire of gastric ghrelin cells. *Mol. Metab.* **2**, 376–392 (2013).

195. Lam, C. K. L., Chari, M., Wang, P. Y. T. & Lam, T. K. T. Central lactate metabolism regulates food intake. *Am. J. Physiol. Metab.* **295**, E491–E496 (2008).

196. Schultes, B. *et al.* Lactate infusion during euglycemia but not hypoglycemia reduces subsequent food intake in healthy men. *Appetite* **58**, 818–821 (2012).

197. Geary, N. Glucagon and the Control of Appetite. in *Handbook of Experimental Pharmacology* (ed. Lefebvre, P.) vol. 123/III 223–238 (Springer-Verlag, 1996).

198. Austin, J. & Marks, D. Hormonal Regulators of Appetite. *Int. J. Pediatr. Endocrinol.* **2009**, 1–9 (2009).

199. Yaribeygi, H., Farrokhi, F. R., Butler, A. E. & Sahebkar, A. Insulin resistance: Review of the underlying molecular mechanisms. *J. Cell. Physiol.* **234**, 8152–8161 (2019).

200. Petersen, M. C. & Shulman, G. I. Mechanisms of Insulin Action and Insulin Resistance. *Physiol. Rev.* **98**, 2133–2223 (2018).

201. Haeusler, R. A., McGraw, T. E. & Accili, D. Biochemical and cellular properties of insulin receptor signalling. *Nat. Rev. Mol. Cell Biol.* **19**, 31–44 (2018).

202. Rhea, E. M., Rask-Madsen, C. & Banks, W. A. Insulin transport across the blood-brain barrier can occur independently of the insulin receptor. *J. Physiol.* **596**, 4753–4765 (2018).

203. Baskin, D. G., Figlewicz, D. P., Woods, S. C., Porte, D. & Dorsa, D. M. Insulin in the brain. *Annu. Rev. Physiol.* **49**, 335–47 (1987).

204. Schwartz, M. W., Woods, S. C., Porte, D., Seeley, R. J. & Baskin, D. G. Central nervous system control of food intake. *Nature* **404**, 661–671 (2000).

205. Benoit, S. C. *et al.* The Catabolic Action of Insulin in the Brain Is Mediated by Melanocortins. *J. Neurosci.* **22**, 9048–9052 (2002).

206. Sipols, A. J., Baskin, D. G. & Schwartz, M. W. Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. *Diabetes* **44**, 147–151 (1995).

207. Schneider, E. *et al.* The effect of intranasal insulin on appetite and mood in women with and without obesity: an experimental medicine study. *Int. J. Obes.* 1–9 (2022).

208. Habegger, K. M. *et al.* The metabolic actions of glucagon revisited. *Nat. Rev. Endocrinol.* **6**, 689–697 (2010).

209. Sandoval, D. A. & D'Alessio, D. A. Physiology of Proglucagon Peptides: Role of Glucagon and GLP-1 in Health and Disease. *Physiol. Rev.* **95**, 513–548 (2015).

210. Jones, B. J., Tan, T. & Bloom, S. R. Minireview: Glucagon in Stress and Energy Homeostasis. *Endocrinology* **153**, 1049–1054 (2012).

211. Christophe, J. Glucagon and its receptor in various tissues. *Ann. N. Y. Acad. Sci.* **805**, 31–42; discussion 42-3 (1996).

212. Müller, T. D., Finan, B., Clemmensen, C., DiMarchi, R. D. & Tschöp, M. H. The New Biology and Pharmacology of Glucagon. *Physiol. Rev.* **97**, 721–766 (2017).

213. Geary, N., Le Sauter, J. & Noh, U. Glucagon acts in the liver to control spontaneous

meal size in rats. *Am. J. Physiol. Integr. Comp. Physiol.* **264**, R116–R122 (1993).

214. Geary, N. & Smith, G. P. Selective hepatic vagotomy blocks pancreatic glucagon's satiety effect. *Physiol. Behav.* **31**, 391–4 (1983).

215. Geary, N., Kissileff, H. R., Pi-Sunyer, F. X. & Hinton, V. Individual, but not simultaneous, glucagon and cholecystokinin infusions inhibit feeding in men. *Am. J. Physiol. Integr. Comp. Physiol.* **262**, R975–R980 (1992).

216. Izzi-Engbeaya, C. *et al.* Acute Effects of Glucagon on Reproductive Hormone Secretion in Healthy Men. *J. Clin. Endocrinol. Metab.* **105**, 1899–1905 (2020).

217. Idle, J. R. & Gonzalez, F. J. Metabolomics. *Cell Metab.* **6**, 348–351 (2007).

218. Gomes, C., Almeida, J. A., Franco, O. L. & Petriz, B. Omics and the molecular exercise physiology. in *Advances in Clinical Chemistry* vol. 96 55–84 (Elsevier Inc., 2020).

219. Schranner, D., Kastenmüller, G., Schönfelder, M., Römisch-Margl, W. & Wackerhage, H. Metabolite Concentration Changes in Humans After a Bout of Exercise: a Systematic Review of Exercise Metabolomics Studies. *Sport. Med. - Open* **6**, 11 (2020).

220. San-Millán, I. *et al.* Metabolomics of Endurance Capacity in World Tour Professional Cyclists. *Front. Physiol.* **11**, (2020).

221. Berton, R. *et al.* Metabolic time-course response after resistance exercise: A metabolomics approach. *J. Sports Sci.* **35**, 1211–1218 (2017).

222. Coelho, W. *et al.* Investigating the Cellular and Metabolic Responses of World-Class Canoeists Training: A Sportomics Approach. *Nutrients* **8**, 719 (2016).

223. Howe, C. *et al.* Untargeted Metabolomics Profiling of an 80.5 km Simulated Treadmill Ultramarathon. *Metabolites* **8**, 14 (2018).

224. Contrepois, K. *et al.* Molecular Choreography of Acute Exercise. *Cell* **181**, 1112-1130.e16 (2020).

225. Yan, B. *et al.* Metabolomic investigation into variation of endogenous metabolites in professional athletes subject to strength-endurance training. *J. Appl. Physiol.* **106**, 531–538 (2009).

226. Morville, T., Sahl, R. E., Moritz, T., Helge, J. W. & Clemmensen, C. Plasma Metabolome Profiling of Resistance Exercise and Endurance Exercise in Humans.

Cell Rep. **33**, 108554 (2020).

227. Melanson, E. L., Keadle, S. K., Donnelly, J. E., Braun, B. & King, N. A. Resistance to exercise-induced weight loss: compensatory behavioral adaptations. *Med. Sci. Sports Exerc.* **45**, 1600–9 (2013).

228. Vieira, A. F., Costa, R. R., Macedo, R. C. O., Coconcelli, L. & Kruel, L. F. M. Effects of aerobic exercise performed in fasted v. fed state on fat and carbohydrate metabolism in adults: a systematic review and meta-analysis. *Br. J. Nutr.* **116**, 1153–1164 (2016).

229. Westerterp, K. R. Physical activity and physical activity induced energy expenditure in humans: measurement, determinants, and effects. *Front. Physiol.* **4**, 1–11 (2013).

230. Deighton, K., Zahra, J. C. & Stensel, D. J. Appetite, energy intake and resting metabolic responses to 60min treadmill running performed in a fasted versus a postprandial state. *Appetite* **58**, 946–954 (2012).

231. Cheng, M. H.-Y., Bushnell, D., Cannon, D. T. & Kern, M. Appetite regulation via exercise prior or subsequent to high-fat meal consumption. *Appetite* **52**, 193–198 (2009).

232. Gonzalez, J. T., Veasey, R. C., Rumbold, P. L. S. & Stevenson, E. J. Breakfast and exercise contingently affect postprandial metabolism and energy balance in physically active males. *Br. J. Nutr.* **110**, 721–732 (2013).

233. Borer, K. T., Wuorinen, E., Chao, C. & Burant, C. Exercise energy expenditure is not consciously detected due to oro-gastric, not metabolic, basis of hunger sensation. *Appetite* **45**, 177–181 (2005).

234. Broad, A. A., Howe, G. J., McKie, G. L., Vanderheyden, L. W. & Hazell, T. J. The effects of a pre-exercise meal on postexercise metabolism following a session of sprint interval training. *Appl. Physiol. Nutr. Metab.* **45**, 411–420 (2020).

235. McIver, V. J., Mattin, L. R., Evans, G. H. & Yau, A. M. W. Diurnal influences of fasted and non-fasted brisk walking on gastric emptying rate, metabolic responses, and appetite in healthy males. *Appetite* **143**, 104411 (2019).

236. Bachman, J. L., Deitrick, R. W. & Hillman, A. R. Exercising in the Fasted State Reduced 24-Hour Energy Intake in Active Male Adults. *J. Nutr. Metab.* **2016**, 1–7 (2016).

237. Griffiths, A. *et al.* Appetite and energy intake responses to breakfast consumption

and carbohydrate supplementation in hypoxia. *Appetite* **147**, 104564 (2020).

238. Farah, N. M. F. & Gill, J. M. R. Effects of exercise before or after meal ingestion on fat balance and postprandial metabolism in overweight men. *Br. J. Nutr.* **109**, 2297–2307 (2013).

239. Edinburgh, R. M. *et al.* Skipping Breakfast Before Exercise Creates a More Negative 24-hour Energy Balance: A Randomized Controlled Trial in Healthy Physically Active Young Men. *J. Nutr.* **149**, 1326–1334 (2019).

240. Hackett, D. & Hagstrom, A. Effect of Overnight Fasted Exercise on Weight Loss and Body Composition: A Systematic Review and Meta-Analysis. *J. Funct. Morphol. Kinesiol.* **2**, 43 (2017).

241. Kondo, T. *et al.* Exercise-induced nausea is exaggerated by eating. *Appetite* **36**, 119–125 (2001).

242. Rothschild, J. A., Kilding, A. E. & Plews, D. J. Pre-Exercise Nutrition Habits and Beliefs of Endurance Athletes Vary by Sex, Competitive Level, and Diet. *J. Am. Coll. Nutr.* **0**, 1–12 (2020).

243. Jeukendrup, A. E. Carbohydrate intake during exercise and performance. *Nutrition* **20**, 669–677 (2004).

244. Pfeiffer, B. *et al.* Nutritional intake and gastrointestinal problems during competitive endurance events. *Med. Sci. Sports Exerc.* **44**, 344–51 (2012).

245. de Oliveira, E. P. & Burini, R. C. Carbohydrate-dependent, exercise-induced gastrointestinal distress. *Nutrients* **6**, 4191–4199 (2014).

246. van Loon, L. J. C., Greenhaff, P. L., Constantin-Teodosiu, D., Saris, W. H. M. & Wagenmakers, A. J. M. The effects of increasing exercise intensity on muscle fuel utilisation in humans. *J. Physiol.* **536**, 295–304 (2001).

247. Gonzalez, J. T., Betts, J. A. & Thompson, D. Carbohydrate availability as a regulator of energy balance with exercise. *Exerc. Sport Sci. Rev.* **47**, 215–222 (2019).

248. Hopkins, M., Blundell, J. E. & King, N. A. Individual variability in compensatory eating following acute exercise in overweight and obese women. *Br. J. Sports Med.* **48**, 1472–1476 (2014).

249. Scanes, C. G. Carbohydrate Metabolism. in *Sturkie's Avian Physiology* 421–441 (Elsevier, 2015).

Chapter 2: The effect of a single bout of continuous aerobic exercise on glucose, insulin and glucagon concentrations compared to resting conditions in adults without diabetes: a systematic review and meta-analysis

2.1 Introduction

A single bout of aerobic exercise often results in a temporary suppression of appetite; a phenomenon referred to as 'exercise-induced anorexia'[1]. Research investigating this response has largely focused on the acute effect of aerobic exercise on the gastrointestinal hormones acyl-ghrelin, glucagon-like peptide 1 (GLP-1) and peptide YY (PYY). Acyl-ghrelin, which is secreted from cells within the stomach, possesses orexigenic properties that increase appetite and energy intake[2]. In contrast, GLP-1 and PYY are released from enteroendocrine L-cells of the gastrointestinal tract and exhibit glucoregulatory and anorexigenic functions[3,4]. A meta-analysis published by Schubert et al.[5] reported that acute exercise decreases acyl-ghrelin concentrations by ~17% and increases GLP-1 and PYY concentrations by ~13% and ~10%, respectively. Changes in acyl-ghrelin, GLP-1 and PYY concentrations are thus considered a principal mechanism underlying exercise-induced anorexia[6]. Despite these findings, changes in appetite during exercise do not always correlate with changes in acyl-ghrelin, GLP-1, and PYY[7–14] (see also chapter 1). Other mechanisms, acting independently or simultaneously, may therefore be involved in exercise-induced anorexia.

Acute aerobic exercise can modulate the release of the pancreatic hormones insulin and glucagon, as well as circulating glucose concentrations[15,16]. Insulin and glucagon have well-established roles in glucose homeostasis but are also known to suppress appetite[17,18]. Similarly, acute perturbations in systemic glucose concentrations have been reported to predict appetite[19]. It is therefore plausible that changes in glucose, insulin, and/or glucagon induced by exercise may also contribute to the changes in appetite experienced during exercise.

Despite its widespread investigation, conflicting results on the magnitude and/or direction of glucose, insulin, and glucagon responses to acute exercise bouts have been reported[15,16,20–23]. Additionally, these studies are conducted in both the fed and fasted state, likely contributing to this inconsistency observed between studies. The influence of this study design choice on the glucose, insulin, and glucagon response to acute exercise has however been little explored. Furthermore, previous meta-analyses investigating the effects of aerobic exercise on glycaemic parameters have been largely limited to individuals with type 2 diabetes[24–26]; the results of which cannot readily be applied to individuals without diabetes due to underlying differences in physiology between these two populations[27–29].

I therefore conducted a systematic review and meta-analysis to quantify the glucose, insulin, and glucagon response to a single bout of continuous aerobic exercise. Furthermore, we aimed to investigate if exercise performed in the fed or fasted state influenced glucose, insulin, and glucagon responses using sub-group meta-analyses. The findings from this chapter will help to provide a better understanding of the changes in insulin, glucagon and glucose to acute aerobic exercise, the influence of fed and fasted exercise on these responses, and determine the plausibility that glucose, insulin, and/or glucagon contribute to exercise-induced anorexia.

2.2 Methods

2.2.1 Registration

This Review and meta-analysis was registered at PROSPERO (registration number: CRD42020191345).

2.2.2 Eligibility criteria

Population: Randomised, crossover study in adults (≥ 18 years) of any body mass index (BMI) value or fitness level. Participants that were pregnant, smoking, currently taking medication that might have interfered with glucose, insulin or glucagon concentrations, impaired glucose metabolism, or had a history of chronic disease, including type 1 and type 2 diabetes, were excluded.

Intervention: A single bout of continuous aerobic exercise. The duration of the exercise must have been greater or equal to 30 minutes and have been performed at a fixed intensity on a treadmill or cycle ergometer. Using a treadmill or cycle ergometer allows exercise intensity to be tightly controlled, guarantees compliance with the protocol relative to self-paced exercise, ensures relative intensity is consistent across participants, and thus permits comparisons within and between studies.

Comparator: A time-matched, resting control arm had to have been performed to negate the effects of time on outcomes; a problem inherent to single trial studies only comparing pre-and post-exercise concentrations. Resting and exercise arms had to also have been energy-matched (participants in both arms had to have consumed the same meal at the same timepoint).

Outcome: Studies measuring glucose and/or insulin and/or glucagon concentrations.

Studies which were not written in the English language, not published in peer-reviewed journals or included a clamp and/or infusion procedure prior to and/or during the exercise period were excluded. These exclusion criteria were chosen to prevent self-reported participant characteristics or health conditions from confounding the glucose, insulin, and/or glucagon response to exercise.

2.2.3 Information sources and search strategy

CENTRAL, CINAHL, Embase, Global Health, HMIC, Medline, PubMed, PsycINFO, ScienceDirect, Scopus and Web of Science databases were searched from inception to May 2020.

Full details of the search strategy are provided in Appendix 2.1. No limits were used during any database search.

2.2.4 Selection process

Database results were imported into Covidence systematic review software (Veritas Health Innovation, Australia). Titles and abstracts were independently reviewed and classified as 'yes', 'no' or 'maybe'. Papers classified as 'yes' or 'maybe' proceeded to the full-text screening stage. Full-text papers were then classified as 'yes' or 'no' independently, with those classified as 'yes' proceeding to the data extraction stage. Any disagreements in paper classification were collectively discussed before coming to an agreement regarding the eligibility of the paper.

2.2.5 Data collection

Data were extracted into an electronic spreadsheet (Excel 2016, Microsoft Corporation, USA) under the following columns: author; year of publication; sample size; participant characteristics; intervention characteristics; pre- and post-exercise concentrations of glucose and/or insulin and/or glucagon concentrations; and corresponding concentrations of glucose and/or insulin and/or glucagon concentrations in the resting control arm. WebPlotDigitizer Version 4.2 (Ankit Rohatgi, USA) was used to extract data from articles that only presented data in graphical form. If not all error bars were presented, homoscedasticity was assumed and the variance from the timepoint within the same experimental arm was imputed.

When glucose, insulin and/or glucagon data were not reported in the text (but methods stated measurements had been taken), methodology and/or participant characteristics were not described sufficiently to determine study eligibility, or data were displayed inadequately (e.g. clustering of data points, overlapping of error bars, collating sub-groups), corresponding authors were contacted. If the author did not respond, or could not provide the required data, the study was excluded.

Following data extraction, glucose, insulin and glucagon values were converted to SI units (glucose: mmol/L; insulin: pmol/L; glucagon: ng/L). If standard errors or 95% confidence intervals were provided, these were converted to a standard deviation. For each outcome, change scores for exercise and resting arms were calculated by subtracting pre-exercise concentrations from post-exercise concentrations. Mean differences (MDs) between resting

and exercise arms were then calculated by subtracting the resting change score from the exercise change score. A positive MD represented an increase in the outcome with exercise, whereas a negative MD represented a decrease with exercise. When within-participant correlation coefficients were not available, a correlation coefficient of 0.5 was assumed in order to calculate variance and standard error[30]. Sensitivity analyses were performed using correlation coefficients of 0.3, 0.7 and 0.9 to assess the robustness of findings to this assumption.

Studies which reported participants undertaking multiple exercise interventions but only one resting arm were combined into a single change score[31]. Exercise intervention characteristics (duration and intensity) were then averaged. Studies which did not report exercise intensity relative to maximal aerobic capacity ($\dot{V}O_2$ max) were converted using equations reported previously[32,33].

2.2.6 Data items

Eligible outcomes were defined as follows:

Glucose: measured in plasma or serum before and immediately after (± 5 minutes exercise cessation) a single bout of continuous aerobic exercise.

Insulin: measured in plasma or serum before and immediately after (± 5 minutes exercise cessation) a single bout of continuous aerobic exercise.

Glucagon: measured in plasma or serum before and immediately after (± 5 minutes exercise cessation) a single bout of continuous aerobic exercise.

These two timepoints were selected in order to evaluate the immediate effect of aerobic exercise on glucose, insulin, and glucagon concentrations that may be lost if investigating a longer time period.

2.2.7 Risk of bias assessment

Risk of bias was assessed using the Revised Cochrane Risk of Bias Tool for Randomized trials (RoB 2.0) with additional considerations for cross-over trials. These additional considerations include carryover effects, period effects, and concerns that trials may report only analyses based on the first period. Risk of bias was assessed using the following domains: bias arising from the randomization process; bias due to deviations from intended intervention; bias due to missing outcome data; bias in measurement of the outcome; and bias in selection of the reported result. No studies were excluded based on risk of bias assessment.

2.2.8 Data synthesis

Data were entered into Stata 16 (StataCorp, USA) for analysis. Data included: participant characteristics (metabolic state [fed or fasted], sample size, % males, age, BMI, $\dot{V}O_2$ max), exercise characteristics (mode [cycle ergometer or treadmill], duration, intensity), mean difference and corresponding standard error.

Fed exercise was defined as exercise performed within 6 hours of meal ingestion. Fasted exercise was defined as exercise performed 6 hours after last meal ingestion.

Overall effect sizes for each outcome were calculated using a random-effects model and with the Sidik-Jonkman approach being employed[34]. All simple effect sizes were presented as (unstandardised) MDs and using SI units to facilitate interpretability of results. A random-effects model was chosen over a fixed-effects model to account for differences in participant characteristics and methodology between studies[35]. This model assumes a distribution of true effect sizes across studies and provides an estimate of the average intervention effect of this distribution [35,36]. Results of syntheses were presented using forest plots. Heterogeneity was assessed using the chi-squared (Q) and I^2 statistic. A Q value above the degrees of freedom (df) for the estimate and an I^2 statistic >50% indicated large heterogeneity between studies.

To investigate the influence of fed and fasted exercise on MDs, a sub-group meta-analysis was performed. This sub-group analysis is presented as the main result for each outcome. Publication bias was assessed using visual inspection of contour-enhanced funnel plots[37] and statistically by Egger's regression test. Trim and fill analyses were used when publication bias was suspected to explore its impact on MDs. Statistical significance was set at $P<0.05$ in a Z test analysis. Z tests were used to examine if MDs were significantly different from zero. Results are displayed as overall MDs with 95% confidence intervals (CI).

2.2.9 Certainty of evidence assessment

The certainty of evidence was assessed using the strategy recommended by the Grading of Recommendations Assessment Development and Evaluation (GRADE) working group [38]. The certainty of evidence was assessed using the following domains: risk of bias; inconsistency; indirectness; imprecision; and publication bias. The estimated effect for each outcome was then classified as very low (true effect is probably markedly different from the estimated effect), low (true effect might be markedly different from the estimated effect), moderate (true effect is probably close to the estimated effect) or high quality (true effect is similar to the estimated effect).

2.3 Results

2.3.1 Study selection

Database searches identified 17141 potentially eligible papers. Title and abstract screening resulted in the exclusion of 16780 papers, resulting in 361 papers being assessed for eligibility by full-text inspection. Screening of full texts identified 42 papers which were eligible to be included in the Review and Meta-analysis. Due to several papers containing multiple studies, a total of 51 separate studies were included in the analysis. Consequently, each outcome comprised the following number of studies and total participants – glucose: 45 studies, 391 participants; insulin: 38 studies, 377 participants; glucagon: 5 studies, 47 participants. This process is summarised in Figure 2.1.

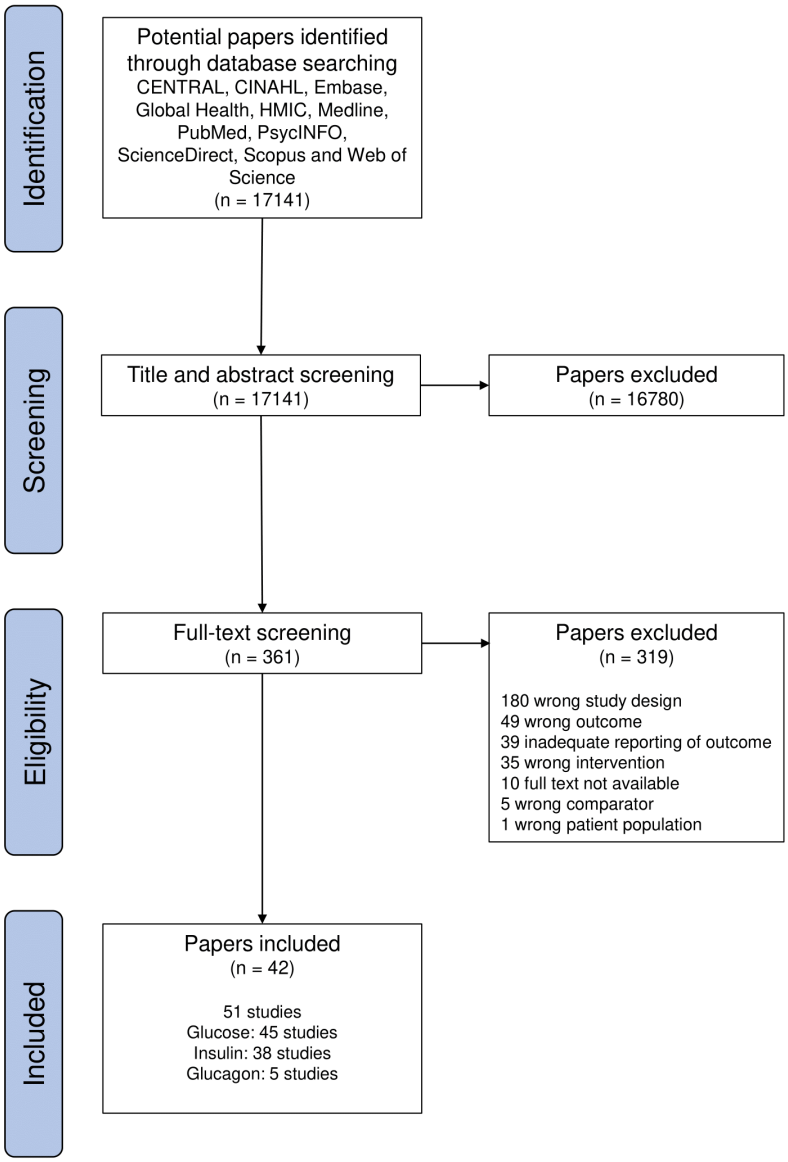


Figure 2.1: Flow diagram of paper selection.

2.3.2 Study characteristics

Details of the included studies are displayed in Table 2.1.

Table 2.1: Participant characteristics, intervention characteristics and outcome measurements for all included studies.

Study	Participant characteristics	Intervention characteristics	Glucose (mmol/L)	Insulin (pmol/L)	Glucagon (ng/L)
Bahr et al.[50]	12 males Fasted Age: 23.0 ± 1.7 $\dot{V}O_2$ max: 52 ± 3.6	Cycle ergometer 63 minutes 62% $\dot{V}O_2$ max	CON: 0.04 ± 0.44 EX: -0.73 ± 0.53	NM	NM
Balaguera-Cortes et al.[21]	10 males Fasted Age: 21.3 ± 1.4 BMI: 23.7 ± 2.0 $\dot{V}O_2$ max: 58.1 ± 7.3	Treadmill 45 minutes 70% $\dot{V}O_2$ max	CON: 0.00 ± 0.46 EX: 0.20 ± 0.44	CON: -10.66 ± 24.67 EX: 6.71 ± 19.64	NM
Bergfors et al.[51]	10 males Fasted Age: 26.7 ± 6.6 BMI: 23.1 ± 2.2	Cycle ergometer 37 minutes 60% $\dot{V}O_2$ max	CON: -0.10 ± 0.32 EX: 0.00 ± 0.55	CON: -4.20 ± 16.85 EX: -19.80 ± 14.32	NM
Broom et al.[11a]	9 males Fasted Age: 21.4 ± 1.7 BMI: 24.5 ± 2.4 $\dot{V}O_2$ max: 58 ± 6	Treadmill 55 minutes 52% $\dot{V}O_2$ max	CON: -0.19 ± 0.87 EX: -0.47 ± 0.72	CON: -0.34 ± 36.37 EX: -47.36 ± 62.09	NM
Broom et al.[11b]	9 males Fasted Age: 23.2 ± 2.1 BMI: 22.7 ± 1.5 $\dot{V}O_2$ max: 63 ± 6	Treadmill 68 minutes[c] 70% $\dot{V}O_2$ max	CON: -0.18 ± 0.20 EX: 0.60 ± 0.58	CON: -5.24 ± 10.37 EX: 13.41 ± 18.53	NM
Burns et al.[52]	9 males; 9 females Fasted Age: 24.8 ± 3.8 BMI: 22.9 ± 2.7 $\dot{V}O_2$ max: 57.7 ± 7.4	Treadmill 60 minutes 75% $\dot{V}O_2$ max	CON: -0.09 ± 0.67 EX: 1.37 ± 1.51	CON: -3.46 ± 43.25 EX: -20.06 ± 55.44	NM
Charlot et al.[53]	9 males Fed Age: 21.9 ± 1.8 BMI: 22.7 ± 1.6 $\dot{V}O_2$ max: 49 ± 9	Cycle ergometer 75 minutes 70% $\dot{V}O_2$ max	CON: -0.26 ± 0.59 EX: -0.95 ± 0.81	NM	NM

Clegg et.[54]	8 males Fasted Age: 22.9 ± 2.8	Cycle ergometer 60 minutes 35% $\dot{V}O_2$ max[d]	CON: -0.16 ± 0.34 EX: -0.36 ± 0.28	NM	NM
Douglas et al.[10a]	11 males, 11 females Fasted Age: 37.5 ± 15.2 BMI: 22.4 ± 1.5 $\dot{V}O_2$ max: 43.6 ± 12.2	Treadmill 60 minutes 60% $\dot{V}O_2$ max	CON: -0.18 ± 0.19 EX: 0.27 ± 0.64	CON: -4.66 ± 7.98 EX: -1.68 ± 13.01	NM
Douglas et al.[10b]	14 males, 11 females Fasted Age: 45.0 ± 12.4 BMI: 29.2 ± 2.9 $\dot{V}O_2$ max: 34.7 ± 8.9	Treadmill 60 minutes 60% $\dot{V}O_2$ max	CON: -0.16 ± 0.28 EX: 0.29 ± 0.45	CON: -1.53 ± 12.27 EX: 3.89 ± 21.09	NM
Edinburgh et al.[55]	10 males Fasted Age: 23.0 ± 3.0 BMI: 23.3 ± 1.8 $\dot{V}O_2$ max: 52.7 ± 8.9	Cycle ergometer 60 mins 63% $\dot{V}O_2$ max	CON: -0.03 ± 0.17 EX: -0.20 ± 0.53	CON: -2.45 ± 3.27 EX: -2.33 ± 7.49	NM
Enevoldsen et al.[56]	6 males Fed Age: 25 (23-28)[e]	Cycle ergometer 60 minutes 50% $\dot{V}O_2$ max	CON: -1.70 ± 0.93 EX: -2.57 ± 0.71	CON: -145.00 ± 111.48 EX: -220.00 ± 51.32	NM
Ezell et al.[57a]	5 females Fed Age: 25.6 ± 7.8 BMI: 20.6 ± 2.1 $\dot{V}O_2$ max: 33.0 ± 7.2	Cycle ergometer 60 minutes 63% $\dot{V}O_2$ max	CON: 0.44 ± 0.62 EX: 0.10 ± 0.72	CON: -83.40 ± 125.43 EX: -105.60 ± 72.20	NM
Ezell et al.[57b]	5 females Fed Age: 26.2 ± 6.3 BMI: 30 ± 6.0 $\dot{V}O_2$ max: 22.1 ± 6.8	Cycle ergometer 60 minutes 63% $\dot{V}O_2$ max	CON: -0.21 ± 0.51 EX: -0.60 ± 0.71	CON: -112.80 ± 81.91 EX: -130.20 ± 115.22	NM
Ezell et al.[57f]	5 females Fed Age: 22.6 ± 2.3 BMI: 22.7 ± 3.0 $\dot{V}O_2$ max: 30 ± 6.5	Cycle ergometer 60 minutes 63% $\dot{V}O_2$ max	CON: -0.01 ± 0.59 EX: -0.37 ± 0.49	CON: -34.20 ± 17.59 EX: -33.60 ± 27.37	NM
Farah & Gill[58]	10 males Fed Age: 28.1 ± 10.7	Treadmill 60 minutes 50% $\dot{V}O_2$ max	CON: -1.12 ± 1.03 EX: -0.12 ± 0.68	CON: -198.60 ± 199.56 EX: -231.24 ± 100.78	NM

	BMI: 29.0 ± 2.8 $\dot{V}O_2$ max: 39.1 ± 5.4				
Gonzalez et al.[59a]	11 males Fed Age: 23.2 ± 4.3 BMI: 24.5 ± 2.0 $\dot{V}O_2$ max: 53.1 ± 5.5	Treadmill 59 minutes 61% $\dot{V}O_2$ max	CON: 0.57 ± 0.29 EX: 0.60 ± 0.81	CON: -70.30 ± 63.69 EX: -158.64 ± 100.94	NM
Gonzalez et al.[59b]	11 males Fasted Age: 23.2 ± 4.3 BMI: 24.5 ± 2.0 $\dot{V}O_2$ max: 53.1 ± 5.5	Treadmill 59 minutes 61% $\dot{V}O_2$ max	CON: 0.07 ± 0.19 EX: 0.52 ± 0.26	CON: -9.04 ± 26.15 EX: -52.82 ± 22.28	NM
Goto et al.[60]	9 males Fasted Age: 24.0 ± 2.1 BMI: 22.1 ± 1.8	Cycle 30 minutes 60% $\dot{V}O_2$ max	CON: -0.08 ± 0.47 EX: 0.38 ± 0.44	NM	NM
Hagobian et al.[9a]	11 males Fasted Age: 22 ± 2 BMI: 26 ± 4 $\dot{V}O_2$ max: 42.9 ± 6.5	Cycle ergometer 82 minutes 70% $\dot{V}O_2$ max	NM	CON: -26.40 ± 32.67 EX: -30.60 ± 31.63	NM
Hagobian et al.[9b]	10 females Fasted Age: 21 ± 2 BMI: 24 ± 2 $\dot{V}O_2$ max: 39.9 ± 5.5	Cycle ergometer 84 minutes 70% $\dot{V}O_2$ max	NM	CON: -15.00 ± 26.09 EX: -24.00 ± 7.87	NM
Hardman & Aldred[61]	6 males, 6 females Fed Age: 26.0 ± 5.2 BMI: 23.95 ± 1.6 $\dot{V}O_2$ max: 48.2 ± 11.9	Treadmill 90 minutes 40% $\dot{V}O_2$ max	NM	CON: -11.88 ± 35.62 EX: -61.38 ± 67.22	NM
Højbjerre et al.[62a]	8 males Fasted Age: 26.0 ± 2.0 BMI: 22.8 ± 1.4 $\dot{V}O_2$ max: 57.1 ± 4.2	Cycle ergometer 60 minutes 55% $\dot{V}O_2$ max	CON: -0.01 ± 0.21 EX: -0.35 ± 0.52	NM	NM
Højbjerre et al.[62b]	8 males Fasted Age: 26.3 ± 2.3 BMI: 28.0 ± 0.8 $\dot{V}O_2$ max: 54.6 ± 6.2	Cycle ergometer 60 minutes 55% $\dot{V}O_2$ max	CON: -0.10 ± 0.13 EX: -0.26 ± 0.39	NM	NM

Isacco et al.[63a]	10 females Fed Age: 22.9 ± 3.5 BMI: 22.0 ± 3.2 $\dot{V}O_2$ max: 54.8 ± 5.4	Cycle ergometer 45 minutes 65% $\dot{V}O_2$ max	CON: 0.29 ± 0.54 EX: -0.54 ± 1.04	CON: -121.40 ± 143.92 EX: -85.19 ± 103.27	NM
Isacco et al.[63b]	11 females Fed Age: 21.2 ± 2.0 BMI: 22.6 ± 2.0 $\dot{V}O_2$ max: 50.4 ± 7.6	Cycle ergometer 45 minutes 65% $\dot{V}O_2$ max	CON: 0.01 ± 0.45 EX: -0.16 ± 0.90	CON: -29.56 ± 59.64 EX: -55.99 ± 48.94	NM
King et al.[64]	14 males Fasted Age: 21.9 ± 1.9 BMI: 23.4 ± 2.2 $\dot{V}O_2$ max: 55.9 ± 6.7	Treadmill 60 minutes 45% $\dot{V}O_2$ max	CON: 0.01 ± 0.60 EX: 0.03 ± 0.56	CON: 5.42 ± 28.47 EX: -9.78 ± 23.87	NM
Knudsen et al.[29]	7 Males Fasted Age: 57.0 ± 3.7 BMI: 26.8 ± 5.0 $\dot{V}O_2$ max: 36.4 ± 5.8	Cycle ergometer 60 minutes 55% $\dot{V}O_2$ max[d]	NM	CON: -2.81 ± 6.18 EX: -4.10 ± 4.61	NM
Larsen et al.[23]	12 males Fasted Age: 48.0 ± 5.0 BMI: 29.9 ± 1.9 $\dot{V}O_2$ max: 31.0 ± 8.0	Cycle ergometer 50 minutes 78% $\dot{V}O_2$ max	NM	CON: -9.64 ± 12.05 EX: -20.56 ± 12.05	CON: -6.50 ± 5.25 EX:17.44 ± 8.58
Lee et al.[65]	12 males Fasted Age: 36.9 ± 7.6 $\dot{V}O_2$ max: 26.3 ± 7.5	Treadmill 45 minutes 60% $\dot{V}O_2$ max	CON: -0.26 ± 0.46 EX: 0.01 ± 0.76	NM	NM
Marion-Latard et al.[66]	6 males Fed Age: 30.7 ± 6.9 BMI: 31.8 ± 2.5 $\dot{V}O_2$ max: 33.2 ± 4.7	Cycle ergometer 60 minutes 50% $\dot{V}O_2$ max	CON: 0.22 ± 0.49 EX: -0.20 ± 0.92	CON: -30.48 ± 33.74 EX: -32.22 ± 30.51	NM
Mattin et al.[67]	12 males Fasted Age: 26.0 ± 5.0 BMI: 25.5 ± 3.5 $\dot{V}O_2$ max: 42.2 ± 6.6	Cycle ergometer 60 minutes 55% $\dot{V}O_2$ max[c]	CON: -0.12 ± 0.29 EX: 0.14 ± 0.29	CON: 0.29 ± 24.87 EX: 2.32 ± 30.42	NM
Mc Clean et al.[68]	10 males Fed Age: 21.5 ± 2.5	Treadmill 60 minutes 35% $\dot{V}O_2$ max[d]	CON: 0.25 ± 0.38	NM	NM

	BMI: 23.6 ± 1.6 $\dot{V}O_2$ max: 58.5 ± 7.1		EX: 0.51 ± 0.34		
Morris et al.[69]	6 males Fed Age: 30.0 ± 8.0 BMI: 23.1 ± 1.1 $\dot{V}O_2$ max: 49 ± 7	Cycle ergometer 60 minutes 50% $\dot{V}O_2$ max	CON: -0.38 ± 0.88 EX: -0.22 ± 0.78	CON: -4.04 ± 24.47 EX: -10.44 ± 17.86	NM
Numao et al.[70]	8 Males Fasted Age: 24.9 ± 1.7 BMI: 21.9 ± 1.4 $\dot{V}O_2$ max: 52.8 ± 5.1	Cycle ergometer 60 minutes 50% $\dot{V}O_2$ max	CON: -0.10 ± 0.28 EX: -0.50 ± 0.28	CON: -10.90 ± 9.48 EX: -24.30 ± 17.82	NM
Nyhoff et al.[22]	11 females Fed Age: 24.3 ± 4.6 BMI: 37.3 ± 7.0 $\dot{V}O_2$ max: 25.2 ± 4.6	Treadmill 55 minutes 55% $\dot{V}O_2$ max	CON: -0.05 ± 0.69 EX: -0.31 ± 0.66	CON: -16.20 ± 172.79 EX: -108.80 ± 126.64	CON: -4.84 ± 4.78 EX: 15.10 ± 4.78
Petridou et al. [71]	11 males Fasted Age: 21.7 ± 2.0 BMI: 22.5 ± 1.6	Cycle ergometer 45 minutes 40% $\dot{V}O_2$ max[d]	CON: -0.14 ± 0.70 EX: -0.21 ± 0.70	CON: -13.80 ± 55.41 EX: -4.56 ± 62.56	NM
Rattray & Smee[72]	10 males, 10 females Fasted Age: 25.6 ± 5.4 $\dot{V}O_2$ max: 49.6 ± 8.1	Cycle ergometer 60 minutes 60% $\dot{V}O_2$ max[d]	CON: -0.75 ± 0.68 EX: -0.47 ± 0.96	NM	NM
Ronsen et al.[73]	9 males Fed Age: 21-27[e]; $\dot{V}O_2$ max: 69.1 ± 11.1	Cycle ergometer 65 mins 75% $\dot{V}O_2$ max	NM	CON: -32.17 ± 70.47 EX: -127.08 ± 19.74	NM
Ronsen et al.[74]	9 males Fed $\dot{V}O_2$ max: 69.1 ± 11.1	Cycle ergometer 65 mins 75% $\dot{V}O_2$ max	CON: 0.14 ± 0.57 EX: -1.27 ± 0.63	NM	NM
Schlierf et al.[75]	12 males Fed Age: 25 (21-37)[e]	Cycle ergometer 90 mins 40% $\dot{V}O_2$ max	CON: 0.55 ± 0.61 EX: 0.89 ± 0.62	CON: -10.80 ± 57.47 EX: -68.40 ± 75.65	NM
Shambrook et al.[76]	10 males Fed Age: 37.3 ± 7.3 BMI: 29.3 ± 6.5 $\dot{V}O_2$ max: 33.7 ± 7.4	Cycle ergometer 30 minutes 42% $\dot{V}O_2$ max[c]	CON: -0.58 ± 0.73 EX: -1.14 ± 0.64	NM	NM
Shambrook et al.[77]	8 males, 2 females	Treadmill	CON: 0.27 ± 0.28		

	Fed Age: 50.0 ± 12.6 BMI: 29.0 ± 5.4 $\dot{V}O_2$ max: 32.6 ± 6.5	30 minutes 63% $\dot{V}O_2$ max[d]	EX: -0.85 ± 0.37	NM	NM
Siopi et al.[78]	14 males Fasted Age: 41.0 ± 7.0 BMI: 28.1 ± 4.2 $\dot{V}O_2$ max: 37.0 ± 4.1	Treadmill 36 mins 40% $\dot{V}O_2$ max[d]	CON: 0.06 ± 0.55 EX: 0.00 ± 0.40	CON: -18.00 ± 33.41 EX: 0.00 ± 43.27	NM
Stokes et al.[79]	8 males Fasted Age: 22.0 ± 1.0 $\dot{V}O_2$ max: 53.0 ± 6.0	Cycle ergometer 30 minutes 70% $\dot{V}O_2$ max	CON: 0.06 ± 0.33 EX: -0.05 ± 0.38	NM	NM
Tobin et al.[28]	7 males Fed Age: 58.0 ± 3.2 BMI: 28.0 ± 2.4 $\dot{V}O_2$ max: 33.6 ± 6.4	Cycle ergometer 60 minutes 53 $\dot{V}O_2$ max	CON: 0.00 ± 0.63 EX: 0.51 ± 0.74	CON: 16.12 ± 76.28 EX: -12.14 ± 78.69	NM
Ueda et al.[20]	10 males Fed Age: 23.4 ± 4.3 BMI: 22.5 ± 1.0 $\dot{V}O_2$ max: 45.9 ± 8.5	Cycle ergometer 30 minutes 63% $\dot{V}O_2$ max[c]	CON: -0.13 ± 0.89 EX: -1.85 ± 1.24	CON: -21.42 ± 71.10 EX: -182.24 ± 55.07	NM
Ueda et al.[16a]	7 males Fed Age: 22.4 ± 4.2 BMI: 22.4 ± 2.4 $\dot{V}O_2$ max: 46.6 ± 3.9	Cycle ergometer 60 minutes 50% $\dot{V}O_2$ max	CON: -0.18 ± 0.74 EX: -0.12 ± 0.56	CON: -57.72 ± 86.12 EX: -84.84 ± 101.50	CON: 3.64 ± 61.70 EX: 52.35 ± 62.09
Ueda et al.[16b]	7 males Fed Age: 22.9 ± 3.4 BMI: 30.0 ± 3.1 $\dot{V}O_2$ max: 34.0 ± 6.3	Cycle ergometer 60 minutes 50% $\dot{V}O_2$ max	CON: -0.16 ± 0.38 EX: 0.09 ± 0.45	CON: -144.72 ± 153.07 EX: -159.30 ± 182.50	CON: 4.77 ± 66.35 EX: 23.56 ± 46.21
Vendelbo et al.[80]	8 males Fasted Age: 25.5 ± 12.2 BMI: 23.8 ± 5.5	Cycle ergometer 60 minutes 65% $\dot{V}O_2$max	CON: 0.00 ± 0.31 EX: 0.40 ± 0.56	CON: 1.00 ± 18.55 EX: 11.00 ± 32.62	NM
Willis et al.[15]	10 males Fasted Age: 26.0 ± 2.0 BMI: 25.6 ± 1.7 $\dot{V}O_2$ max: 49.8 ± 5.3	Treadmill 50 minutes[c] 65% $\dot{V}O_2$ max[c]	CON: -0.01 ± 1.32 EX: 0.94 ± 1.32	CON: 0.06 ± 19.30 EX: 2.33 ± 19.30	CON: -6.48 ± 14.78 EX: 25.32 ± 23.85

Data expressed as mean ± SD; Participant characteristic (units): age (years), BMI (kg/m^2) and $\dot{V}O_2$ max (ml/min/kg); CON, control arm; EX, exercise arm; NM, not measured or data could not be extracted; [a,b,f] after author names denotes sub-studies; [c]averaged value across two sub-studies; [d]converted to $\dot{V}O_2$ max; [e]only range provided.

2.3.3 Risk of bias analysis

The results of the risk of bias assessment for each outcome are presented in Appendix 2.2. Most studies measuring glucose (93%) and insulin (97%) concentrations were classified as possessing an unclear risk of bias overall. All studies measuring glucagon concentrations were classified as having an unclear risk of bias overall.

2.3.4 Meta-analysis

2.3.4.1 Glucose

The results of the meta-analysis revealed that aerobic exercise non-significantly decreased glucose concentrations compared to resting conditions irrespective if exercise was performed in the fed or fasted state (MD: -0.05 mmol/L; 95% CI, -0.22 to 0.13 mmol/L; P = 0.589; n = 45; Figure 2.2). I^2 (91.08%) and Q (401.33, df = 44, P<0.001) statistics highlighted large heterogeneity between studies.

Sub-group meta-analysis of fed and fasted exercise highlighted a significant difference in MDs between fed and fasted aerobic exercise (P = 0.013). Fed aerobic exercise significantly decreased glucose concentrations (MD: -0.27 mmol/L; 95% CI, -0.55 to -0.00 mmol/L; P = 0.049; n = 22) and fasted aerobic exercise non-significantly increased glucose concentrations (MD: 0.15 mmol/L; 95% CI, -0.04 to 0.34 mmol/L; P = 0.122; n = 23) relative to resting conditions. Sub-group analysis resulted in only a small reduction in the I^2 statistic (fed: 89.72%; fasted: 87.75%).

Visual inspection of the contour-enhanced funnel plot implied a symmetrical distribution, suggesting no evidence of publication bias (Figure 2.3A). This was supported by results from Egger's regression test (P = 0.604).

2.3.4.2 Insulin

The results of the meta-analysis revealed that aerobic exercise significantly decreased insulin concentrations relative to resting conditions irrespective if exercise was performed in the fed or fasted state (MD: -18.07 pmol/L; 95% CI, -30.47 to -5.66 pmol/L; P = 0.004; n = 38; Figure 2.4). I^2 (95.39%) and Q (190.11, df = 37, P<0.001) statistics highlighted large heterogeneity among studies.

Sub-group meta-analysis of fed and fasted exercise highlighted a significant difference in MDs between fed and fasted aerobic exercise (P = 0.002). Fed aerobic exercise significantly

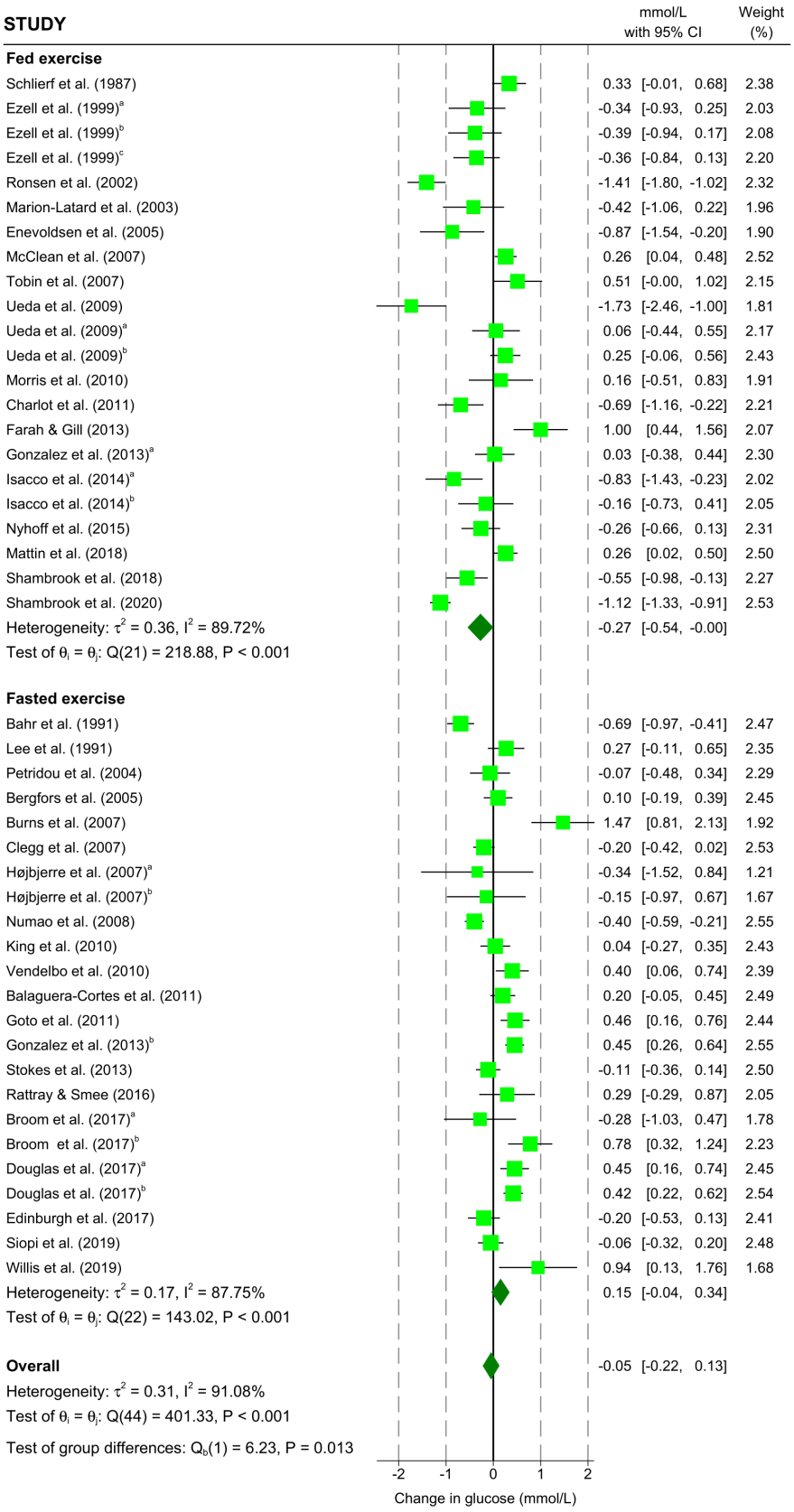


Figure 2.2: Sub-group forest plot of simple effect sizes (fed exercise vs fasted exercise) for studies assessing the effect of a single bout of continuous aerobic exercise on glucose concentrations (mmol/L). Data are presented as mean difference ± 95% CI. Random-effects Sidik-Jonkman model. [a,b,c] denotes sub-studies.

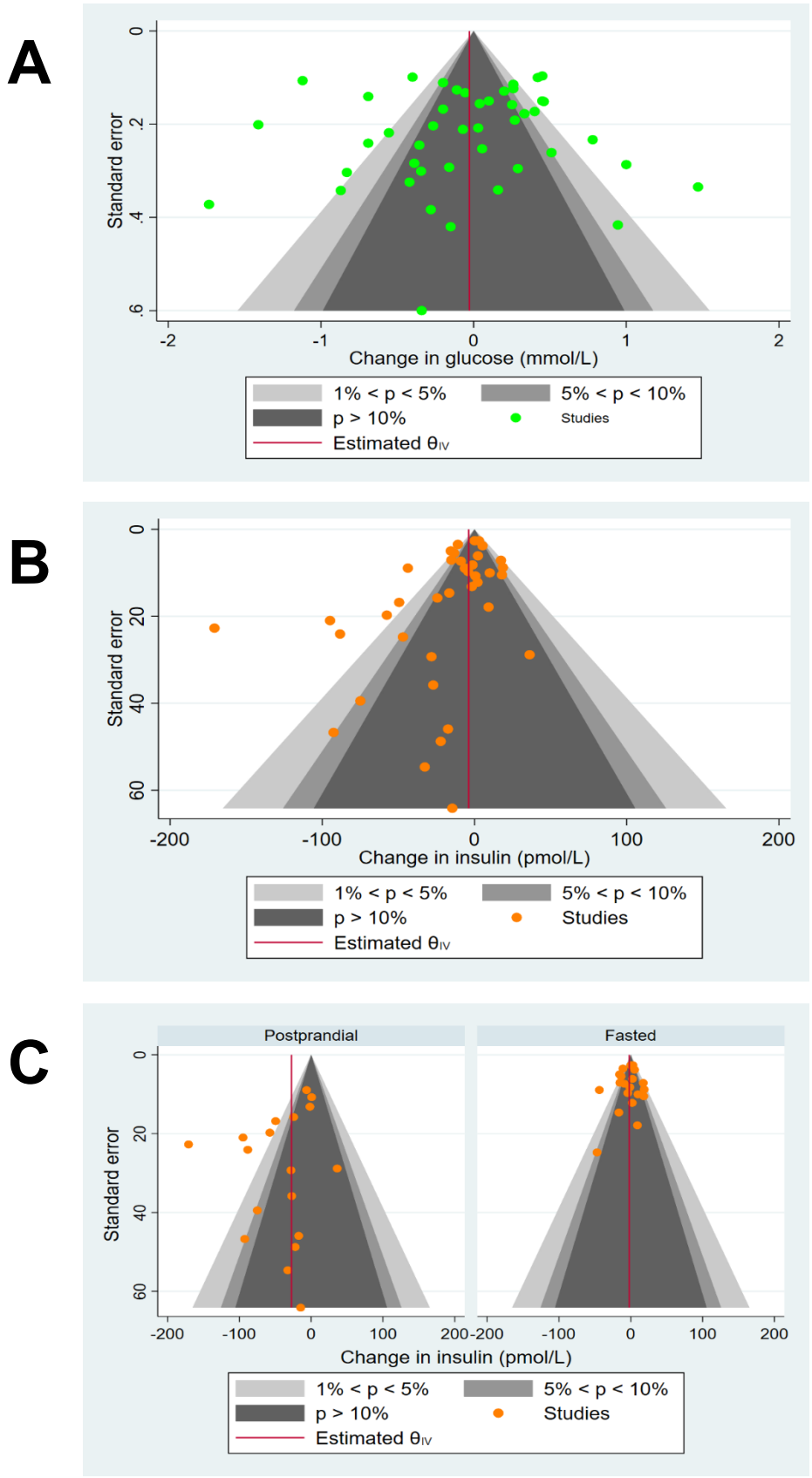


Figure 2.3: Contour-enhanced funnel plots for (A) glucose, (B) insulin, and (C) insulin by metabolic state. Significance contours were added at the 1% (light grey), 5% (medium grey) and 10% (dark grey) level.

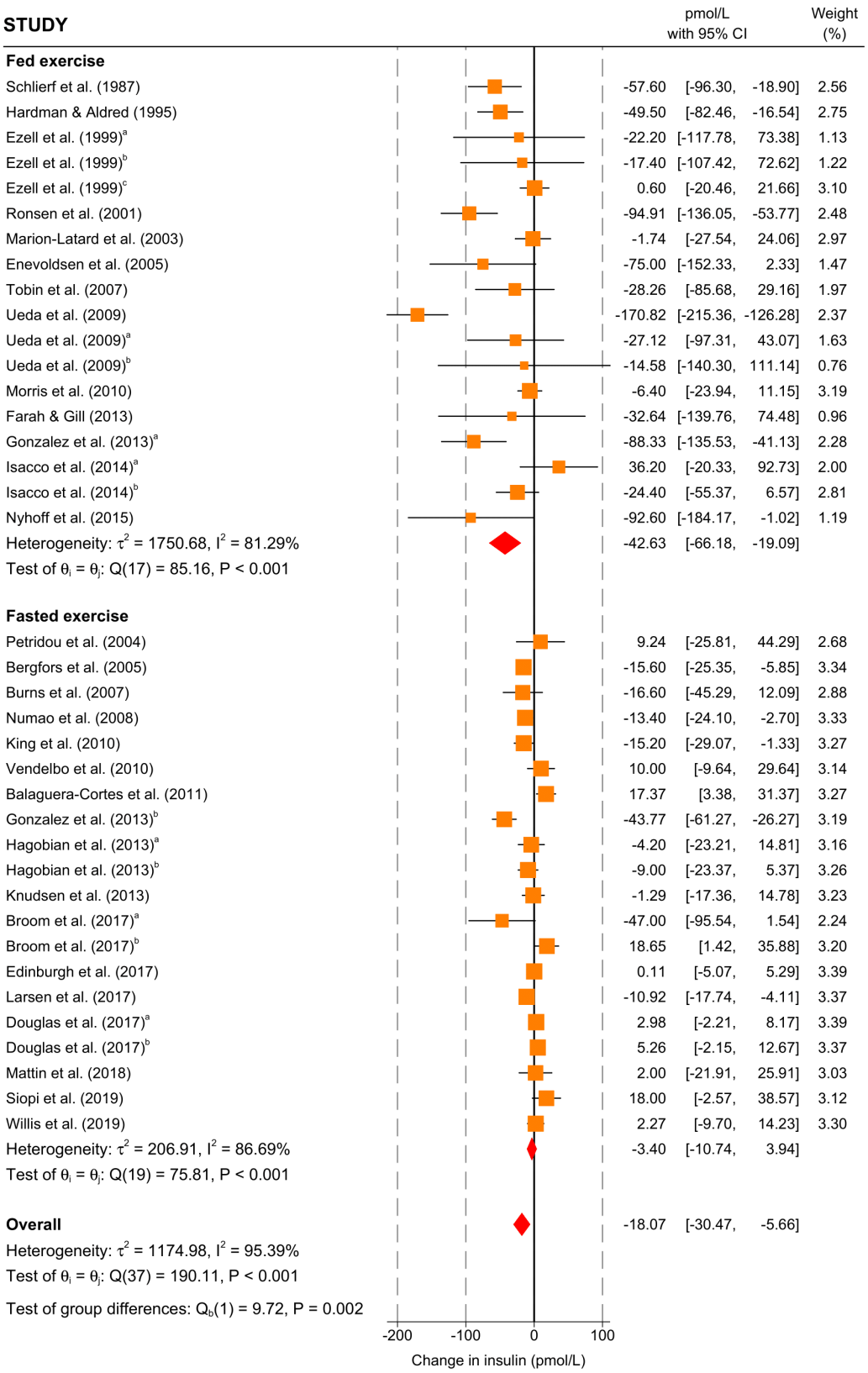


Figure 2.4: Sub-group forest plot of simple effect sizes (fed exercise vs fasted exercise) for studies assessing the effect of a single bout of continuous aerobic exercise on insulin concentrations (pmol/L). Data are presented as mean difference ± 95% CI. Random-effects Sidik-Jonkman model. [a,b,c] denotes sub-studies.

decreased insulin concentrations (MD: -42.63 pmol/L; 95% CI, -66.18 to -19.09 pmol/L; P<0.001; n = 18), whereas fasted aerobic exercise non-significantly decreased insulin concentrations (MD: -3.40 pmol/L; 95% CI, -10.74 to 3.94; P = 0.370; n = 20) compared to resting conditions. Sub-group analysis resulted in only a small reduction in the I^2 statistic (fed: 81.29%; fasted: 86.69%).

Visual inspection of the contour-enhance funnel plot showed a distribution to the left, suggesting publication bias (Figure 2.3B). However, studies appear to be missing from non-significant (dark grey) and significant (light grey and white) regions, indicating that funnel plot asymmetry maybe due to other factors such as heterogeneity. Based on the results of the sub-group meta-analysis showing a significant difference in MDs between fed and fasted exercise, separate contour-enhanced funnel plots were generated for each metabolic state (Figure 2.3C). Funnel plots for fed and fasted exercise displayed an approximal symmetrical distribution, which was supported by Egger's regression test with metabolic state (fed or fasted exercise) included as moderator (P = 0.404).

2.3.4.3 Glucagon

The results of the meta-analysis revealed that aerobic exercise significantly increased glucagon concentrations compared to resting conditions (MD: 24.60 ng/L; 95% CI, 16.25 to 32.95 ng/L; P<0.001; n = 5; Figure 2.5). I^2 (79.36%) and Q (6.23, df = 4, P = 0.183) statistics highlighted large heterogeneity between studies.

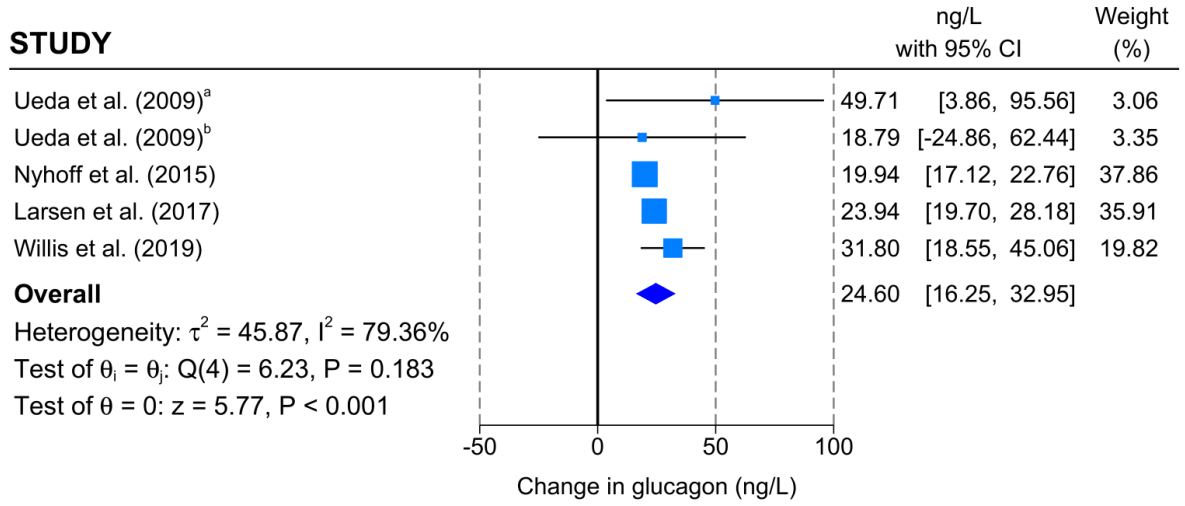


Figure 2.5: Forest plot of simple effect sizes for studies assessing the effect of a single bout of continuous aerobic exercise on glucagon concentrations (ng/L). Data are presented as mean difference ± 95% CI. Random-effects Sidik-Jonkman model. [a,b] denotes sub-studies.

Due to the small number of studies reporting glucagon concentrations, sub-group meta-analysis was not performed, and a contour-enhanced funnel plot was not created.

2.3.4.4 Sensitivity analyses

Sensitivity analyses employing within-participant correlation coefficients of 0.3, 0.7 and 0.9 did not affect the significance of the MDs for insulin, glucagon or glucose (Appendix 2.3).

2.3.5 Quality of evidence

The effect estimates for insulin, glucagon and glucose outcomes were all categorised as moderate quality. Insulin, glucagon and glucose were all downgraded by one level due to inconsistency of results, as the large heterogeneity observed for all three outcomes could not be explained by sub-group analyses or meta-regression. A summary of findings is presented in Table 2.2.

Table 2.2: Summary of findings for glucose, insulin and glucagon outcomes.

Acute continuous aerobic exercise compared with resting conditions in adults without diabetes

Patient or population: adults without diabetes

Setting: laboratory environment

Intervention: acute continuous aerobic exercise

Comparison: rest

Outcomes	**Relative effect** (95% CI)	**Number of participants** (studies)	**Certainty of evidence** (GRADE)	Comments
Glucose (mmol/L)	MD 0.05 mmol/L lower with exercise (0.22 lower to 0.13 higher)	391 participants (45 studies)	⊕⊕⊕⊖ Moderate[a]	Glucose concentrations moderated by metabolic state
Insulin (pmol/L)	MD 18.07 pmol/L lower with exercise (5.66 lower to 30.47 lower)	377 participants (38 studies)	⊕⊕⊕⊖ Moderate[b]	Insulin concentrations moderated by metabolic state
Glucagon (ng/L)	MD 24.60 ng/L higher with exercise (16.25 higher to 32.95 higher)	47 participants (5 studies)	⊕⊕⊕⊖ Moderate[c]	

CI: confidence interval; MD: mean difference.

GRADE Working Group grades of evidence

High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.

Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a]There was considerable heterogeneity (I^2 = 91.08%) that could not be explained by sub-group analyses or meta-regression.

[b]There was considerable heterogeneity (I^2 = 95.39%) that could not be explained by sub-group analyses or meta-regression.

[c]There was considerable heterogeneity (I^2 = 79.36%) that could not be explained by sub-group analyses or meta-regression.

2.4 Discussion

The aim of this review was to determine the effect of a single bout of continuous aerobic exercise on circulating glucose, insulin and glucagon concentrations in adults without diabetes, and the influence of whether exercise was performed in the fed or fasted state on these effects. Our results reveal that a single bout of aerobic exercise significantly decreases glucose and insulin concentrations when performed in the fed state, but not when performed in the fasted state. Our results also show that acute aerobic exercise significantly increases glucagon concentrations.

2.4.1 The effect of single bout of continuous aerobic exercise on glucose concentrations

Acute aerobic exercise appeared to result in no meaningful change in glucose concentrations compared to resting conditions when the metabolic state (fed or fasted exercise) was not accounted for. However, when accounting for metabolic state, fed aerobic exercise resulted in a significant reduction in glucose concentrations. This reduction is likely due to the induction of glucose transporter translocation and glucose transporter activity in skeletal muscle by exercise [39]. The upregulation of glucose transporter translocation and activity may not be secondary to insulin action, as exercise-stimulated glucose uptake has been demonstrated to occur independently of insulin [40], and glucose concentrations decreased in the context of decreasing insulin concentrations during fed aerobic exercise. Alternatively, exercise can increase insulin-dependent glucose uptake, possibly via a reduction in intramuscular glycogen and/or increase in AS160 phosphorylation [41]. The decrease in insulin concentrations (despite a reduction in glucose concentrations) may therefore reflect an increase in insulin sensitivity instead. Regardless of the mechanism responsible, this reduction is likely facilitated by the increase in microvascular recruitment and blood flow to skeletal muscle (thus increasing glucose delivery) caused by exercise [40]. In contrast, no significant change in glucose concentrations during fasted aerobic exercise was detected. This is likely due to glucose concentrations already being low following an overnight fast [27], and that participants were individuals without diabetes, therefore making any further reduction difficult. The overall absence of a large decrease or increase in glucose concentrations does however highlight the high degree to which glucose concentrations are homeostatically regulated in non-diabetic populations.

With regards to exercise-induced anorexia, it appears unlikely that the changes in glucose concentrations during exercise influence subjective appetite. Firstly, the change in glucose concentrations only appears to be present when exercise is performed in the fed state, despite exercise-induced anorexia being observed in both the fed and fasted state[10,42]. Secondly, the direction of change that occurs with (fasted) exercise is in the opposite direction to what would be expected if glucose was involved in exercise-induced anorexia (a decrease in glucose would likely increase subjective appetite[19,43]).

2.4.2 The effect of single bout of continuous aerobic exercise on insulin concentrations

Acute aerobic exercise resulted in a significant reduction in circulating insulin concentrations relative to resting conditions irrespective when performed in the fed but fasted state. This reduction in insulin concentrations may partly reflect the decrease in glucose concentrations observed with fed aerobic exercise, which may be due to the stimulation of insulin-independent glucose uptake pathways in skeletal muscle by exercise [44]. Alternatively or additively, the reduction in insulin concentrations with fed exercise may be caused by an increase in insulin clearance [45], or an increase in insulin delivery (blood flow x blood insulin concentration) as a result of exercise-induced increases in skeletal muscle perfusion, decreasing insulin requirements and thus output [46]. In contrast, acute aerobic exercise undertaken in the fasted state resulted in a non-significant reduction in insulin concentrations. Short-term fasting (<24 hours) is well known to decrease insulin concentrations [27] and insulin levels would likely be at their lowest following >6 hours of fasting in non-diabetic individuals. Therefore, aerobic exercise performed in the fasted state is unlikely to prompt further reductions, especially when compared to fasted resting conditions.

Like glucose, the results from this analysis do not support a role for changes in insulin concentrations in exercise-induced anorexia. Again, this is largely due to the observed change in insulin with exercise (decreased concentrations) occurring in the opposite direction to what would be expected if insulin played a role in the suppression of appetite observed during exercise (increased insulin concentrations are associated with a reduction in appetite[18]).

2.4.3 The effect of single bout of continuous aerobic exercise on glucagon concentrations

To our knowledge, this is the first study to quantify the changes in glucagon concentrations during exercise using a meta-analytical approach. The results from our analysis showed that acute aerobic exercise increased glucagon concentrations relative to resting conditions. Importantly, all five studies reported an increase in glucagon concentrations independent of metabolic state. This increase may be necessary to stimulate hepatic gluconeogenesis in order to provide substrate for contracting muscles and maintain euglycaemia [47]; thus facilitating the absence of any large deviations in glucose concentrations. Despite the

consistency of the glucagon response to acute aerobic exercise, the findings from this analysis should be treated with caution due to the small number of studies included. Future work should explore the effect of metabolic state (fed vs fasted) on changes in glucagon concentrations during acute aerobic exercise considering the limited number of studies currently available.

Unlike glucose and insulin, acute exercise results in an increase in glucagon concentrations; a response which is congruent with a role in exercise-induced anorexia. While the mechanism underlying glucagon-induced appetite is not fully established, it is hypothesised to operate via the liver-vagus-hypothalamus axis[48]. Nevertheless, glucagon concentrations are rarely measured during acute exercise studies, and consequently there are no reports of its association with appetite during or post-exercise. Future work investigating acute exercise and appetite should look to prioritize glucagon measurements (considering the consistent glucagon response to acute aerobic exercise) to evaluate its role in exercise-induced anorexia. These gaps in the current literature will be addressed in Chapter 4 of this thesis.

2.4.4 Limitations

There are several limitations to the present review and meta-analysis. Firstly, the application of these results is restricted to individuals who possess the same characteristics as those defined by the inclusion and exclusion criteria (adults without diabetes). The glucose, insulin and glucagon response to acute aerobic exercise in other populations cannot be assumed from our findings. Likewise, the results cannot be applied to other exercise modalities, such as high-intensity interval training or resistance training. The large heterogeneity observed in all three analyses is another limitation of the current review and meta-analysis but was expected considering the diversity in participant characteristics and experimental methodology used in acute exercise studies. Glucose, insulin and glucagon outcomes were consequently downgraded by one level using the GRADE approach and classified as moderate quality due to the large heterogeneity observed. The present review is also limited by the small number of studies measuring glucagon concentrations, which prevented sub-group meta-analysis from being performed. A further limitation is the use of pre- and post-exercise measurements to summarise the effect of acute exercise. While these measures represent the effect of acute exercise on glucose, insulin and glucagon concentrations at the point of exercise completion, they do not account for the temporal changes during exercise. Furthermore, these measures do not consider the effect of acute exercise on glucose, insulin and glucagon concentrations in the post-exercise period. Lastly, the majority of studies measuring glucose and/or insulin and/or glucagon concentrations were classified as having an unclear risk of bias overall. This was largely due to inadequate reporting of the randomisation process. Future investigations in this field should therefore report methodology in sufficient detail as described in the recent PRESENT checklist proposed by Betts et al. [49].

2.4.5 Summary

This systematic review and meta-analysis found that, when not accounting for metabolic state, a single bout of continuous aerobic exercise had no significant effect on glucose concentrations, but significantly decreased insulin (~20 pmol/L on average) and significantly increased glucagon concentrations (~25 ng/L on average) relative to resting conditions in healthy adults. Sub-group analyses, however, revealed that the glucose and insulin responses were significantly moderated by metabolic state. A single bout of continuous aerobic exercise significantly decreased glucose (~0.3 mmol/L on average) and insulin (~40 pmol/L on average) concentrations when performed in the fed state (within 6 hours of meal ingestion), but had no significant effect in the fasted state (at least 6 hours after last meal ingestion) relative to resting conditions. When considering higher concentrations of glucose, insulin, and glucagon are associated with a suppression of appetite, and glucagon was the only outcome to experience an increase in response to acute exercise, our findings support a potential role of glucagon in exercise-induced anorexia. Future experimental work should attempt to evaluate the causal relationship between the increase in glucagon and decrease in subjective appetite observed in response to acute exercise. This will be investigated in Chapter 4.

2.6 References

1. King, N. A., Burley, V. J. & Blundell, J. E. Exercise-induced suppression of appetite: effects on food intake and implications for energy balance. *Eur. J. Clin. Nutr.* **48**, 715–24 (1994).

2. Müller, T. D. *et al.* Ghrelin. *Mol. Metab.* **4**, 437–460 (2015).

3. Karra, E., Chandarana, K. & Batterham, R. L. The role of peptide YY in appetite regulation and obesity. *J. Physiol.* **587**, 19–25 (2009).

4. Müller, T. D. *et al.* Glucagon-like peptide 1 (GLP-1). *Mol. Metab.* **30**, 72–130 (2019).

5. Schubert, M. M., Sabapathy, S., Leveritt, M. & Desbrow, B. Acute Exercise and Hormones Related to Appetite Regulation: A Meta-Analysis. *Sport. Med.* **44**, 387–403 (2014).

6. Martins, C., Morgan, L. M., Bloom, S. R. & Robertson, M. D. Effects of exercise on gut peptides, energy intake and appetite. *J. Endocrinol.* **193**, 251–258 (2007).

7. Broom, D. R., Batterham, R. L., King, J. A. & Stensel, D. J. Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. *Am. J. Physiol. Integr. Comp. Physiol.* **296**, R29–R35 (2009).

8. Unick, J. L. *et al.* Acute effect of walking on energy intake in overweight/obese

women. *Appetite* **55**, 413–419 (2010).

9. Hagobian, T. A. *et al.* Effects of acute exercise on appetite hormones and ad libitum energy intake in men and women. *Appl. Physiol. Nutr. Metab.* **38**, 66–72 (2013).

10. Douglas, J. A. *et al.* Acute effects of exercise on appetite, ad libitum energy intake and appetite-regulatory hormones in lean and overweight/obese men and women. *Int. J. Obes.* **41**, 1737–1744 (2017).

11. Broom, D. R. *et al.* Acute effect of exercise intensity and duration on acylated ghrelin and hunger in men. *J. Endocrinol.* **232**, 411–422 (2017).

12. Holliday, A. & Blannin, A. Appetite, food intake and gut hormone responses to intense aerobic exercise of different duration. *J. Endocrinol.* **235**, 193–205 (2017).

13. Deighton, K., Karra, E., Batterham, R. L. & Stensel, D. J. Appetite, energy intake, and PYY 3–36 responses to energy-matched continuous exercise and submaximal high-intensity exercise. *Appl. Physiol. Nutr. Metab.* **38**, 947–952 (2013).

14. Deighton, K., Batterham, R. L. & Stensel, D. J. Appetite and gut peptide responses to exercise and calorie restriction. The effect of modest energy deficits. *Appetite* **81**, 52–59 (2014).

15. Willis, S. A. *et al.* Effect of exercise intensity on circulating hepatokine concentrations in healthy men. *Appl. Physiol. Nutr. Metab.* **44**, 1065–1072 (2019).

16. Ueda, S. *et al.* Changes in gut hormone levels and negative energy balance during aerobic exercise in obese young males. *J. Endocrinol.* **201**, 151–159 (2009).

17. Jones, B. J., Tan, T. & Bloom, S. R. Minireview: Glucagon in Stress and Energy Homeostasis. *Endocrinology* **153**, 1049–1054 (2012).

18. Loh, K. *et al.* Insulin controls food intake and energy balance via NPY neurons. *Mol. Metab.* **6**, 574–584 (2017).

19. Wyatt, P. *et al.* Postprandial glycaemic dips predict appetite and energy intake in healthy individuals. *Nat. Metab.* **3**, 523–529 (2021).

20. Ueda, S., Yoshikawa, T., Katsura, Y., Usui, T. & Fujimoto, S. Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. *J. Endocrinol.* **203**, 357–364 (2009).

21. Balaguera-Cortes, L., Wallman, K. E., Fairchild, T. J. & Guelfi, K. J. Energy intake and appetite-related hormones following acute aerobic and resistance exercise. *Appl.*

Physiol. Nutr. Metab. **36**, 958–966 (2011).

22. Nyhoff, L. M. *et al.* Prior exercise does not alter the incretin response to a subsequent meal in obese women. *Peptides* **71**, 94–99 (2015).

23. Larsen, P. S. *et al.* Effects of Aerobic, Strength or Combined Exercise on Perceived Appetite and Appetite-Related Hormones in Inactive Middle-Aged Men. *Int. J. Sport Nutr. Exerc. Metab.* **27**, 389–398 (2017).

24. Schwingshackl, L., Missbach, B., Dias, S., König, J. & Hoffmann, G. Impact of different training modalities on glycaemic control and blood lipids in patients with type 2 diabetes: a systematic review and network meta-analysis. *Diabetologia* **57**, 1789–1797 (2014).

25. Liubaoerjijin, Y., Terada, T., Fletcher, K. & Boulé, N. G. Effect of aerobic exercise intensity on glycemic control in type 2 diabetes: a meta-analysis of head-to-head randomized trials. *Acta Diabetol.* **53**, 769–781 (2016).

26. Grace, A., Chan, E., Giallauria, F., Graham, P. L. & Smart, N. A. Clinical outcomes and glycaemic responses to different aerobic exercise training intensities in type II diabetes: a systematic review and meta-analysis. *Cardiovasc. Diabetol.* **16**, 37 (2017).

27. Groop, L. C., Bonadonna, R., DelPrato, S., Ratheiser, K. & DeFronzo, R. A. Effect of prolonged overnight fasting on energy metabolism in non-insulin-dependent diabetic and non-diabetic subjects. *Acta Endocrinol. (Copenh).* **123**, 30–36 (1990).

28. Tobin, L. W. L., Kiens, B. & Galbo, H. The effect of exercise on postprandial lipidemia in type 2 diabetic patients. *Eur. J. Appl. Physiol.* **102**, 361–370 (2007).

29. Knudsen, S. H., Karstoft, K. & Solomon, T. P. J. Impaired postprandial fullness in Type 2 diabetic subjects is rescued by acute exercise independently of total and acylated ghrelin. *J. Appl. Physiol.* **115**, 618–625 (2013).

30. Follmann, D., Elliott, P., Suh, I. & Cutler, J. Variance imputation for overviews of clinical trials with continuous response. *J. Clin. Epidemiol.* **45**, 769–773 (1992).

31. Borenstein, M., Hedges, L. V., Higgins, J. P. T. & Rothstein, H. R. *Introduction to Meta-Analysis*. (John Wiley & Sons, Ltd, 2009). doi:10.1002/9780470743386.

32. Schneider, D. & Pollack, J. Ventilatory Threshold and Maximal Oxygen Uptake during Cycling and Running in Female Triathletes. *Int. J. Sports Med.* **12**, 379–383 (1991).

33. Arts, F. & Kuipers, H. The Relation Between Power Output, Oxygen Uptake and Heart Rate in Male Athletes. *Int. J. Sports Med.* **15**, 228–231 (1994).

34. Sidik, K. & Jonkman, J. N. A simple confidence interval for meta-analysis. *Stat. Med.* **21**, 3153–3159 (2002).

35. Borenstein, M., Hedges, L. V., Higgins, J. P. T. & Rothstein, H. R. A basic introduction to fixed-effect and random-effects models for meta-analysis. *Res. Synth. Methods* **1**, 97–111 (2010).

36. Riley, R. D., Higgins, J. P. T. & Deeks, J. J. Interpretation of random effects meta-analyses. *BMJ* **342**, d549–d549 (2011).

37. Peters, J. L., Sutton, A. J., Jones, D. R., Abrams, K. R. & Rushton, L. Contour-enhanced meta-analysis funnel plots help distinguish publication bias from other causes of asymmetry. *J. Clin. Epidemiol.* **61**, 991–996 (2008).

38. Guyatt, G. H. *et al.* GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* **336**, 924–926 (2008).

39. Henriksen, E. J. Invited Review: Effects of acute exercise and exercise training on insulin resistance. *J. Appl. Physiol.* **93**, 788–796 (2002).

40. Fritzen, A. M., Lundsgaard, A. & Kiens, B. Tuning fatty acid oxidation in skeletal muscle with dietary fat and exercise. *Nat. Rev. Endocrinol.* (2020) doi:10.1038/s41574-020-0405-1.

41. Cartee, G. D. Mechanisms for greater insulin-stimulated glucose uptake in normal and insulin-resistant skeletal muscle after acute exercise. *Am. J. Physiol. Metab.* **309**, E949–E959 (2015).

42. Matos, V. *et al.* Acute Effects of High-Intensity Interval and Moderate-Intensity Continuous Exercise on GLP-1, Appetite and Energy Intake in Obese Men: A Crossover Trial. *Nutrients* **10**, 889 (2018).

43. Chaput, J.-P. & Tremblay, A. The glucostatic theory of appetite control and the risk of obesity and diabetes. *Int. J. Obes.* **33**, 46–53 (2009).

44. Sylow, L., Kleinert, M., Richter, E. A. & Jensen, T. E. Exercise-stimulated glucose uptake — regulation and implications for glycaemic control. *Nat. Rev. Endocrinol.* **13**, 133–148 (2017).

45. Tuominen, J. A., Ebeling, P. & Koivisto, V. A. Exercise increases insulin clearance in healthy man and insulin-dependent diabetes mellitus patients. *Clin. Physiol.* **17**, 19–30 (1997).

46. Wojtaszewski, J. F. P., Nielsen, J. N. & Richter, E. A. Invited Review: Effect of acute

exercise on insulin signaling and action in humans. *J. Appl. Physiol.* **93**, 384–392 (2002).

47. Trefts, E., Williams, A. S. & Wasserman, D. H. Exercise and the Regulation of Hepatic Metabolism. in *Progress in Molecular Biology and Translational Science* vol. 135 203–225 (2015).

48. Geary, N., Le Sauter, J. & Noh, U. Glucagon acts in the liver to control spontaneous meal size in rats. *Am. J. Physiol. Integr. Comp. Physiol.* **264**, R116–R122 (1993).

49. Betts, J. A. *et al.* PRESENT 2020: Text Expanding on the Checklist for Proper Reporting of Evidence in Sport and Exercise Nutrition Trials. *Int. J. Sport Nutr. Exerc. Metab.* **30**, 2–13 (2020).

50. Bahr, R., Høstmark, A. T., Newsholme, E. A., Grønnerød, O. & Sejersted, O. M. Effect of exercise on recovery changes in plasma levels of FFA, glycerol, glucose and catecholamines. *Acta Physiol. Scand.* **143**, 105–15 (1991).

51. Bergfors, M., Barnekow-Bergkvist, M., Kalezic, N., Lyskov, E. & Eriksson, J. W. Short-term effects of repetitive arm work and dynamic exercise on glucose metabolism and insulin sensitivity. *Acta Physiol. Scand.* **183**, 345–356 (2005).

52. Burns, S. F., Broom, D. R., Miyashita, M., Mundy, C. & Stensel, D. J. A single session of treadmill running has no effect on plasma total ghrelin concentrations. *J. Sports Sci.* **25**, 635–642 (2007).

53. Charlot, K., Pichon, A. & Chapelot, D. Exercise prior to a freely requested meal modifies pre and postprandial glucose profile, substrate oxidation and sympathovagal balance. *Nutr. Metab. (Lond).* **8**, 66 (2011).

54. Clegg, M. *et al.* Exercise and postprandial lipaemia: effects on peripheral vascular function, oxidative stress and gastrointestinal transit. *Lipids Health Dis.* **6**, 30 (2007).

55. Edinburgh, R. M. *et al.* Prior exercise alters the difference between arterialised and venous glycaemia: implications for blood sampling procedures. *Br. J. Nutr.* **117**, 1414–1421 (2017).

56. Enevoldsen, L. H., Simonsen, L. & Bülow, J. Postprandial triacylglycerol uptake in the legs is increased during exercise and post-exercise recovery. *J. Physiol.* **568**, 941–950 (2005).

57. Ezell, D. *et al.* Substrate oxidation and availability during acute exercise in non-obese, obese, and post-obese sedentary females. *Int. J. Obes.* **23**, 1047–1056 (1999).

58. Farah, N. M. F. & Gill, J. M. R. Effects of exercise before or after meal ingestion on fat balance and postprandial metabolism in overweight men. *Br. J. Nutr.* **109**, 2297–2307 (2013).

59. Gonzalez, J. T., Veasey, R. C., Rumbold, P. L. S. & Stevenson, E. J. Breakfast and exercise contingently affect postprandial metabolism and energy balance in physically active males. *Br. J. Nutr.* **110**, 721–732 (2013).

60. Goto, K., Tanaka, K., Ishii, N., Uchida, S. & Takamatsu, K. A single versus multiple bouts of moderate-intensity exercise for fat metabolism. *Clin. Physiol. Funct. Imaging* **31**, 215–220 (2011).

61. Hardman, A. E. & Aldred, H. E. Walking during the postprandial period decreases alimentary lipaemia. *J. Cardiovasc. Risk* **2**, 71–8 (1995).

62. Højbjerre, L., Rosenzweig, M., Dela, F., Bruun, J. M. & Stallknecht, B. Acute exercise increases adipose tissue interstitial adiponectin concentration in healthy overweight and lean subjects. *Eur. J. Endocrinol.* **157**, 613–623 (2007).

63. Isacco, L. *et al.* Exercise per se masks oral contraceptive-induced postprandial lipid mobilization. *Appl. Physiol. Nutr. Metab.* **39**, 1222–1229 (2014).

64. King, J. A., Wasse, L. K., Broom, D. R. & Stensel, D. J. Influence of brisk walking on appetite, energy intake, and plasma acylated ghrelin. *Med. Sci. Sports Exerc.* **42**, 485–92 (2010).

65. Lee, R., Nieman, D., Raval, R., Blankenship, J. & Lee, J. The Effects of Acute Moderate Exercise on Serum Lipids and Lipoproteins in Mildly Obese Women. *Int. J. Sports Med.* **12**, 537–542 (1991).

66. Marion-Latard, F. *et al.* Post-Exercise Increase of Lipid Oxidation after a Moderate Exercise Bout in Untrained Healthy Obese Men. *Horm. Metab. Res.* **35**, 97–103 (2003).

67. Mattin, L., Yau, A., McIver, V., James, L. & Evans, G. 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**, 787 (2018).

68. Mc Clean, C. M. *et al.* The effect of acute aerobic exercise on pulse wave velocity and oxidative stress following postprandial hypertriglyceridemia in healthy men. *Eur. J. Appl. Physiol.* **100**, 225–234 (2007).

69. Morris, C. J. *et al.* Paradoxical Post-Exercise Responses of Acylated Ghrelin and Leptin During a Simulated Night Shift. *Chronobiol. Int.* **27**, 590–605 (2010).

70. Numao, S. *et al.* Effects of Acute Aerobic Exercise on High-Molecular-Weight Adiponectin. *Med. Sci. Sport. Exerc.* **40**, 1271–1276 (2008).

71. Petridou, A. *et al.* Effect of exercise performed immediately before a meal of moderate fat content on postprandial lipaemia. *Br. J. Nutr.* **91**, 683–687 (2004).

72. Rattray, B. & Smee, D. J. The effect of high and low exercise intensity periods on a simple memory recognition test. *J. Sport Heal. Sci.* **5**, 342–348 (2016).

73. Ronsen, O., Haug, E., Pedersen, B. K. & Bahr, R. Increased neuroendocrine response to a repeated bout of endurance exercise. *Med. Sci. Sports Exerc.* **33**, 568–75 (2001).

74. Ronsen, O., Lea, T., Bahr, R. & Pedersen, B. K. Enhanced plasma IL-6 and IL-1ra responses to repeated vs. single bouts of prolonged cycling in elite athletes. *J. Appl. Physiol.* **92**, 2547–2553 (2002).

75. Schlierf, G., Dinsenbacher, A., Kather, H., Kohlmeier, M. & Haberbosch, W. Mitigation of alimentary lipemia by postprandial exercise—Phenomena and mechanisms. *Metabolism* **36**, 726–730 (1987).

76. Shambrook, P. *et al.* Glucose response to exercise in the post-prandial period is independent of exercise intensity. *Scand. J. Med. Sci. Sports* **28**, 939–946 (2018).

77. Shambrook, P. *et al.* A comparison of acute glycaemic responses to accumulated or single bout walking exercise in apparently healthy, insufficiently active adults. *J. Sci. Med. Sport* **23**, 902–907 (2020).

78. Siopi, A. *et al.* Comparison of the Serum Metabolic Fingerprint of Different Exercise Modes in Men with and without Metabolic Syndrome. *Metabolites* **9**, 116 (2019).

79. Stokes, K. A., Gilbert, K. L., Hall, G. M., Andrews, R. C. & Thompson, D. Different responses of selected hormones to three types of exercise in young men. *Eur. J. Appl. Physiol.* **113**, 775–783 (2013).

80. Vendelbo, M. H. *et al.* Exercise and Fasting Activate Growth Hormone-Dependent Myocellular Signal Transducer and Activator of Transcription-5b Phosphorylation and Insulin-Like Growth Factor-I Messenger Ribonucleic Acid Expression in Humans. *J. Clin. Endocrinol. Metab.* **95**, E64–E68 (2010).

Appendix 2.1 Database search strategies

CENTRAL

Searched: 11/05/2020

"exercise OR run OR running OR cycle OR cycling OR walk OR walking in Record Title

AND ""glucose"" OR ""insulin"" OR ""glucagon"" in Title Abstract Keyword

AND rest OR resting OR control OR ctrl OR ""no exercise"" OR sedentary OR crossover OR cross-over OR counterbalanced in Abstract"

CINAHL

Searched: 10/04/2020

"TI (exercise or run or running or cycle or cycling or walk or walking)

AND AB (glucose or insulin or glucagon)

AND AB (rest or resting or control or ctrl or "noexercise" or sedentary or crossover or cross-over or counterbalanced)"

Embase

Searched: 10/04/2020

1. (exercise or run or running or cycle or cycling or walk or walking).ti.

2. (glucose or insulin or glucagon).ab,ti.

3. (rest or resting or control or ctrl or ""no exercise"" or sedentary or crossover or cross-over or counterbalanced).ab.

4. 1 and 2 and 3

Global Health

Searched: 10/04/2020

1. (exercise or run or running or cycle or cycling or walk or walking).ti.

2. (glucose or insulin or glucagon).ab,ti.

3. (rest or resting or control or ctrl or ""no exercise"" or sedentary or crossover or cross-over or counterbalanced).ab.

4. 1 and 2 and 3

HMIC

Searched: 10/04/2020

1. (exercise or run or running or cycle or cycling or walk or walking).ti.

2. (glucose or insulin or glucagon).ab,ti.

3. (rest or resting or control or ctrl or ""no exercise"" or sedentary or crossover or cross-over or counterbalanced).ab.

4. 1 and 2 and 3

Medline

Searched: 10/04/2020

1. (exercise or run or running or cycle or cycling or walk or walking).ti.

2. (glucose or insulin or glucagon).ab,ti.

3. (rest or resting or control or ctrl or ""no exercise"" or sedentary or crossover or cross-over or counterbalanced).ab.

4. 1 and 2 and 3

PubMed

Searched: 31/03/2020

"(((exercise[Title] OR run[Title] OR running[Title] OR cycle[Title] OR cycling[Title] OR walk[Title] OR walking[Title]))

AND (glucose[Title/Abstract] OR insulin[Title/Abstract] OR glucagon[Title/Abstract]))

AND (rest[Title/Abstract] OR resting[Title/Abstract] OR control[Title/Abstract] OR ctrl[Title/Abstract] OR ""no exercise""[Title/Abstract] OR sedentary[Title/Abstract] OR crossover[Title/Abstract] OR cross-over[Title/Abstract] OR counterbalanced[Title/Abstract]) "

PscyInfo

Searched: 10/04/2020

1. (exercise or run or running or cycle or cycling or walk or walking).ti.

2. (glucose or insulin or glucagon).ab,ti.

3. (rest or resting or control or ctrl or ""no exercise"" or sedentary or crossover or cross-over or counterbalanced).ab.

4. 1 and 2 and 3

ScienceDirect

Searched: 11/04/2020

Title: exercise OR run OR running OR cycle OR cycling OR walk OR walking

Title, abstract, keywords: glucose OR insulin OR glucagon

Terms: rest OR resting OR control OR ctrl OR ""no exercise"" OR sedentary OR crossover OR cross-over OR counterbalanced"

Scopus

Searched:07/04/2020

(TITLE(exercise OR run OR running OR cycle OR cycling OR walk OR walking)

AND TITLE-ABS-KEY(glucose OR insulin OR glucagon)

AND TITLE-ABS-KEY(rest OR resting OR control OR ctrl OR ""no exercise"" OR sedentary OR crossover OR cross-over OR counterbalanced))

Web of Science

Searched: 10/04/2020

"TITLE: (exercise OR run OR running OR cycle OR cycling OR walk OR walking)

AND TOPIC: (glucose OR insulin OR glucagon)

AND TOPIC: (rest OR resting OR control OR ctrl OR ""no exercise"" OR sedentary OR crossover OR cross-over OR counterbalanced)"

Appendix 2.2 Risk of bias analysis

	Glucose	Insulin	Glucagon	(1)	(2)	(3)	(4)	(5)	Overall bias
Bahr et al., 1991	✓	✗	✗	?	+	+	+	+	?
Balaguera-Cortes et al., 2011	✓	✓	✗	?	+	+	+	+	?
Bergfors et al., 2005	✓	✓	✗	?	+	+	+	+	?
Broom et al., 2017[a]	✓	✓	✗	?	+	+	+	+	?
Broom et al., 2017[b]	✓	✓	✗	?	+	+	+	+	?
Burns et al., 2007	✓	✓	✗	?	+	+	+	+	?
Charlot et al., 2011	✓	✗	✗	?	-	+	+	+	-
Clegg et al., 2007	✓	✗	✗	?	+	+	+	+	?
Douglas et al., 2017[a]	✓	✓	✗	?	+	+	+	+	?
Douglas et al., 2017[b]	✓	✓	✗	?	+	+	+	+	?
Edinburgh et al., 2017	✓	✓	✗	?	+	+	+	+	?
Enevoldsen et al., 2005	✓	✓	✗	?	+	+	+	+	?
Ezell et al., 1999[a]	✓	✓	✗	?	+	+	+	+	?
Ezell et al., 1999[b]	✓	✓	✗	?	+	+	+	+	?
Ezell et al., 1999[c]	✓	✓	✗	?	+	+	+	+	?
Farah & Gill, 2013	✓	✓	✗	+	+	+	+	+	+
Gonzalez et al., 2013[a]	✓	✓	✗	?	+	+	+	+	?
Gonzalez et al., 2013[b]	✓	✓	✗	?	+	+	+	+	?
Goto et al., 2011	✗	✓	✗	?	+	+	+	+	?
Hagobian et al., 2013[a]	✗	✓	✗	?	+	+	+	+	?
Hagobian et al., 2013[b]	✗	✓	✗	?	+	+	+	+	?
Hardman & Aldred, 1995	✗	✓	✗	?	+	+	+	+	?
Højbjerre et al., 2007[a]	✓	✗	✗	?	+	+	+	+	?
Højbjerre et al., 2007[b]	✓	✗	✗	?	+	+	+	+	?
Isacco et al., 2014[a]	✓	✓	✗	?	+	+	+	+	?
Isacco et al., 2014[b]	✓	✓	✗	?	+	+	+	+	?
King et al., 2010	✓	✓	✗	?	+	+	+	+	?
Knudsen et al., 2013	✗	✓	✗	?	+	+	+	+	?
Lee et al., 1991	✓	✗	✗	?	+	+	+	+	?
Larsen et al. 2017	✗	✓	✓	?	+	+	+	+	?
Marion-Latard et al., 2003	✓	✓	✗	?	+	+	+	+	?
Mattin et al., 2018	✓	✓	✗	?	+	+	+	+	?
McClean et al., 2007	✓	✗	✗	?	+	+	+	+	?
Morris et al., 2010	✓	✓	✗	?	+	+	+	+	?
Numao et al., 2008	✓	✓	✗	?	+	+	+	+	?
Nyhoff et al., 2015	✓	✓	✓	?	+	+	+	+	?
Petridou et al., 2004	✓	✓	✗	?	+	+	+	+	?
Rattray & Smee, 2016	✓	✗	✗	?	+	+	+	+	?
Ronsen et al., 2001	✗	✓	✗	?	+	+	+	+	?
Ronsen et al., 2002	✓	✗	✗	?	+	+	+	+	?
Schlierf et al., 1987	✓	✓	✗	?	+	+	+	+	?

Shambrook et al., 2018	✓	✗	✗	?	+	+	+	+	?
Shambrook et al., 2020	✓	✗	✗	+	+	+	+	+	+
Siopi et al., 2019	✓	✓	✗	?	+	+	+	+	?
Stokes et al., 2013	✓	✗	✗	?	+	+	+	+	?
Tobin et al., 2007	✓	✓	✗	?	+	+	+	+	?
Ueda et al., 2009	✓	✓	✗	?	+	+	+	+	?
Ueda et al., 2009[a]	✓	✓	✓	?	+	+	+	+	?
Ueda et al., 2009[b]	✓	✓	✓	?	+	+	+	+	?
Vendelbo et al., 2010	✓	✓	✗	?	+	+	+	+	?
Willis et al., 2019	✓	✓	✓	?	+	+	+	+	?

(1) Bias arising from the randomization process; (2) Bias due to deviations from intended interventions; (3) Bias due to missing outcome data; (4) Bias in measurement of the outcome; (5) Bias in the selection of the reported result; [-] high risk of bias; [?] some concerns; [+] low risk of bias. [a,b,c] denotes sub-studies.

Appendix 2.3 Sensitivity analyses using variable within-participant correlation coefficients for meta-analyses

Outcome	Within-participant correlation coefficient	Mean effect size	95% confidence interval	P value
Glucose	0.3	-0.04	-0.22 to 0.14	0.641
Glucose	0.5	-0.05	-0.22 to 0.13	0.589
Glucose	0.7	-0.05	-0.23 to 0.12	0.557
Glucose	0.9	-0.05	-0.23 to 0.12	0.548
Insulin	0.3	-16.40	-28.69 to -4.11	0.001
Insulin	0.5	-18.07	-30.47 to -5.66	0.004
Insulin	0.7	-20.11	-32.67 to -7.55	0.002
Insulin	0.9	-21.95	-34.64 to -9.26	0.001
Glucagon	0.3	23.74	15.97 to 31.50	<0.001
Glucagon	0.5	24.60	16.25 to 32.95	<0.001
Glucagon	0.7	26.16	16.78 to 35.54	<0.001
Glucagon	0.9	28.48	17.94 to 39.01	<0.001

Chapter 3: The acute effect of glucagon administration on energy intake and subjective appetite in adults without diabetes: a systematic review and meta-analysis

3.1 Introduction

An acute bout of exercise is commonly accompanied by a temporary suppression of appetite referred to as exercise-induced anorexia[1]. This suppression of appetite is often attributed to increased concentrations of acyl-ghrelin, glucagon-like peptide 1 (GLP-1), and peptide YY (PYY) due to their well-investigated roles in appetite regulation[2,3]. However, acute exercise also results in an increase in systemic glucagon concentrations (see Chapter 2); a hormone that has been implicated in appetite regulation[4].

Glucagon is a 29 amino acid polypeptide synthesised by the alpha cells of the pancreatic islets, which acts via the glucagon receptor (GCGR) to exert various physiological effects[5]. Glucagon is primarily known for its role in glucose homeostasis[6], but has also been identified as a key regulator of amino acid metabolism[7]. Evidence from rodent models has demonstrated that glucagon can also suppress energy intake[8], possibly via direct activation of target tissues such as the hypothalamus[9]. GCGR agonism has consequently been identified as a possible therapeutic target for obesity, and a number of studies have investigated the effects of acute glucagon administration on energy intake in humans. However, the magnitude and/or direction of appetite change following glucagon administration has been mixed[10–12], likely attributable to differences in study design. Indeed, effects attributed to glucagon are frequently confounded by co-infusion of other bioactive peptides, such as somatostatin[13,14]. Moreover, many studies do not include an appropriate control arm, instead favouring pre-post designs that do not exclude the effect of time on observed responses[15–17].

We therefore conducted a systematic review and random-effects meta-analysis to estimate the average effects of acute glucagon administration on energy intake and subjective appetite in adults without diabetes. As acute exercise increases glucagon concentrations, and rodent models suggest an anorectic effect of glucagon, the results of this analysis will help to provide insight into glucagon's potential role in exercise-induced anorexia.

3.2 Methods

3.2.1 Registration

This Review and meta-analysis was registered at PROSPERO (registration number: CRD42021269623).

3.2.2 Eligibility criteria

Population: We included randomised, controlled, single- or double blind, crossover studies in adults (>18 years old) of any body mass index (BMI) value. Studies performed in current smokers, pregnant individuals, or individuals with a history of chronic disease (including type 1 and type 2 diabetes) were excluded.

Intervention: Administration of glucagon via any route (intravenous, intramuscular, intranasal) for less than 24 hours while at rest. Studies which administered glucagon for longer than 24 hours or co-infused pharmacological agents (e.g. somatostatin) were excluded. Studies could be performed in the fasted or postprandial state.

Comparator: To be included, studies must have performed a time-and energy matched control arm that administered an energy-free control agent (e.g. saline) in place of glucagon.

Outcome: Studies measuring energy intake and/or subjective appetite were included.

Studies written in the English language and published in peer-reviewed journals were only considered. Conference abstracts were excluded. If methodology and/or participant characteristics were not described sufficiently to determine study eligibility, corresponding authors were contacted. If the author did not respond, or could not provide the required information, the study was excluded.

3.2.3 Information sources and search strategy

CENTRAL, CINAHL, Embase, MEDLINE, PubMed, and Scopus databases on 24 May 2021. Embase and Medline databases were accessed via Ovid, and the CINAHL database was accessed via EBSCOhost. All databases were searched from inception to 24 May 2021.

The search strategy was developed based on the PICO (population, intervention, comparator, and outcome) format, with additional concepts incorporated to exclude pre-clinical studies. Full details of the search strategy are provided in Appendix 3.1. No limits were used during any database search.

Backward (using Google Scholar) and forward citation searching of eligible papers was also performed by on 23 July 2021.

3.2.4 Selection process

Results of each database search were imported into Covidence systematic review software (Veritas Health Innovation, Australia). Duplicate results were automatically detected and removed by Covidence. Title and abstracts were then independently screened, with each paper being classified as ‘yes’, ’no’ or ‘maybe’. Papers classified as ‘yes’ or ‘maybe’ continued to the full-text screening phase. All disputes (papers with a ‘yes’ or ‘maybe’ *and* a ‘no’ vote) were resolved prior to conducting full-text screening. Full texts of each paper were then

accessed and independently classified as ‘yes’ or ‘no’. Papers classified as ‘yes’ continued to the data extraction phase. Disputes following full-text screening (papers with a ‘yes’ *and* a ‘no’ vote) were resolved via a meeting prior to data extraction.

3.2.5 Data collection

Corresponding authors for all eligible studies were first contacted for raw study data. If authors did not respond or could not provide raw study data, data were extracted from the published manuscript. WebPlotDigitizer Version 4.2 (Ankit Rohatgi, USA) was used to extract data from papers that only presented data in a figure.

When data were displayed inadequately (e.g. clustering of data points, overlapping of error bars) or data were not reported in published manuscript or supplementary materials (despite methods stating measurements had been taken), the paper was no longer considered eligible and excluded from analysis.

Data were collected and stored in an electronic spreadsheet (Excel 2016, Microsoft Corporation, USA). If data were presented from multiple glucagon doses, only data from the highest dose was collected.

3.2.6 Data items

Eligible outcomes were defined as follows:

Energy intake - total *ad libitum* energy intake at the first meal presented to participants following the administration of glucagon and comparator. Measured in kcal, kJ, or grams.

Subjective appetite - assessed at baseline and at least two other timepoints during the glucagon and comparator arms. Alternatively, total or incremental area under the curve (AUC) for the glucagon and comparator arms. Measured by a visual analogue scale (VAS) or other questionnaire assessing a domain relating to appetite (e.g. hunger, pleasantness, prospective consumption, fullness) or a composite appetite score.

The following data items were also collected relating to paper, participant, and intervention characteristics: author(s), year of publication, sample size, proportion of males, participant age, participant BMI, degree of blinding, route of administration, glucagon dose, and duration of administration.

3.2.7 Risk of bias assessment

Risk of bias of included studies was assessed using the Revised Cochrane Risk of Bias Tool for Randomized trials (RoB 2.0) with additional considerations for cross-over trials. Risk of bias was assessed using the following domains: bias arising from the randomization process; bias arising from period and carryover effects; bias due to deviations from intended intervention; bias due to missing outcome data; bias in the measurement of the outcome; and

bias in the selection of the reported result. Risk of bias assessment was performed for each outcome (energy intake, subjective appetite), in which the risk of bias of each individual study was determined by the highest risk of bias level attained in any of the assessed domains. Studies were not excluded based on the risk of bias assessment.

3.2.8 Data synthesis

Data were collated and grouped by outcome (energy intake, subjective appetite). Standard errors and 95% confidence intervals (CIs) were converted to standard deviations. For subjective appetite only, total AUC was calculated for glucagon and comparator arms using the maximum number of timepoints available. If data were extracted from figures using WebPlotDigitizer, standard deviations of AUCs were estimated using the AUC of values depicted by the corresponding top or bottom error bars.

Standardised mean differences (SMDs) were then calculated for each study as described by Higgins et al.[18]. When raw study data were not available, a correlation coefficient of 0.5 was assumed to calculate the standard error of the SMD[19]. Sensitivity analyses using correlation coefficients of 0.3, 0.7, and 0.9 were performed to assess the robustness of findings to this assumption.

A random-effects meta-analysis model was selected as the effect of glucagon administration on outcomes was expected to vary across studies due to differences in participant and intervention characteristics. This model assumes a distribution of true effect sizes across studies and provides an estimate of the mean intervention effect of this distribution[20,21]. Between-study variance (τ^2) was estimated using the Hartung-Knapp-Sidik-Jonkman method[22,23].

Heterogeneity was assessed using the chi-squared (Q) and I^2 statistic. A Q value above the degrees of freedom (df) for the estimate and an I^2 statistic >50% indicated large heterogeneity between studies.

Publication bias was assessed via visual inspection of contour-enhanced funnel plots[24] and statistically by Egger's regression test for outcomes containing at least 10 studies. Trim and fill analyses (L_0 estimator) were used when publication bias was suspected to explore its impact on effect sizes[25].

All analyses were performed in Stata 16 (StataCorp, USA). Meta-analysis was only performed for outcomes with at least five studies[26].

3.2.9 Certainty of evidence assessment

Certainty of evidence was assessed using the GRADE approach[27,28]. Certainty of evidence was assessed using the following domains: study limitations, consistency of effect,

imprecision, indirectness, and publication bias. Estimated effect of each outcome was independently classified as high (true effect is similar to the estimated effect), moderate (true effect is probably close to the estimated effect), low (true effect might be markedly different from the estimated effect), or very low (true effect is probably markedly different from the estimated effect) certainty of evidence.

3.3 Results

3.3.1 Study selection

Database searching found 24,833 potentially eligible papers. Following removal of duplicates, 13,020 papers underwent title and abstract screening, resulting in the removal of 12,744 papers. Consequently, 246 papers underwent full-text screening, yielding 6 eligible papers. Due to one paper containing multiple studies, a total of 7 separate studies were deemed eligible. The following number of studies proved eligible for each outcome: energy intake, 5 studies; subjective appetite, 4 studies. This process is summarised in Figure 3.1.

3.3.2 Study characteristics

Study characteristics of included studies are presented in Table 3.1.

3.3.3 Risk of bias

The results of the risk of bias assessment for each outcome are presented in Appendix 3.2. With regards to overall risk of bias, there were some concerns for all studies included in the review, irrespective of the outcome measured. This was primarily due to inadequate reporting of the randomization and sequence allocation process, or inadequate reporting of the analysis plan.

3.3.4 Meta-analysis

Data used for meta-analysis is presented in Appendix 3.3.

3.3.4.1 Energy intake

Five studies comprising 77 participants (90% males) measured *ad libitum* energy intake following comparator and glucagon administration[11,29–32]. Of these five studies, four used intravenous administration[11,12,29,30] and one used intranasal administration[32]. Average age of participants ranged from 22.0 to 48.5 years, with three studies being conducted in healthy-weight participants (18.5 ≥ BMI < 25.0)[11,12,30] and two studies being conducted in overweight participants (25.0 ≥ BMI < 30.0)[29,32].

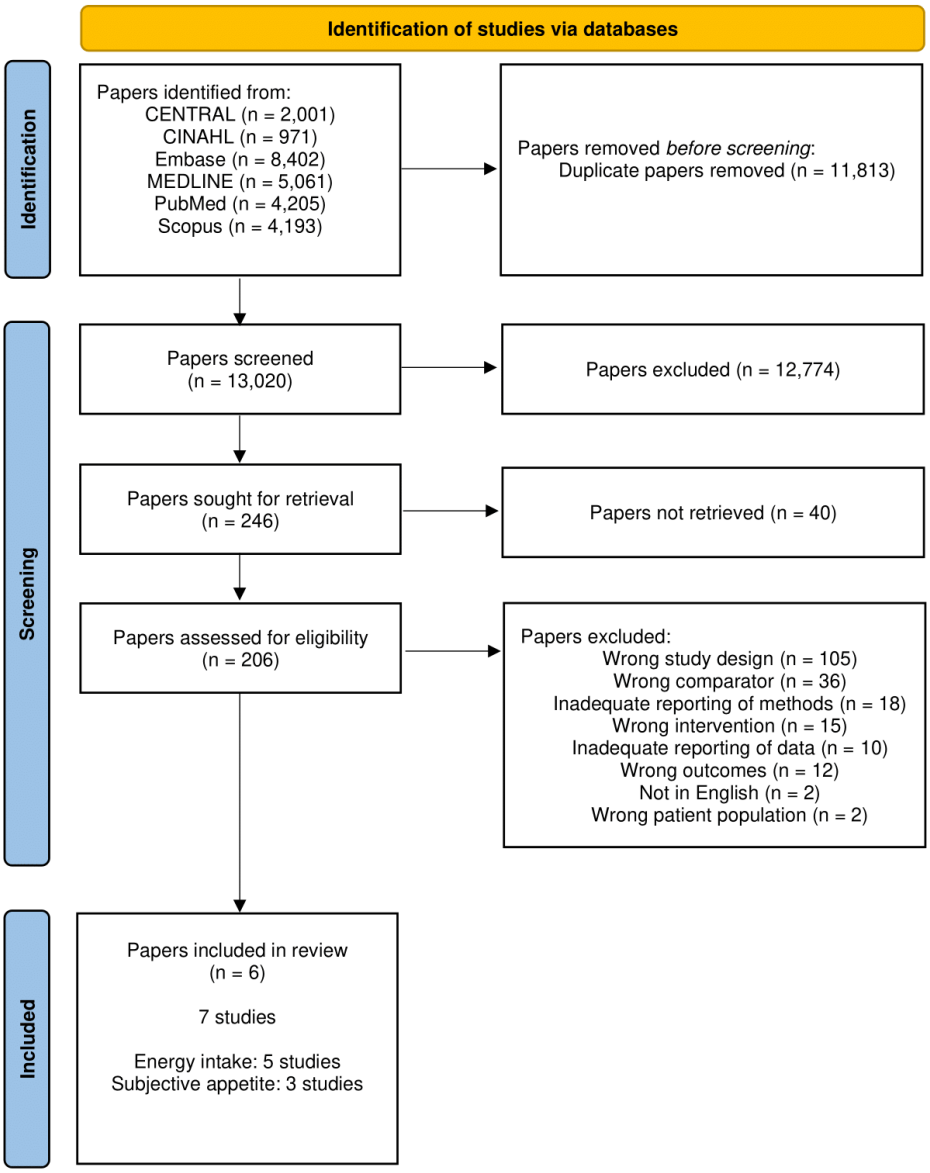


Figure 3.1: Flow diagram of paper selection.

Mean intervention effect of glucagon administration relative to comparator on *ad libitum* meal energy intake was SMD = -0.19 (95% CI, -0.59 to 0.21; P = 0.345; Figure 3.2). I^2 (81.11%) and Q (20.28, df = 4, P<0.001) statistics highlighted large heterogeneity between studies. An assessment of publication bias was not performed due to an insufficient number of studies.

3.3.4.2 Subjective appetite

Four studies comprising 57 participants (73% males) measured subjective appetite following comparator and glucagon administration[10,11]. Of these four studies, two used intramuscular[10] administration and two used intravenous administration[11,12]. Average age of participants

Table 3.1: Population characteristics, intervention characteristics, and outcome measurements for all included studies.

Study ID	**Population**				**Intervention**				**Outcome**	
Author	*Sample size*	*Male, %*	*Age, mean*	*BMI, mean*	*Route*	*Dose*	*Total dose*	*Comparator*	*Energy intake*	*Subjective appetite*
Arafat et al.[10a]	13	46	25.1	21.7	Intramuscular	1 mg bolus at start	1 mg	Saline		✓
Arafat et al.[10b]	11	45	28.4	34.4	Intramuscular	1 mg bolus at start	1 mg	Saline		✓
Bagger et al.[11]	15	100	22.0	23.0	Intravenous	3 ng/kg/min over 270 mins	60658 ng (0.06 mg)	Saline	✓	✓
Cegla et al.[29]	13	69	31.6	27.0	Intravenous	9.8 ng/kg/min over 120 mins	87774 ng (0.09 mg)	Gelofusine	✓	M-IR
Geary et al.[30]	12	100	24.3	21.8	Intravenous	3 ng/kg/min over 10 mins	2097 ng (0.002 mg)	Saline	✓	
Izzi-Engbeaya et al.[12]	18	100	25.1	22.5	Intravenous	7 ng/kg/min over 480 mins	250784 ng (0.25 mg)	Gelofusine	✓	✓
Stahel et al.[32]	19	79	48.5	29.5	Intranasal	0.7 mg bolus at start	0.7 mg	Sterile dilutant	✓	

[a,b]after author names denotes sub-studies. M-IR, measured but inadequately reported. Note: some doses have been converted to ng from pmol to enable comparisons (ng = pmol ÷ 0.2871).

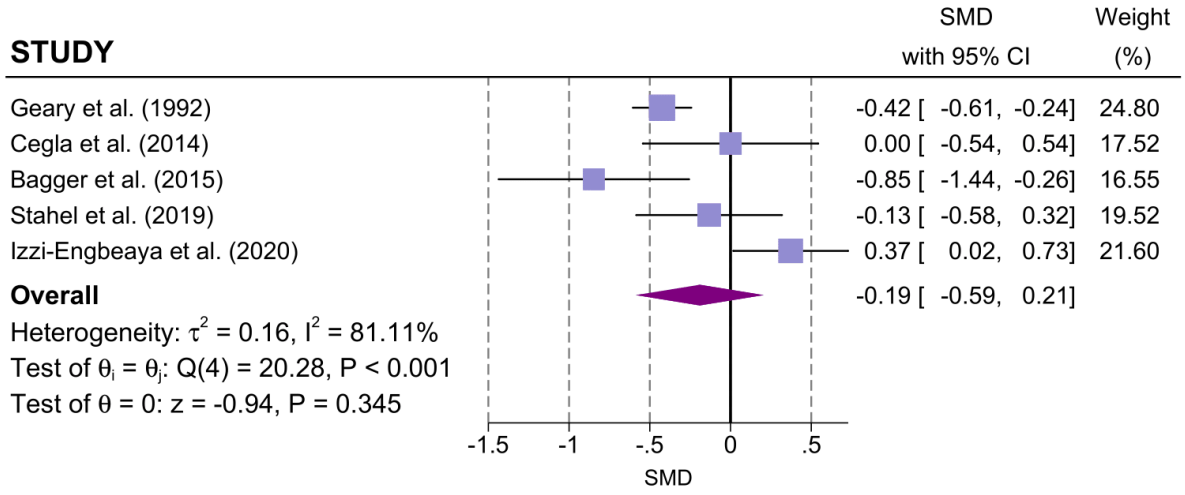


Figure 3.2: Forest plot of standardised mean differences between glucagon administration and comparator for *ad libitum* energy intake. Results produced from a random-effects meta-analysis using the Hartung-Knapp-Sidik-Jonkman method to estimate between-study variance. Data are presented as mean with 95% confidence intervals. CI, confidence interval; SMD, standardised mean difference.

ranged from 22.0 to 28.4 years, with three studies being conducted in healthy-weight participants[10–12] and one study being conducted in obese participants (BMI ≥ 30.0)[10]. Two studies reported subjective satiety[10], one study reported a composite appetite score [11], and one study reported subjective hunger[12].

Due to the limited number of studies, a meta-analysis was not performed. However, two studies reported an increase in subjective appetite following glucagon administration relative to comparator [10,11] and two studies reported a decrease in subjective appetite [10,12] (Appendix 3.3).

3.3.4.3 Sensitivity analyses

Sensitivity analyses employing correlation coefficients of 0.3, 0.7 and 0.9 did not meaningfully alter the mean intervention effect and overall interpretation of glucagon administration on energy intake (Appendix 3.4).

3.3.5 Certainty of evidence

Certainty of evidence for energy intake, energy expenditure, glucose and insulin was rated as low, high, low, and low, respectively. Explanation of judgements alongside certainty of evidence assessments are presented in the summary of findings table (Table 3.2).

3.4 Discussion

This review analysed the evidence on the effect of acute glucagon administration on energy intake and subjective appetite in humans. Meta-analysis of available studies revealed that the

Table 3.2: Summary of findings for energy intake and subjective appetite.

Acute glucagon administration compared with an energy-free control agent in adults without diabetes

Patient or population: adults without diabetes

Setting: laboratory environment

Intervention: acute glucagon administration

Comparison: energy-free control agent

Outcomes	Relative effect (95% CI)	Number of participants (studies)	Quality of the evidence (GRADE)	Comments
Energy intake	SMD -0.19 decrease with glucagon (-0.59 decrease to 0.21 increase)	77 participants (5 studies)	⊕⊕⊖⊖ Low[a]	
Subjective appetite	Not estimated	57 participants (4 studies)	Not graded	Meta-analysis not performed due to limited number of studies

CI: confidence interval; SMD: standardised mean difference.

GRADE Working Group grades of evidence

High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.

Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a]There was considerable heterogeneity (I^2 = 81.11%; 95% CI, 41.18% to 97.87%) and 95% confidence interval contained zero. We therefore downgraded by two levels for inconsistency and imprecision.

effect of acute glucagon administration on energy intake is unclear, in which an orexigenic and anorexigenic effect cannot be excluded. Additionally, too few studies exist to permit a meta-analysis on subjective appetite.

3.4.1 The effect of acute glucagon administration on energy intake and subjective appetite

The results from our analysis highlight that the effect of acute glucagon administration on energy intake in humans is inconclusive. Despite the point estimate for the mean intervention effect suggesting a small anorectic effect, the confidence interval for this effect was large and included both an increase and decrease in energy intake following acute glucagon administration. This uncertainty is also reflected in the effect sizes of the individual studies and the reported effects of acute glucagon administration on subjective appetite. Indeed, no study performed a power calculation based on differences in energy intake between groups (with only two studies stating energy intake as a pre-registered primary outcome[11,12]), likely contributing to the observed imprecision of the mean intervention effect.

Two of the five eligible studies reported a suppression of energy intake with acute glucagon administration relative to placebo[11,30]. Of note, these studies used considerably lower doses

of glucagon, and thus produced systemic concentrations more representative of that seen in day-to-day physiology (~50 pmol/L)[33]. Studies reporting no effect of acute glucagon administration however achieved supraphysiological glucagon concentrations (≥150 pmol/L)[12,29]. This is somewhat counterintuitive as higher doses are associated with increased feelings of nausea [34–38], which would also be expected to contribute to a reduction in energy intake. Similar responses have however been observed in rodents, in which higher glucagon doses resulted in a mitigated (and sometimes abolished) suppression of energy intake relative to moderate doses[9]. The reasons for a possible diminished response at higher glucagon doses is unclear, but may be due GCGR desensitisation[9]. Nevertheless, a suppression of energy intake induced by physiological concentrations of glucagon (either via exogenous or endogenous sources) cannot be excluded.

The effect of acute glucagon administration on subjective appetite is also inconclusive, with half of the included studies reporting a suppression of appetite[10,12] and half reporting an increase in appetite[10,11]. Future work using acute glucagon administration as intervention should therefore consider the inclusion of subjective appetite measures to improve our current understanding.

The undetermined effect of acute glucagon administration on energy intake and subjective appetite unfortunately provides little insight into any potential role of systemic glucagon concentrations in exercise-induced anorexia. It is however important to note that our results do not exclude the possibility that glucagon does contribute to the suppression of appetite experienced during exercise, especially in light of the possibility that reductions in energy intake are only observed at physiological glucagon concentrations. Consequently, the next chapter of this thesis (Chapter 4) will directly measure glucagon concentrations during and following acute exercise, and investigate its association with subjective appetite and energy intake.

3.4.2 Limitations

The present review is subject to several limitations. Firstly, both outcomes were only measured by a small number of studies, reducing the precision of summary effect estimates, and also preventing the assessment of publication bias. Secondly, most studies used supraphysiological glucagon doses, and thus the implications for glucagon's role in appetite under physiological conditions (such as those experienced during acute exercise) is limited. Thirdly, the participants of included studies were predominantly young (<35 years old) males, with less than half of eligible studies being conducted in participants with a BMI ≥25.0. The findings of the present analysis may therefore not be applicable to all populations, particularly those more likely to be treated with anti-obesity agents. Finally, the present analysis only

focuses on acute effects of glucagon administration on energy intake and subjective appetite, and thus any observed effects cannot be extrapolated to chronic administration.

3.4.3 Summary

Overall, the effect of acute glucagon administration on energy intake and subjective appetite remains unclear. Consequently, an evaluation of the role of glucagon in exercise-induced anorexia cannot be made using the results of the present study. Future work should look to clarify the effect of acute glucagon administration on energy intake and appetite using adequately powered studies in which energy intake is the primary outcome. Furthermore, these studies should incorporate doses that result in physiological glucagon concentrations in order to evaluate the potential appetite-suppressive effects of exercise-induced increases in systemic glucagon concentrations.

3.5 References

1. King, N. A., Burley, V. J. & Blundell, J. E. Exercise-induced suppression of appetite: effects on food intake and implications for energy balance. *Eur. J. Clin. Nutr.* **48**, 715–24 (1994).
2. Martins, C., Morgan, L. M., Bloom, S. R. & Robertson, M. D. Effects of exercise on gut peptides, energy intake and appetite. *J. Endocrinol.* **193**, 251–258 (2007).
3. Schubert, M. M., Sabapathy, S., Leveritt, M. & Desbrow, B. Acute Exercise and Hormones Related to Appetite Regulation: A Meta-Analysis. *Sport. Med.* **44**, 387–403 (2014).
4. Geary, N. Glucagon and the Control of Appetite. in *Handbook of Experimental Pharmacology* (ed. Lefebvre, P.) vol. 123/III 223–238 (Springer-Verlag, 1996).
5. Müller, T. D., Finan, B., Clemmensen, C., DiMarchi, R. D. & Tschöp, M. H. The New Biology and Pharmacology of Glucagon. *Physiol. Rev.* **97**, 721–766 (2017).
6. Sandoval, D. A. & D'Alessio, D. A. Physiology of Proglucagon Peptides: Role of Glucagon and GLP-1 in Health and Disease. *Physiol. Rev.* **95**, 513–548 (2015).
7. Holst, J. J., Wewer Albrechtsen, N. J., Pedersen, J. & Knop, F. K. G. Glucagon and Amino Acids Are Linked in a Mutual Feedback Cycle: The Liver–α-Cell Axis. *Diabetes* **66**, 235–240 (2017).
8. Geary, N., Le Sauter, J. & Noh, U. Glucagon acts in the liver to control spontaneous meal size in rats. *Am. J. Physiol. Integr. Comp. Physiol.* **264**, R116–R122 (1993).
9. Inokuchi, A., Oomura, Y. & Nishimura, H. Effect of intracerebroventricularly infused glucagon on feeding behavior. *Physiol. Behav.* **33**, 397–400 (1984).

10. Arafat, A. M. *et al.* The Impact of Insulin-Independent, Glucagon-Induced Suppression of Total Ghrelin on Satiety in Obesity and Type 1 Diabetes Mellitus. *J. Clin. Endocrinol. Metab.* **98**, 4133–4142 (2013).

11. Bagger, J. I. *et al.* Effect of Oxyntomodulin, Glucagon, GLP-1, and Combined Glucagon +GLP-1 Infusion on Food Intake, Appetite, and Resting Energy Expenditure. *J. Clin. Endocrinol. Metab.* **100**, 4541–4552 (2015).

12. Izzi-Engbeaya, C. *et al.* Acute Effects of Glucagon on Reproductive Hormone Secretion in Healthy Men. *J. Clin. Endocrinol. Metab.* **105**, 1899–1905 (2020).

13. Genuth, S. M. & Hoppel, C. L. Acute hormonal effects on carnitine metabolism in thin and obese subjects: Responses to somatostatin, glucagon, and insulin. *Metabolism* **30**, 393–401 (1981).

14. Calles-Escandón, J. Insulin dissociates hepatic glucose cycling and glucagon-induced thermogenesis in man. *Metabolism* **43**, 1000–1005 (1994).

15. Samols, E., Marri, G. & Marks, V. Promotion of insulin secretion by glucagon. *Lancet* **286**, 415–416 (1965).

16. Crockford, P. M., Porte, D., Wood, F. C. & Williams, R. H. Effect of glucagon on serum insulin, plasma glucose and free fatty acids in man. *Metabolism* **15**, 114–122 (1966).

17. Arafat, M. A. *et al.* Glucagon inhibits ghrelin secretion in humans. *Eur. J. Endocrinol.* **153**, 397–402 (2005).

18. Higgins, J., Eldridge, S. & Li, T. Chapter 23: Including variants on randomized trials. in *Cochrane Handbook for Systematic Reviews of Interventions version* (eds. Higgins, J. et al.) (2021).

19. Follmann, D., Elliott, P., Suh, I. & Cutler, J. Variance imputation for overviews of clinical trials with continuous response. *J. Clin. Epidemiol.* **45**, 769–773 (1992).

20. Borenstein, M., Hedges, L. V., Higgins, J. P. T. & Rothstein, H. R. A basic introduction to fixed-effect and random-effects models for meta-analysis. *Res. Synth. Methods* **1**, 97–111 (2010).

21. Riley, R. D., Higgins, J. P. T. & Deeks, J. J. Interpretation of random effects meta-analyses. *BMJ* **342**, d549–d549 (2011).

22. Hartung, J. & Knapp, G. On tests of the overall treatment effect in meta-analysis with normally distributed responses. *Stat. Med.* **20**, 1771–1782 (2001).

23. Sidik, K. & Jonkman, J. N. A simple confidence interval for meta-analysis. *Stat. Med.*

21, 3153–3159 (2002).

24. Peters, J. L., Sutton, A. J., Jones, D. R., Abrams, K. R. & Rushton, L. Contour-enhanced meta-analysis funnel plots help distinguish publication bias from other causes of asymmetry. *J. Clin. Epidemiol.* **61**, 991–996 (2008).

25. Duval, S. & Tweedie, R. A Nonparametric "Trim and Fill" Method of Accounting for Publication Bias in Meta-Analysis. *J. Am. Stat. Assoc.* **95**, 89–98 (2000).

26. Jackson, D. & Turner, R. Power analysis for random-effects meta-analysis. *Res. Synth. Methods* **8**, 290–302 (2017).

27. Guyatt, G. H. *et al.* GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* **336**, 924–926 (2008).

28. The GRADE Working Group. *GRADE handbook for grading quality of evidence and strength of recommendations*. (2013).

29. Cegla, J. *et al.* Coinfusion of Low-Dose GLP-1 and Glucagon in Man Results in a Reduction in Food Intake. *Diabetes* **63**, 3711–3720 (2014).

30. Geary, N., Kissileff, H. R., Pi-Sunyer, F. X. & Hinton, V. Individual, but not simultaneous, glucagon and cholecystokinin infusions inhibit feeding in men. *Am. J. Physiol. Integr. Comp. Physiol.* **262**, R975–R980 (1992).

31. Salem, V. *et al.* Glucagon increases energy expenditure independently of brown adipose tissue activation in humans. *Diabetes, Obes. Metab.* **18**, 72–81 (2016).

32. Stahel, P., Lee, S. J., Sud, S. K., Floh, A. & Dash, S. Intranasal glucagon acutely increases energy expenditure without inducing hyperglycaemia in overweight/obese adults. *Diabetes, Obes. Metab.* **21**, 1357–1364 (2019).

33. Rix, I., Nexøe-Larsen, C., Bergmann, N., Lund, A. & Knop, F. Glucagon Physiology. in *Endotext* (eds. Feingold, K., Anawalt, B. & Boyce, A.) (MDText.com, 2000).

34. Larsen, S., Osnes, M. & Christensen, M. S. The Effect of Glucagon, Glucagon-(l-21)-Peptide, and Placebo on Duodenal Pressure Activity in Healthy Subjects. *Scand. J. Gastroenterol.* **21**, 634–640 (1986).

35. Schjoldager, B., Lawaetz, O. & Christiansen, J. Effect of Pancreatic Glucagon and Its 1-21 Fragment on Gastric Emptying in Man. *Scand. J. Gastroenterol.* **23**, 726–730 (1988).

36. Arvat, E. *et al.* Glucagon is an ACTH secretagogue as effective as hCRH after intramuscolar administration while it is ineffective when given intravenously in normal

subjects. *Pituitary* **3**, 169–73 (2000).

37. Lockton, J. A. & Poucher, S. M. Single dose glucagon (0.5 mg IV bolus) administration in healthy human volunteers is a robust model for assessment of glycogenolysis. *J. Pharmacol. Toxicol. Methods* **55**, 86–90 (2007).

38. Whytock, K. L. *et al.* Prolonged Glucagon Infusion Does Not Affect Energy Expenditure in Individuals with Overweight/Obesity: A Randomized Trial. *Obesity* **29**, 1003–1013 (2021).

Appendix 3.1: Database search strategies

CENTRAL

#1 (glucagon):ti,ab,kw AND (infus* OR admin* OR intravenous OR dos* OR saline OR subcutaneous OR intramuscular OR nasal):ti,ab,kw AND (energy OR intake OR food OR meal OR appetite OR eat* OR hunger OR hungry OR expenditure OR calorie* OR kcal OR joule* OR kJ OR insulin OR glucose OR glyc*):ti,ab,kw AND (human* OR participant* OR subject* OR patient* OR volunteer* OR men OR women OR man OR woman OR male* OR female* OR individual* OR recruit* OR trial OR crossover OR cross over OR cross-over OR consent OR assign* OR allocate* OR placebo):ti,ab,kw

#2 (review OR meta analysis OR meta-analysis OR case study OR case studies):ti OR (databases adj4 searched):ab OR (rat OR rats or mouse OR mice OR swine OR porcine OR murine OR sheep OR lamb OR pigs OR piglets OR rabbit OR rabbits OR cat OR cats OR dog OR dogs OR cattle OR bovine OR monkey OR monkeys OR trout OR chick OR chicks OR broiler OR broilers OR carp):ti

#3 MeSH descriptor: [Animals] in all MeSH products

#4 MeSH descriptor: [Models, Animal] explode all trees

#5 #1 NOT #2 NOT #3 NOT #4"

CINAHL

"AB glucagon

AND AB (infus* OR admin* OR intravenous OR dos* OR saline OR subcutaneous OR intramuscular OR nasal)

AND AB (energy OR intake OR food OR meal OR appetite OR eat* OR hunger OR hungry OR expenditure OR calorie* OR kcal OR joule* OR kJ OR insulin OR glucose OR glyc*)

AND AB (human* OR participant* OR subject* OR patient* OR volunteer* OR men OR women OR man OR woman OR male* OR female* OR individual* OR recruit* OR trial OR crossover OR cross over OR cross-over OR consent OR assign* OR allocate* OR placebo)

NOT TI (review OR meta analysis OR meta-analysis OR case study OR case studies)

NOT AB databases adj4 searched NOT TI (rat OR rats or mouse OR mice OR swine OR porcine OR murine OR sheep OR lamb OR pigs OR piglets OR rabbit OR rabbits OR cat OR cats OR dog OR dogs OR cattle OR bovine OR monkey OR monkeys OR trout OR chick OR chicks OR broiler OR broilers OR carp)

NOT MW (animal* OR animal studies OR animal model*)"

Embase

1. glucagon.ab,kw,ti.

2. (infus* or admin* or intravenous or dos* or saline or subcutaneous or intramuscular or nasal).ab,kw,ti.

3. (energy or intake or food or meal or appetite or eat* or hunger or hungry or expenditure or calorie* or kcal or joule* or kJ or insulin or glucose or glyc*).ab,kw,ti.

4. (human* or participant* or subject* or patient* or volunteer* or men or women or man or woman or male* or female* or individual* or recruit* or trial or crossover or cross over or cross-over or consent or assign* or allocate* or placebo).ab,kw,ti.

5. (review or meta analysis or meta-analysis or case study).ti.

6. (databases adj4 searched).ab.

7. (rat or rats or mouse or mice or swine or porcine or murine or sheep or lamb or pigs or piglets or rabbit or rabbits or cat or cats or dog or dogs or cattle or bovine or monkey or monkeys or trout or chick or chicks or broiler or broilers or carp).ti.

8. (animal* or animal studies or animal model*).sh.

9. (1 and 2 and 3 and 4) not 5 not 6 not 7 not 8

Medline

1. glucagon.ab,kw,ti.

2. (infus* or admin* or intravenous or dos* or saline or subcutaneous or or intramuscular or nasal).ab,kw,ti.

3. (energy or intake or food or meal or appetite or eat* or hunger or hungry or expenditure or calorie* or kcal or joule* or kJ or insulin or glucose or glyc*).ab,kw,ti.

4. (human* or participant* or subject* or patient* or volunteer* or men or women or man or woman or male* or female* or individual* or recruit* or trial or crossover or cross over or cross-over or consent or assign* or allocate* or placebo).ab,kw,ti.

5. (review or meta analysis or meta-analysis or case study).ti.

6. (databases adj4 searched).ab.

7. (rat or rats or mouse or mice or swine or porcine or murine or sheep or lamb or pigs or piglets or rabbit or rabbits or cat or cats or dog or dogs or cattle or bovine or monkey or monkeys or trout or chick or chicks or broiler or broilers or carp).ti.

8. (animal* or animal studies or animal model*).sh.

9. (1 and 2 and 3 and 4) not 5 not 6 not 7 not 8

PubMed

(((((((glucagon[Title/Abstract])

AND (infus*[Title/Abstract] OR admin*[Title/Abstract] OR intravenous[Title/Abstract] OR dos*[Title/Abstract] OR saline[Title/Abstract] OR subcutaneous[Title/Abstract] OR intramuscular[Title/Abstract] OR nasal[Title/Abstract]))

AND (energy[Title/Abstract] OR intake[Title/Abstract] OR food[Title/Abstract] OR meal[Title/Abstract] OR appetite[Title/Abstract] OR eat*[Title/Abstract] OR hunger[Title/Abstract] OR hungry[Title/Abstract] OR expenditure[Title/Abstract] OR

calorie*[Title/Abstract] OR kcal[Title/Abstract] OR joule*[Title/Abstract] OR kJ[Title/Abstract] OR insulin[Title/Abstract] OR glucose[Title/Abstract] OR glyc*[Title/Abstract]))

AND (human*[Title/Abstract] OR participant*[Title/Abstract] OR subject*[Title/Abstract] OR patient*[Title/Abstract] OR volunteer*[Title/Abstract] OR men[Title/Abstract] OR women[Title/Abstract] OR man[Title/Abstract] OR woman[Title/Abstract] OR male*[Title/Abstract] OR female*[Title/Abstract] OR individual*[Title/Abstract] OR recruit*[Title/Abstract] OR trial[Title/Abstract] OR crossover[Title/Abstract] OR cross over[Title/Abstract] OR cross-over[Title/Abstract] OR consent[Title/Abstract] OR assign*[Title/Abstract] OR allocate*[Title/Abstract] OR placebo[Title/Abstract]))

NOT (review[Title] OR meta analysis[Title] OR meta-analysis[Title] OR case study[Title] OR case studies[Title]))

NOT (databases adj4 searched[Title/Abstract]))

NOT (rat[Title] OR rats[Title] OR mouse[Title] OR mice[Title] OR swine[Title] OR porcine[Title] OR murine[Title] OR sheep[Title] OR lamb[Title] OR pigs[Title] OR piglets[Title] OR rabbit[Title] OR rabbits[Title] OR cat[Title] OR cats[Title] OR dog[Title] OR dogs[Title] OR cattle[Title] OR bovine[Title] OR monkey[Title] OR monkeys[Title] OR trout[Title] OR chick[Title] OR chicks[Title] OR broiler[Title] OR broilers[Title] OR carp[Title]))

NOT (animal* OR animal studies OR animal model*[MeSH Terms])

Scopus

(TITLE-ABS-KEY (glucagon)

AND TITLE-ABS-KEY (infus* OR admin* OR intravenous OR dos* OR saline OR subcutaneous OR intramuscular OR nasal)

AND TITLE-ABS-KEY (energy OR intake OR food OR meal OR appetite OR eat* OR hunger OR hungry OR expenditure OR calorie* OR kcal OR joule* OR kj OR insulin OR glucose OR glyc*)

AND TITLE-ABS-KEY (human* OR participant* OR subject* OR patient* OR volunteer* OR men OR women OR man OR woman OR male* OR female* OR individual* OR recruit* OR trial OR crossover OR cross AND over OR cross-over OR consent OR assign* OR allocate* OR placebo)

AND NOT TITLE (review OR meta AND analysis OR meta-analysis OR case AND study OR case AND studies)

AND NOT ABS (databases AND adj4 AND searched)

AND NOT TITLE (rat OR rats OR mouse OR mice OR swine OR porcine OR murine OR sheep OR lamb OR pigs OR piglets OR rabbit OR rabbits OR cat OR cats OR dog OR dogs OR cattle OR bovine OR monkey OR monkeys OR trout OR chick OR chicks OR broiler OR broilers OR carp))

Appendix 3.2: Risk of bias analysis

Energy intake

Study	Bias arising from the randomization process	Bias arising from period and carryover effects	Bias due to deviations from intended intervention	Bias due to missing outcome data	Bias in the measurement of the outcome	Bias in the selection of the reported result	Overall risk of bias
Bagger et al.[11]	Some concerns	Some concerns	Low risk	Low risk	Low risk	Low risk	Some concerns
Cegla et al.[29]	Some concerns	Some concerns	Low risk	Low risk	Low risk	Some concerns	Some concerns
Geary et al.[30]	Some concerns	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
Izzi-Engbeaya et al.[12]	Some concerns	Some concerns	Low risk	Low risk	Low risk	Low risk	Some concerns
Stahel et al.[32]	Some concerns	Low risk	Low risk	Low risk	Low risk	Low risk	Some concerns

Appetite

Study	Bias arising from the randomization process	Bias arising from period and carryover effects	Bias due to deviations from intended intervention	Bias due to missing outcome data	Bias in the measurement of the outcome	Bias in the selection of the reported result	Overall risk of bias
Arafat et al.[10a]	Some concerns	Some concerns	Low risk	Low risk	Low risk	Some concerns	Some concerns
Arafat et al.[10b]	Some concerns	Some concerns	Low risk	Low risk	Low risk	Some concerns	Some concerns
Bagger et al.[11]	Some concerns	Some concerns	Low risk	Low risk	Low risk	Low risk	Some concerns
Izzi-Engbeaya et al.[12]	Some concerns	Some concerns	Low risk	Low risk	Low risk	Low risk	Some concerns

Appendix 3.3: Data used for meta-analysis

Energy intake

Author	Sample size	Comparator		Glucagon		Data source
		Mean	SD	Mean	SD	
Bagger et al.[11]	15	811	147	686	148	Primary reference (Table 2)
Cegla et al.[29]	13	1086	397	1086	349	Primary reference (In text)
Geary et al.[30]	12	803	314	672	302	Primary reference (Table 4)
Izzi-Engbeaya et al.[12]	17	1069	371	1213	402	Correspondence with authors
Stahel et al.[32]	19	1206	309	1165	309	Primary reference (In text)

Subjective appetite

Author	Sample size	Control		Glucagon		Data source
		Mean	SD	Mean	SD	
Arafat et al.[10a]	13	189*	62	284*	50	Primary reference (Figure 1D) - extracted using WebPlotDigitizer
Arafat et al.[10b]	11	274*	125	212*	33	Primary reference (Figure 1D) - extracted using WebPlotDigitizer
Bagger et al.[11]	15	46	33	57	33	Primary reference (Table 2)
Izzi-Engbeaya et al.[12]	16	3110	795	2865	719	Correspondence with authors

*satiety score measured (inverse of appetite)

Appendix 3.4: Sensitivity analyses using variable within-participant correlation coefficients for meta-analyses

Energy intake

Within-participant correlation coefficient	Mean intervention effect	95% confidence interval	P-value
0.3	-0.18	-0.58, 0.22	0.375
0.5	-0.19	-0.59, 0.21	0.345
0.7	-0.20	-0.60, 0.19	0.314
0.9	-0.22	-0.61, 0.18	0.284

Chapter 4: The metabolic interplay between dietary carbohydrate intake and exercise and its role in appetite-regulation and energy intake

4.1 Introduction

Chronic exercise interventions typically produce only modest weight loss, especially when compared to dietary energy restriction[1]. This relative lack of efficacy is primarily attributed to compensatory eating behaviours, in which the energy expended through exercise is negated by an increase in energy intake[2]. Subjective appetite responses and compensatory energy intake following acute exercise however display marked inter-individual variability[3,4]. Understanding the mechanisms that regulate exercise-induced changes in appetite and energy intake would facilitate the development of targeted therapeutics for obesity.

Exercise-induced appetite responses have largely been attributed to changes in the systemic concentrations of gastrointestinal hormones implicated in appetite regulation, including glucagon-like peptide 1 (GLP-1), peptide YY (PYY), and (active) ghrelin[5]. However, acute exercise exerts changes beyond gastrointestinal hormone release, producing large-scale shifts in metabolism that may also drive exercise-induced changes in appetite and energy intake[6]. This includes an increase in systemic concentrations of the pancreatic hormone glucagon (as discussed and investigated in Chapters 1-3).

Evidence is growing that the metabolic effects of acute exercise are dependent on the pre-exercise nutritional state[7–9]. Recreational exercisers often consume carbohydrate in the immediate period prior to exercise in order to increase substrate availability for skeletal muscle activity and consequently exercise performance[10]. Acute carbohydrate ingestion also modulates GLP-1, PYY, and ghrelin concentrations[11], as well as producing extensive shifts in the plasma metabolite profile[12]. The concurrent provision of carbohydrate and exercise may therefore produce independent or interactive hormonal and metabolite responses, which could possess important downstream implications for appetite regulation and energy intake.

Our work therefore aimed to determine the interactive effects of carbohydrate and exercise on the plasma hormonal and metabolite responses and explore potential mediators of exercise-induced changes in appetite and energy intake across nutritional states.

4.2 Methods

4.2.1 Ethics

This study was granted ethical approval (South West - Frenchay Research Ethics Committee; 19/SW/007) and conducted in line with the Declaration of Helsinki. All participants provided

written informed consent prior to enrolment. This study is registered at clinicaltrials.gov (NCT04019418).

4.2.2 Participants

This study recruited 12 healthy male participants aged 18 to 40 years old with a body mass index (BMI) of 18 to 30 kg/m^2 (inclusive). Females were excluded due to the influence of the menstrual cycle on appetite control, gastrointestinal hormone release, and exercise metabolism[13–15].

Participants had an age of 24 ± 5 years (mean ± SD) with a BMI of 21.9 ± 2.1 kg/m^2 and a maximal oxygen uptake ($\dot{V}O_2$ max) of 40.2 ± 8.7 ml/kg/min.

4.2.3 Study design

This study was a semi-double blind, randomised, four-period crossover, placebo-controlled design.

All participants first attended a screening visit to assess eligibility. Eligible participants returned to undertake a $\dot{V}O_2$ max test to determine absolute exercise intensity for study visits. Participants then completed four study visits in a randomised order: (i) control beverage and rest session; (ii) control beverage and exercise session; (iii) carbohydrate beverage and rest session; and (iv) carbohydrate beverage and exercise session. Study visits were separated by a minimum of three days.

The study design is described as semi-double blind as both participants and researchers were blinded to the nature of the beverage, but not to the rest or exercise session.

All study visits and assessments were performed at the NIHR Imperial Clinical Research Facility at Hammersmith Hospital (London, UK).

4.2.3.1 Screening visit

Participants attended a screening visit following a ≥ 4 hour fast. A blood test (glucose and full blood count), height and weight measurements, and an electrocardiogram (ECG) were performed to determine participant eligibility. Participants were excluded if they had had an abnormal ECG, blood values outside the reference range, a BMI of <18 or >30 kg/m^2, a history of metabolic disease, and/or started a new medication within the last 3 months likely to interfere with energy metabolism, appetite regulation and hormonal balance.

4.2.3.2 $\dot{V}O_2$ max assessment

All assessments were performed on an ergometrics 900 bicycle ergometer (ergoline, Germany). Participants began the assessment by cycling at 50 watts, after which the intensity was increased by 25 watts every three minutes until volitional exhaustion. Pulmonary gas

measurements (oxygen consumption and carbon dioxide production) were taken throughout the assessment via a Quark CPET metabolic cart (COSMED, Italy). If participants did not achieve a respiratory quotient ≥1.1 at the point of volitional exhaustion, the assessment was repeated at a separate study visit. After completion of the assessment, participants were presented with an *ad libitum* meal identical to that received during study visits to facilitate familiarisation.

4.2.3.3 Study visits

Participants were asked to refrain from strenuous exercise, caffeine, and alcohol intake, and to standardise their evening meal the day prior to each study visit. Participants were also asked to fast overnight from 20:00 the evening prior to each study visit (drinking water was permitted).

Participants arrived at the research facility at 09:00 ± 1 hour upon which an intravenous cannula was inserted into the antecubital vein to permit serial blood sampling. Participants were also asked to void their bladder, with all urine thereafter collected for the remainder of the study visit. Following the collection of baseline measurements, participants either consumed a control or carbohydrate beverage. Participants were given five minutes to consume the beverage, after which participants either rested or exercised for 30 minutes. Following completion of the rest or exercise session, participants remained rested for a further 90 minutes at which point an *ad libitum* meal was provided.

Venous blood samples and 100 mm visual analogue scales (VAS) were collected at baseline and at 15-minute intervals following beverage ingestion (T = 15, 30, 45, 60, 75, 90, 105, 120). Pulmonary gas measurements were also performed for 15-minute intervals throughout the visit (baseline, T = 15-30, 45-60, 105-120). A schematic of a typical study visit is shown in Figure 4.1.

4.2.4 Interventions

4.2.4.1 Beverages

The control beverage consisted of 300ml of water only. The carbohydrate beverage constituted 300ml of water with 75g of maltodextrin (equating to 75g of carbohydrate; MyProtein, UK). This amount of carbohydrate has previously been shown to modulate gastrointestinal hormone release[16]. Carbohydrate was provided in liquid form and beverages given in opaque bottles to facilitate blinding.

4.2.4.2 Rest and exercise sessions

Participants laid semi-recumbently on a bed for 30 minutes for the rest session. For the exercise session, participants exercised on an ergometrics 900 bicycle ergometer (ergoline,

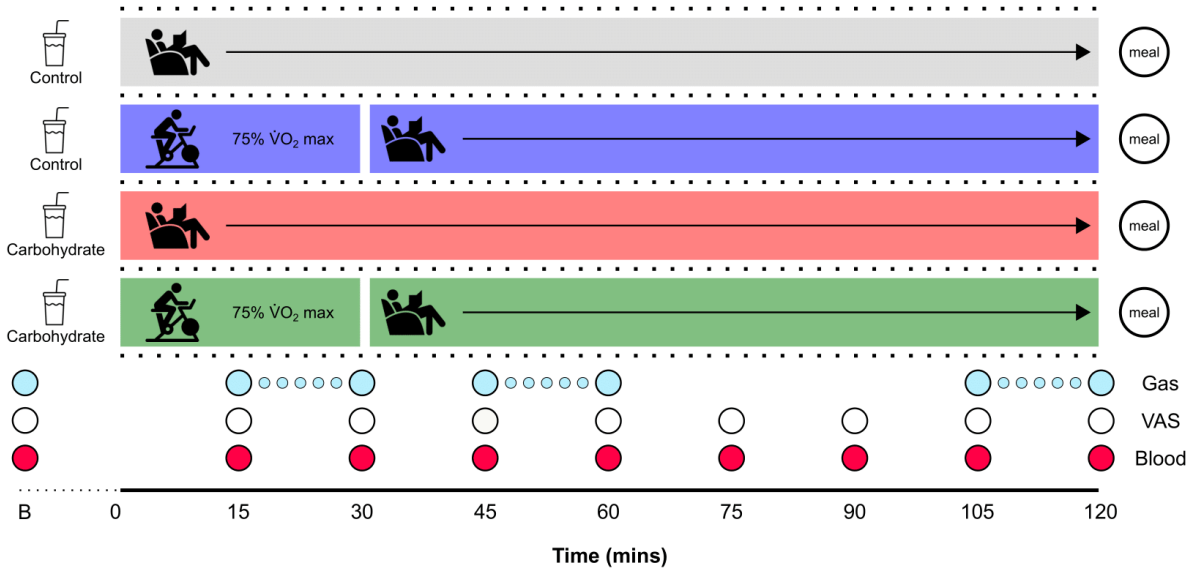


Figure 4.1: Overview of study interventions including serial blood sampling scheme, visual analogue scale assessments, and pulmonary gas measurements. B, baseline.

Germany) for 30 minutes at 75% $\dot{V}O_2$ max at a cadence ≥ 80 pedal revolutions per minute. This exercise intensity has previously been shown to modulate gastrointestinal hormone release[17].

4.2.4.3 Beverage-exercise interval

Most studies investigating the effect of carbohydrate provision prior to exercise on energy intake have involved participants undertaking exercise several hours following carbohydrate ingestion[18–20]. The influence of carbohydrate ingestion on physiological responses such as subjective appetite, GLP-1 and PYY release is transient[21], and thus largely attenuated by the time exercise is performed. As a result, any potential interaction between acute carbohydrate ingestion and exercise on these responses is lost. We therefore used a beverage-exercise interval of five minutes to ensure that changes in appetite-related hormones due to both carbohydrate ingestion and exercise coincided.

4.2.4.4 Ad libitum meal

The *ad libitum* meal consisted of dried durum wheat semolina pasta (Tesco, UK), tomato and herb pasta sauce (Tesco, UK), and olive oil (Tesco, UK). This meal possessed an energy density of 5.3 kJ/g (1.3 kcal/g) and contained 21.2 g of carbohydrate, 3.0 g of fat, and 3.3 g of protein per 100 g.

Participants were given 20 minutes to consume the meal and were asked to eat until 'comfortably full'. Participants were also asked to refrain from using mobile telephones, laptops or other distractions during this period.

4.2.5 Measurements

4.2.5.1 Primary outcome measures

Blood samples were analysed for GLP-1 and PYY using previously established in-house radioimmunoassays[22,23] at all time points. Active ghrelin was measured using a commercial ELISA (EZGRA-88K, Merck, Germany) at T = 0, 30, 60, and 120 only.

4.2.5.2 Secondary outcome measures

Blood samples were analysed for glucose using a colorimetric enzymatic activity assay (GL364, Randox, UK) at all time points. Insulin (HI-14K, Merck, Germany) and glucagon (GL-32K, Merck, Germany) were measured using commercial radioimmunoassays at T = 0, 15, 30, 45, 60, 90, and 120.

All commercial assays were performed as specified by the manufacturer's instructions.

Pulmonary gas measurements (oxygen consumption and carbon dioxide production) and urea content of urine were used to calculate substrate oxidation (carbohydrate and fat oxidation)[24] and total energy expenditure[25]. Carbohydrate and fat balance were calculated by subtracting oxidation from intake (excluding intake from the *ad libitum* meal).

Meal energy intake was calculated by measuring the difference in mass of the *ad libitum* meal prior to and following consumption. Total energy intake was calculated by adding the energy content of beverages to meal energy intake. Energy balance was calculated by subtracting total energy expenditure from total energy intake.

Subjective appetite and nausea were measured by VAS. Subjective appetite was assessed using four questions relating to hunger, pleasantness, prospective consumption, and fullness. These scales were then converted into a single composite appetite score (CAS) using the formula: CAS = [hunger + pleasantness + prospective consumption + (100 – fullness)]/4

4.2.5.3 ^{1}H NMR metabolic profiling analysis of blood plasma

Water-suppressed ^{1}H NMR spectroscopy was performed at 310 K using a 600 MHz Bruker Avance III NMR spectrometer (*in vitro* diagnostics research (IVDr)) equipped with a 5-mm BBI Z-gradient probe, high-order shims, and automated tuning and matching (Bruker Biospin GmBH, Rheinstetten, Germany). Previous to the analysis, a quantitative calibration was performed using the standard high-throughput protocol[26]. Bruker IVDr (B.I.) methods were used to extract lipoprotein data (B.I. LISA^{TM}) and to quantify small molecule metabolites (B.I. Quant-PS 2.0^{TM})[27]. For each sample, three experiments were acquired in automation: two 1D ^{1}H NMR spectra were acquired using standard one-dimensional pulse sequences, the first one with saturation of the water resonance only (noesygppr1d, standard Bruker pulse program), and the second one with the same feature and with a block to filter out

macromolecular/protein signals from the spectrum (Carr–Purcell–Meiboom–Gill, cpmgpr1d); $^{1}H-^{1}H$ 2D *J*-resolved (*J*-Res) experiment with water suppression was also acquired (jresgpprqf). The parameter sets used for acquisition and processing were according to the literature[26].

4.2.6 Randomisation and blinding

Randomisation sequences were generated using an online randomisation tool (randomizer.org). Sequences were then placed into opaque envelopes, sealed, and allocated to participants prior to their first study visit.

Beverages were created and labelled (A or B) by an external researcher prior to study visits. Beverage contents was revealed after VAS scoring, assays, and statistical analysis had been performed.

4.2.7 Statistical analysis

4.2.7.1 Robust linear mixed model analysis

Time-averaged area under the curve (AUC) was calculated for all longitudinal outcomes and used for subsequent robust linear mixed effect model analysis. Unadjusted values were used for meal energy intake, total energy intake, total energy expenditure, carbohydrate balance, fat balance, and energy balance.

Robust linear mixed effects models were fitted using the R package 'robustlmm'[28] to evaluate the independent and interactive effects of carbohydrate and exercise on all outcomes. Fixed effects included in the model were carbohydrate and exercise, and an interaction between carbohydrate and exercise. Random effects included in the model were participant, an interaction between participant and carbohydrate, and an interaction between participant and exercise. Random effects with zero variance were removed from the model. The Satterthwaite's degrees of freedom method implemented in the R package 'lmerTest'[29] was used to derive P values for fixed effects. If a significant interaction effect between carbohydrate and exercise was detected, multiple comparisons of estimated marginal means were performed using the R package 'emmeans'[30] with the Tukey adjustment being applied. Independent effects were defined as a significant main effect of carbohydrate and/or exercise in the absence of a significant interaction effect. Interactive effects were defined as a significant interaction effect[31]. Statistical significance was set at $P < 0.05$.

4.2.7.2 NMR data processing and statistical analysis

The multivariate data analysis was performed on the 1D ^{1}H CPMG spectra. Each spectrum was automatically phased and baseline corrected using Topspin 3.5 pl 7 (Bruker BioSpin GmbH, Rheinstetten, Germany), and then digitized over the range δ -0.5 to 10 and imported

into MATLAB (2014a, MathWorks, Natick, U.S.A.). The spectra were referenced to the doublet of the anomeric proton signal of a-glucose at δ 5.23. The spectral regions corresponding to the internal standard (δ -0.5 to 0.6), water (δ 4.3 to 5.1) and noise (δ 5.4 to 5.7, 5.8 to 6.7, and 8.5 to 10.00) were excluded to give full resolution spectra (~11.7 K spectral data points or variables per spectrum). Prior to multivariate data analysis, the spectra were normalized using the probabilistic quotient method (PQN)[32].

The data set was auto-scaled and modelled using Partial Least Squares Discriminant Analysis (PLS-DA) in a Monte Carlo Cross-Validation (MCCV) framework and repeated measures (RM) design. Storey-Tibshirani method of correction for multiple testing was used, where variables with $q \leq 0.05$ were considered as significant in the MCCV framework. Goodness of fit (R^2Y) was calculated using the training data, and the goodness of prediction (Q^2Y) from test data[33]. For discriminant analysis, the number of the intervention was used as dummy variable at each time point (Y = 1, 2, 3 and 4).

Relevant features from the NMR targeted data (e.g. B.I. LISA™, and B.I. Quant-PS 2.0™) were also subjected to linear mixed models, correlation networks, and partial least squares regression.

4.2.7.3 Identification of metabolites for untargeted data

Subset Optimization by Reference Matching (STORM) was used for the identification of significant metabolites using the correlation structure of 1D ^{1}H-NMR data[34]. *J*-Res spectra were also used for identification purposes. Internal and external databases such as the Human Metabolome Data Base (HMDB; http://hmdb.ca/) and/or the Biological Magnetic Resonance Data Bank (BMRB; http://www.bmrb.wisc.edu) were used for confirmation of assignments.

4.2.7.4 Correlation network analysis

Correlation networks were created for outcomes measured at T = 0, 30, and 120 using the R package 'rmcorr'[35]. Only outcomes with a significant main effect of carbohydrate and/or exercise, and/or a significant interaction effect were included. Correlations with a coefficient ≥ 0.6 and P value < 0.05 (after correction with Q = 5%[36]) were displayed graphically using the R package 'circlize'[37]. A cut-off of ≥ 0.6 was selected as this indicated a strong relationship between the two variables[38], and therefore more likely to be biologically meaningful.

4.2.7.5 Partial least squares regression

Partial least squares regression was performed using the R package 'mixOmics'[39] using all outcomes measured in plasma/serum with a significant main effect of carbohydrate and/or exercise, and/or a significant interaction effect. Leave-one-out cross-validation (LOOCV) was employed to select the number of latent variables for each model. Root mean square error of

prediction (RMSEP) and goodness of fit (R^2) were calculated via LOOCV using the selected number of latent variables. All variables were scaled and centred prior to analysis.

4.2.7.6 Sample size determination

A formal sample size calculation was not performed as the interactive effect of carbohydrate and exercise has not been previously investigated. Prior studies have however demonstrated that 12 participants are sufficient to show that carbohydrate ingestion[21] and exercise[17] influence gastrointestinal hormone release. Furthermore, 12 participants have been argued to be sufficient with respect to precision about the mean and variance when no prior information is available[40].

4.3 Results

4.3.1 Carbohydrate and exercise independently and interactively modulate the hormonal milieu

Initially, we performed robust linear mixed model analyses to identify the independent and interactive effects of carbohydrate and exercise on the hormonal milieu (Figure 4.2). Our results revealed that carbohydrate and exercise independently increased the anorexigenic hormones GLP-1 and PYY (Figures 4.2A and 4.2B), while also acting independently to decrease circulating levels of orexigenic hormone (active) ghrelin (Figure 4.2C). We also investigated the interactive effects of carbohydrate and exercise on insulin, glucagon, and glucose concentrations due to the posited role of glucose homeostasis in appetite regulation[41]. Carbohydrate and exercise exhibited antagonistic and interactive effects on the insulin response, in which carbohydrate increased and exercise decreased circulating levels, and with exercise producing larger reductions following carbohydrate ingestion (Figure 4.2D). Conversely, carbohydrate and exercise independently increased glucagon concentrations (Figure 4.2E). Glucose concentrations were unaffected by exercise, but as expected showed a marked increase following carbohydrate ingestion (Figure 4.2F).

4.3.2 The influence of exercise on energy intake is dependent on carbohydrate intake

The independent and interactive effects of carbohydrate and exercise on appetite, energy balance, and substrate oxidation are presented in Figure 4.3. Carbohydrate and exercise showed independent appetite-suppressive effects (Figure 4.3E). However, changes in appetite did not correspond with subsequent *ad libitum* meal energy intake. Instead, meal energy intake exhibited a differential response to exercise dependent on carbohydrate provision, with exercise increasing energy intake without carbohydrate ingestion, but decreasing energy intake when carbohydrate was provided (Figure 4.3A). As meal energy intake was the sole component of energy balance that was allowed to vary in our study design (pre-exercise energy intake and exercise energy expenditure were fixed), meal energy intake

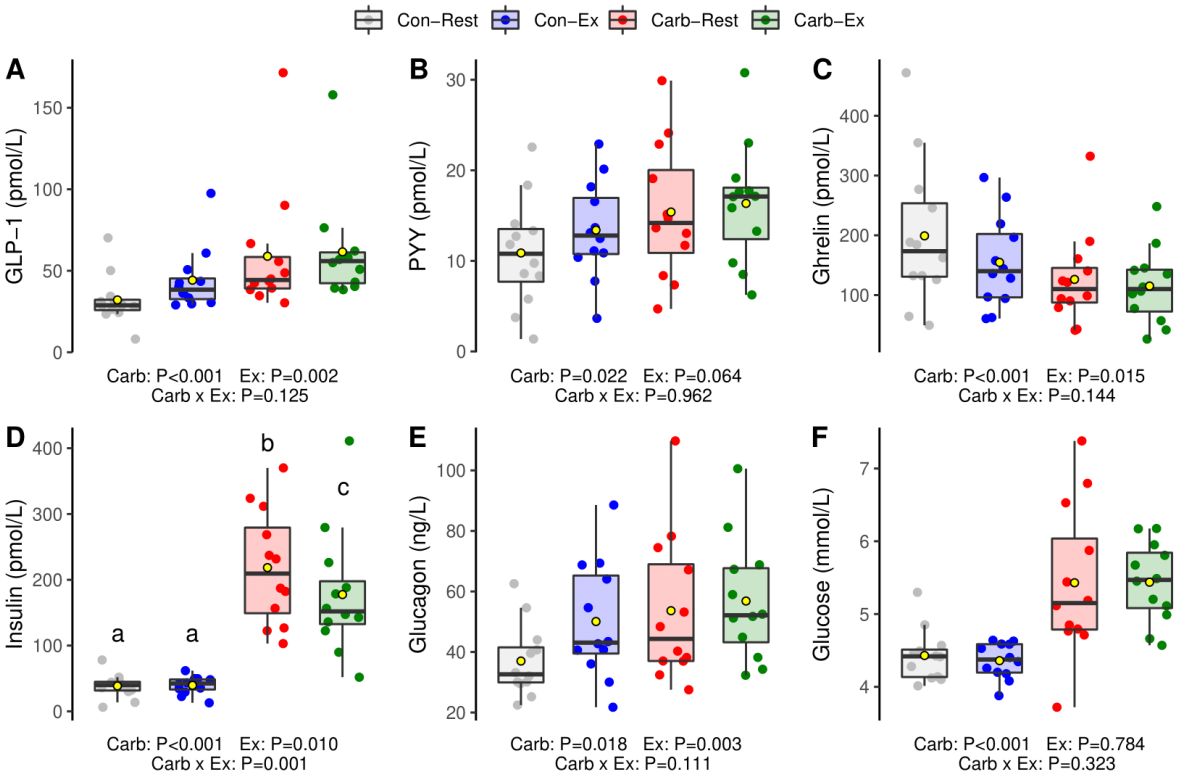


Figure 4.2: The effect of dietary carbohydrate and exercise on gastrointestinal hormone release and glucose homeostasis. Data are time-averaged AUC for all outcomes. Individual dots represent individual participant values, with yellow dots representing intervention means. P values from robust linear mixed models for main effects of carbohydrate (Carb) and exercise (Ex), and the interaction between carbohydrate and exercise (Carb x Ex) are presented.

exerted a large influence over energy balance. Similar responses in energy balance were consequently observed with carbohydrate ingestion (Figure 4.3D). However, exercise without carbohydrate ingestion resulted in the lowest acute energy balance despite the increase in meal energy intake (Figure 4.3D).

4.3.3 Carbohydrate and exercise generate distinct metabolic phenotypes

Data obtained from untargeted metabolomic analyses were modelled using repeated measures partial least squares discriminant analysis (RM-PLS-DA) to distinguish metabolic phenotypes between study interventions at pre-defined time points (T = 0, T = 30, T = 120). The metabolic phenotype was highly distinguishable between all study interventions immediately post exercise (T = 30), characterised by high a degree of explained variance ($R^2Y \geq 0.99$) and capability of prediction ($Q^2Y > 0.7$ with the exception of Con-Ex vs Carb-Ex; Figure 4.4A). Differences between study interventions persevered until the end of the study visit (T = 120) but were less pronounced (Figure 4.4B). No differences between study interventions were detected at baseline (T = 0).

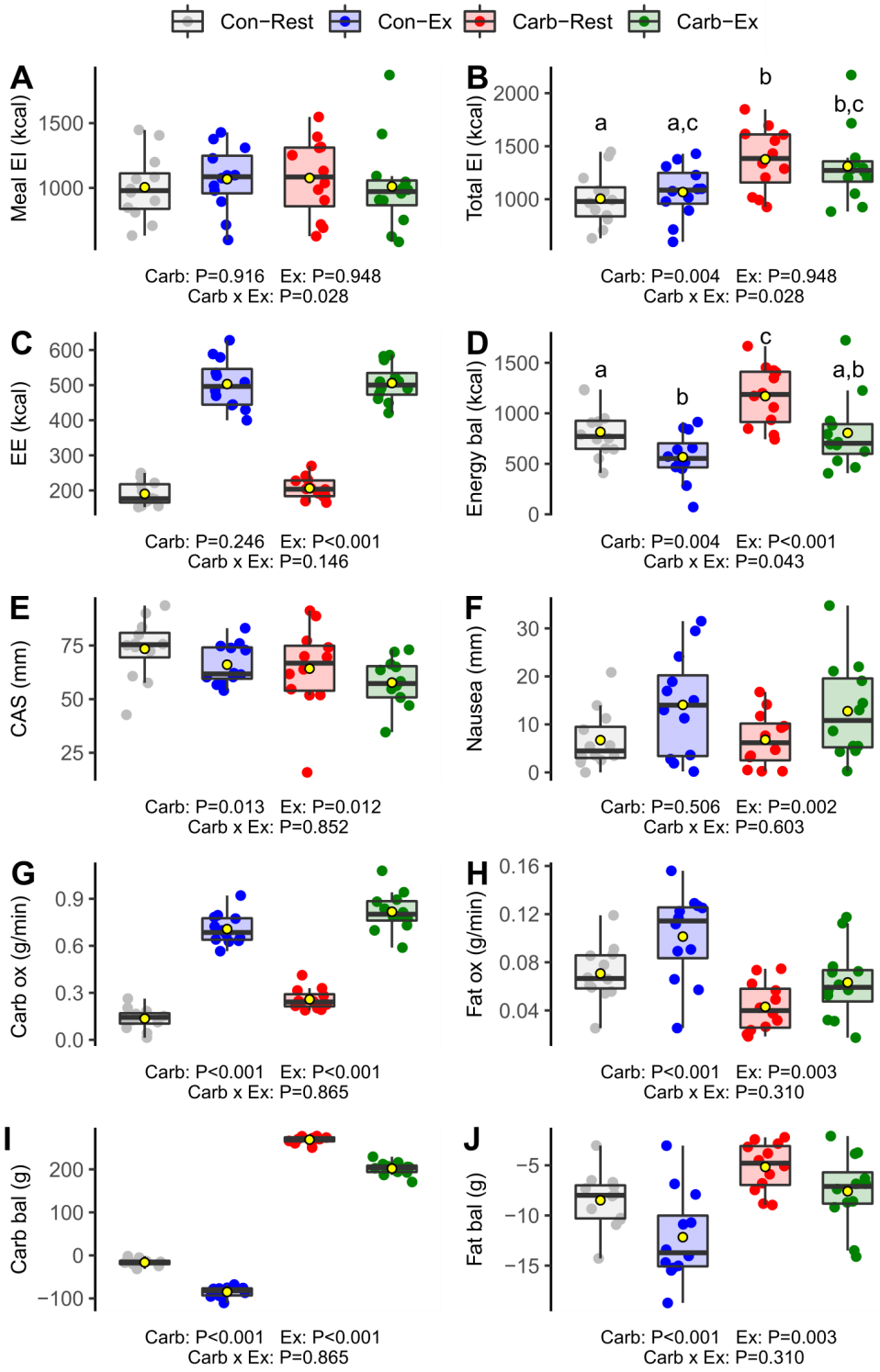


Figure 4.3: The effect of dietary carbohydrate and exercise on components of energy balance and substrate oxidation. Data are time-averaged AUC for CAS, nausea, carb oxidation (carb ox), and fat oxidation (fat ox) only. Individual dots represent individual participant values, with yellow dots representing intervention means. P values from robust linear mixed models for main effects of carbohydrate (Carb) and exercise (Ex), and the interaction between carbohydrate and exercise (Carb x Ex) are presented. Interventions with different letters are significantly different from each other (P< 0.05). CAS, composite appetite score. EE, energy expenditure. EI, energy intake.

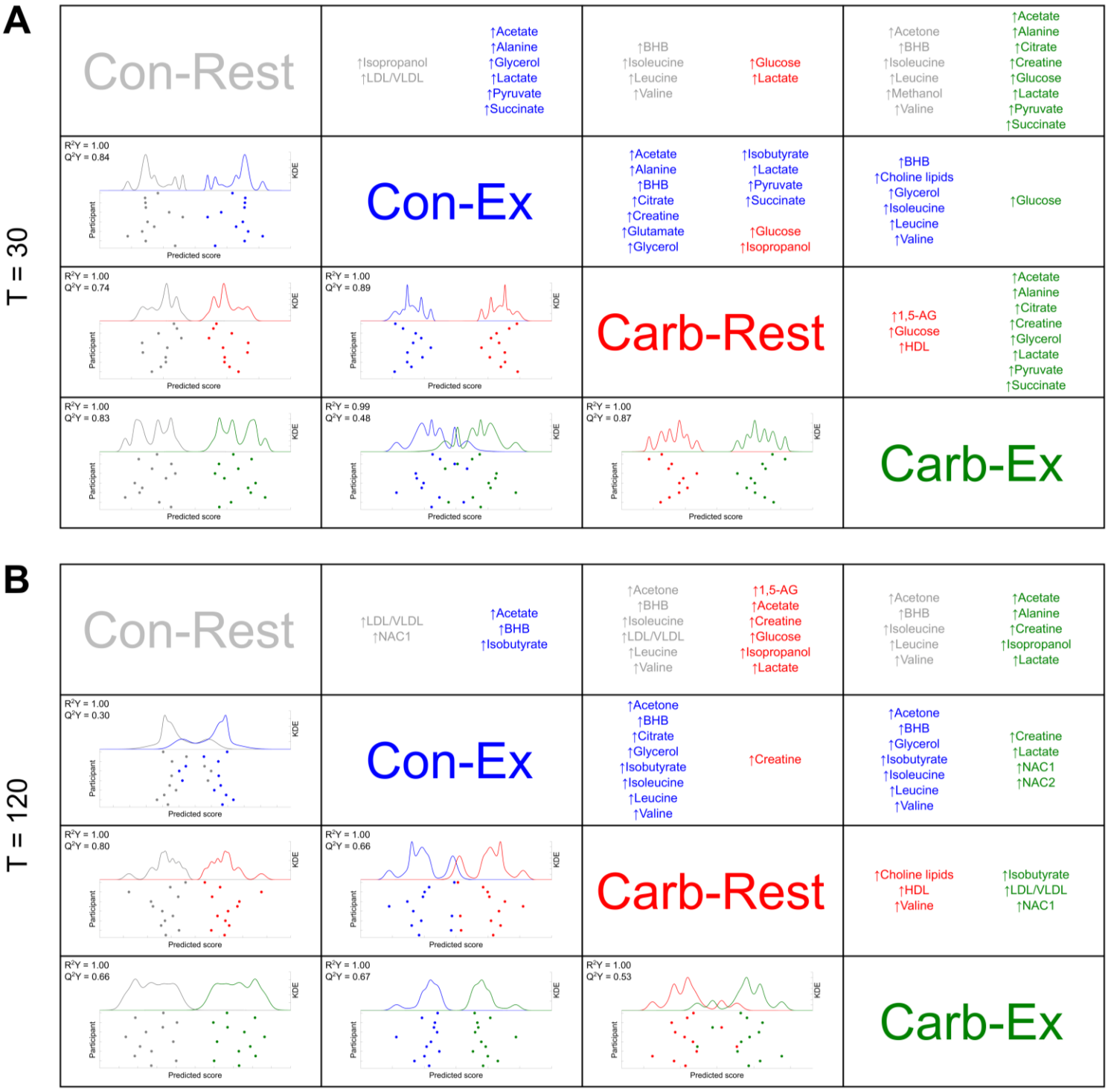


Figure 4.4: PLS-DA score plot and metabolite matrix. (A) Comparisons between study interventions at T = 30. (B) Comparisons between study interventions at T = 120. Lower matrix displays score plots derived from partial least-squares discriminant analysis demonstrating differentiation in metabolic profiles between study interventions (Con-Rest: grey; Con-Ex: blue; Carb-Rest: Red; Carb-Ex: green). Individual dots represent individual participant metabolic profiles. All models include kernel density estimates (KDE) of the predicted scores for both study interventions under comparison. Upper matrix displays differences in metabolites between study interventions. R^2Y, explained variance. Q^2Y, capability of prediction.

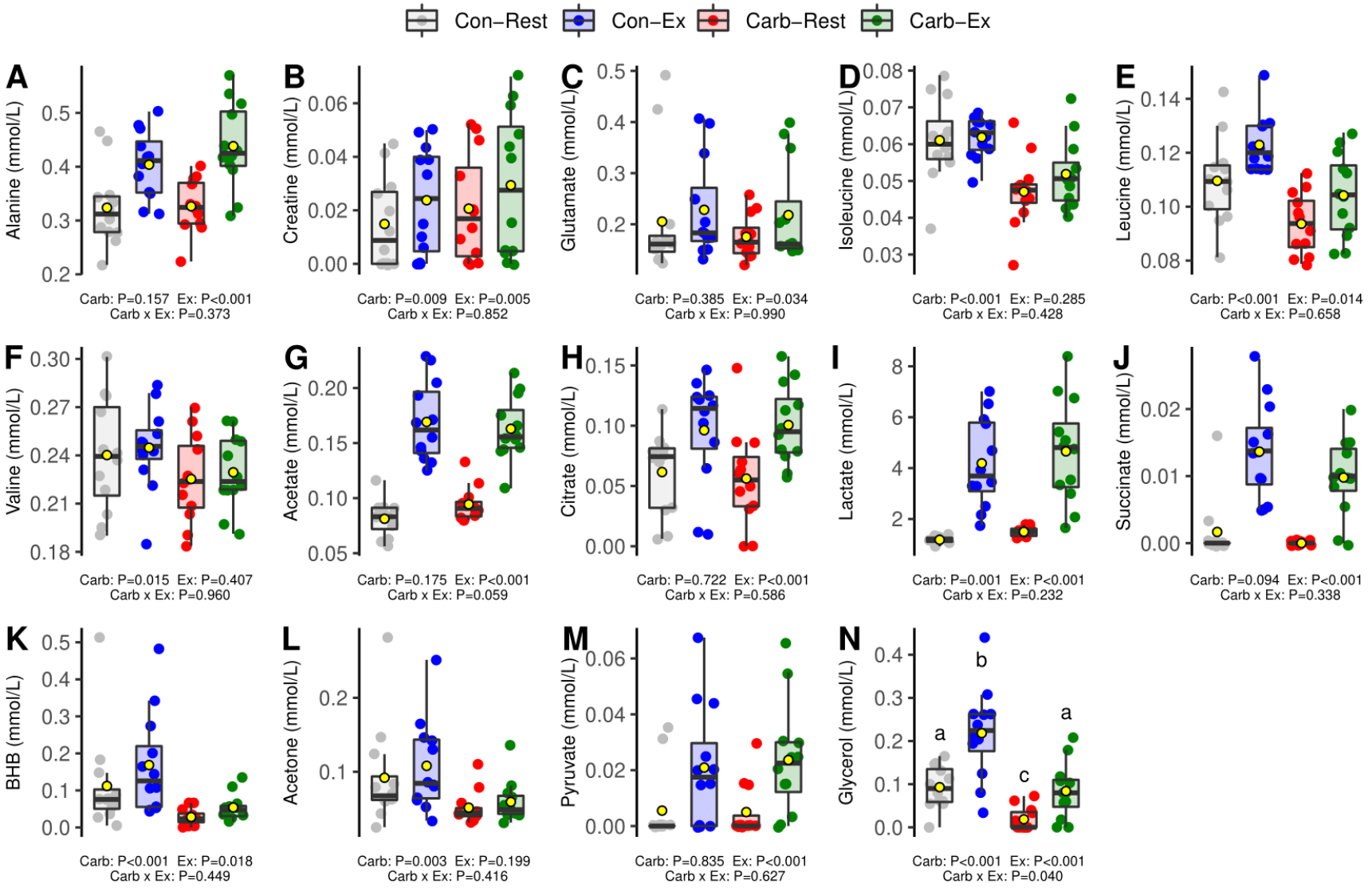


Figure 4.5: The effect of dietary carbohydrate and exercise on circulating small metabolites. Data are time-averaged AUC for all outcomes. Individual dots represent individual participant values, with yellow dots representing intervention means. P values from robust linear mixed models for main effects of carbohydrate (Carb) and exercise (Ex), and the interaction between carbohydrate and exercise (Carb x Ex) are presented. Interventions with different letters are significantly different from each other (P< 0.05). BHB, 3-hydroxybutyrate.

Untargeted metabolomic analysis identified 23 unique small metabolites that showed significant differences over time between study interventions (Figure 4.4A and 4.4B), with targeted metabolomic data[27] available for 14 of these metabolites (Figure 4.5). Carbohydrate ingestion produced differential amino acid and amino acid derivative responses characterised by an increase in creatine concentrations (Figure 4.5B) and a simultaneous decrease in branched chain amino acid (isoleucine, leucine, and valine) concentrations (Figures 4.5D-F). Exercise increased concentrations of creatine and the amino acids alanine and glutamate (Figures 4.5A-C). Concentrations of the TCA cycle intermediates citrate and succinate, as well as acetate and lactate also increased in response to exercise, with only lactate increasing in response to carbohydrate ingestion (Figures 4.5G-J). Exercise resulted in increased concentrations of 3-hydroxybutyrate (BHB) and the metabolic precursor pyruvate (Figures 4.5K and 4.5M). Ketogenesis was however suppressed by carbohydrate ingestion as demonstrated by decreased BHB and acetone concentrations (Figures 4.5K and 4.5L). Carbohydrate and exercise exerted antagonistic and interactive effects on glycerol concentrations, with carbohydrate ingestion suppressing and exercise elevating concentrations, and with exercise generating higher concentrations when carbohydrate was not ingested (Figure 4.5N).

Untargeted metabolomic analysis also revealed differences between interventions in ^{1}H-NMR peaks assigned to HDL and LDL/VLDL (Figures 4.4A and 4.4B). Consequently, targeted metabolomic data[27] for the lipoprotein response was analysed, with data presented for main lipoprotein fractions and parameters (Figure 4.6). Carbohydrate ingestion resulted in an increase in high-density lipoprotein cholesterol (HDL-C; Figure 4.6I), whereas exercise resulted in an increase in intermediate- density lipoprotein particle number (IDL-P) and cholesterol concentrations (IDL-C), as well as an increase in apolipoprotein A2 (Apo-A2; Figures 4.6D and 4.6E). No other main effects of carbohydrate and exercise, or any interaction between carbohydrate and exercise, was detected.

4.3.4 Changes in circulating acetate, lactate, and PYY are associated with exercise-induced appetite suppression

To identify temporal relationships between outcomes, we used repeated measure correlation analyses to create within-intervention correlation networks (Figure 4.7A). These analyses highlighted distinct relationships between outcomes that were dependent on carbohydrate intake and exercise. Acetate, alanine, Apo-A2, carbohydrate oxidation, total energy expenditure, lactate, and valine showed a high degree of connectivity (≥15 total number of connections) across multiple study interventions (Figure 4.7E). Irrespective of carbohydrate intake, higher concentrations of acetate, lactate, and PYY were associated with decreased appetite during exercise study interventions (Figures 4.7B and 4.7D). Furthermore, of these

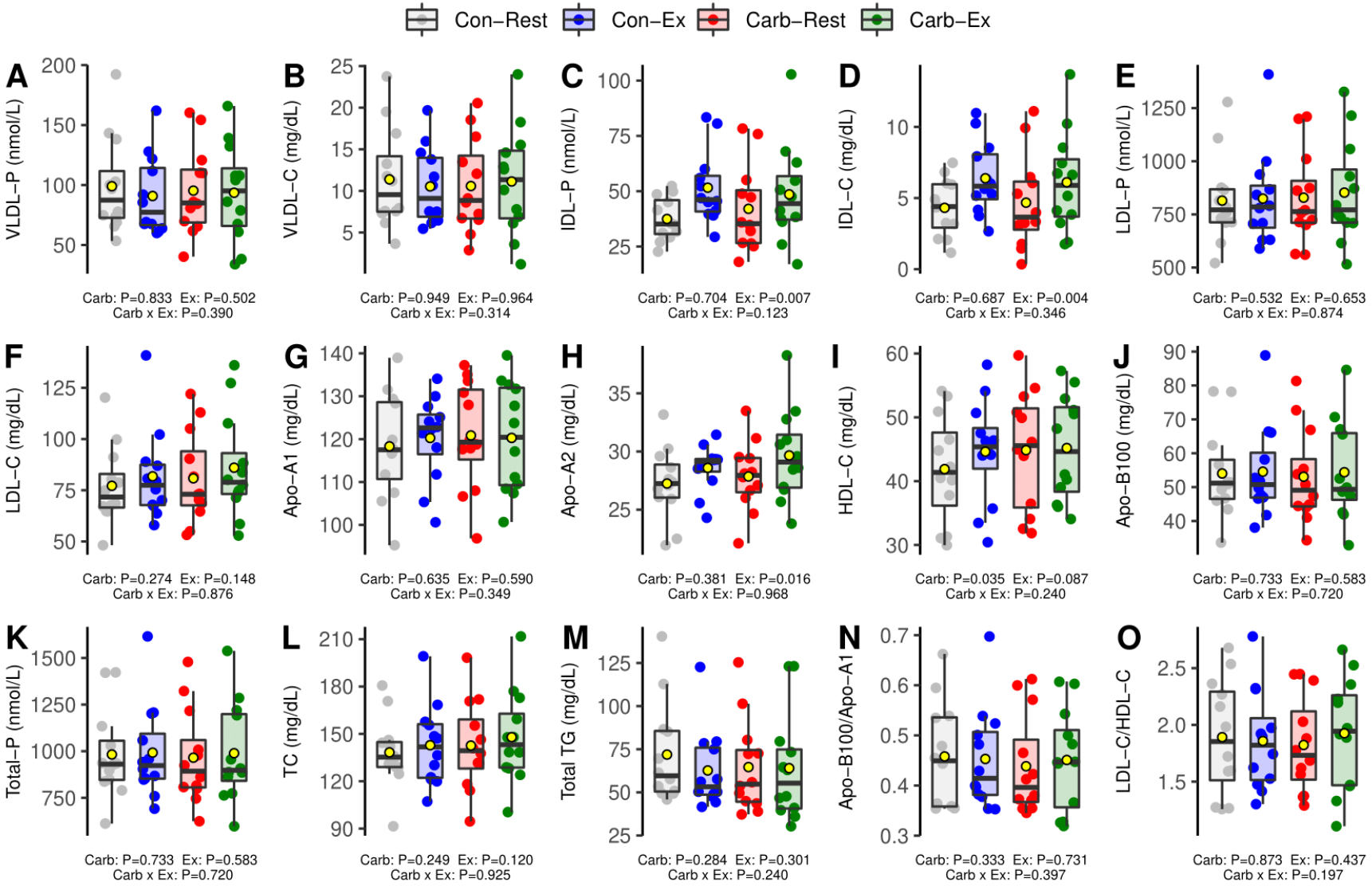


Figure 4.6: The effect of dietary carbohydrate and exercise on main lipoprotein fractions and parameters. Data are time-averaged AUC for all outcomes. Individual dots represent individual participant values, with yellow dots representing intervention means. P values from robust linear mixed models for main effects of carbohydrate (Carb) and exercise (Ex), and the interaction between carbohydrate and exercise (Carb x Ex) are presented. TC, total cholesterol. TG, total triglycerides. Total-P, total particle number.

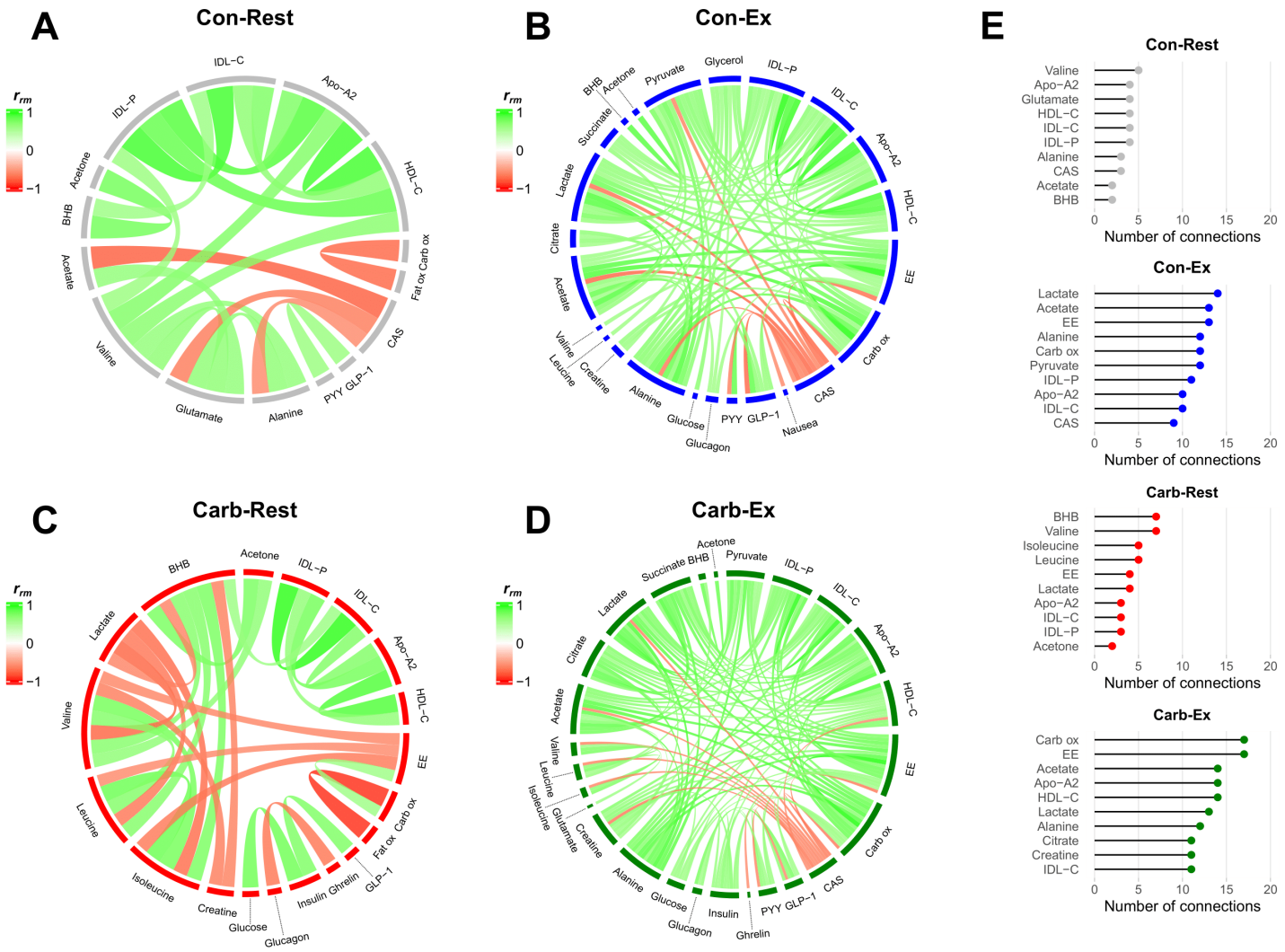


Figure 4.7: Correlation networks of temporal measurements across study interventions. Repeated measures correlation networks for (A) Con-Rest, (B) Con-Ex, (C) Carb-Rest, and (D) Carb-Ex. (E) The top 10 outcomes with the highest number of connections for each study intervention. Outcomes linked by a green line showed a significant positive correlation, outcomes linked by a red line showed a significant negative correlation. Only outcomes measured at T = 0, 30, and 120 with a significant main effect of carbohydrate and/or exercise, and/or a significant interaction effect were included in the analyses. Correlations with a coefficient ≥0.6 and adjusted P value <0.05 are displayed. r_{rm}, repeated measures correlation

outcomes, only higher concentrations of acetate were associated with decreased appetite in the resting state (Figure 4.7A). However, glucagon did not show any strong association with appetite across study interventions despite positive associations with GLP-1 and PYY during Carb-Ex (Figure 4.7D), and a negative association with ghrelin during Carb-Rest (Figure 4.7C). GLP-1 and PYY showed positive correlations across most study interventions (Figures 4.7A, 4.7B, and 4.7D). GLP-1 also exhibited strong positive correlations with insulin following carbohydrate ingestion (Figures 4.7C and 4.7D), and with total energy expenditure and carbohydrate oxidation during exercise study interventions (Figure 4.7B and 4.7D). The only identified hormone-metabolite relationships were positive correlations between GLP-1 and acetate as well as between GLP-1 and lactate during exercise without carbohydrate ingestion (Figure 4.7B).

4.3.5 Identification of metabolic predictors of *ad libitum* energy intake

Congruent with prior research[42], subjective appetite did not correlate with subsequent *ad libitum* meal energy intake within study interventions (data not shown). While subjective appetite may reflect eating latency[43], it does not appear to be predictive of energy intake, and therefore any observed relationship with subjective appetite may not necessarily be present for energy intake.

We therefore investigated the capacity of the preceding metabolic environment during the 120-minute study visit period (represented as time-averaged AUC) to predict subsequent *ad libitum* meal energy intake within each study intervention using partial least squares regression (Figure 4.8). For all study interventions, one latent variable was created, with variable importance in projection (VIP) scores >1[44] used to identify outcomes important in the prediction of subsequent *ad libitum* energy intake (Figure 4.8A). VIP scores identified GLP-1 and creatine to be important predictors of subsequent energy intake, exhibiting VIP scores >1 in three of the study interventions. Similarly, glucose, alanine, citrate, succinate, acetone, pyruvate, glycerol, and Apo-A2 also appeared to be important predictors, possessing a VIP score >1 in two of the study interventions. Of these metabolites, only succinate was an important predictor of meal energy intake in both exercise interventions irrespective of carbohydrate intake. Glucagon was not found to be an important predictor of energy intake during any study intervention. Follow-up within-intervention simple univariable regression analyses were also performed to explore the direction of relationship between important predictors (VIP >1) and meal energy intake (Figure 4.8B).

4.4 Discussion

Dietary carbohydrate and exercise both exert profound changes on human metabolism that have important implications for appetite-regulation and energy intake. Here, we show that

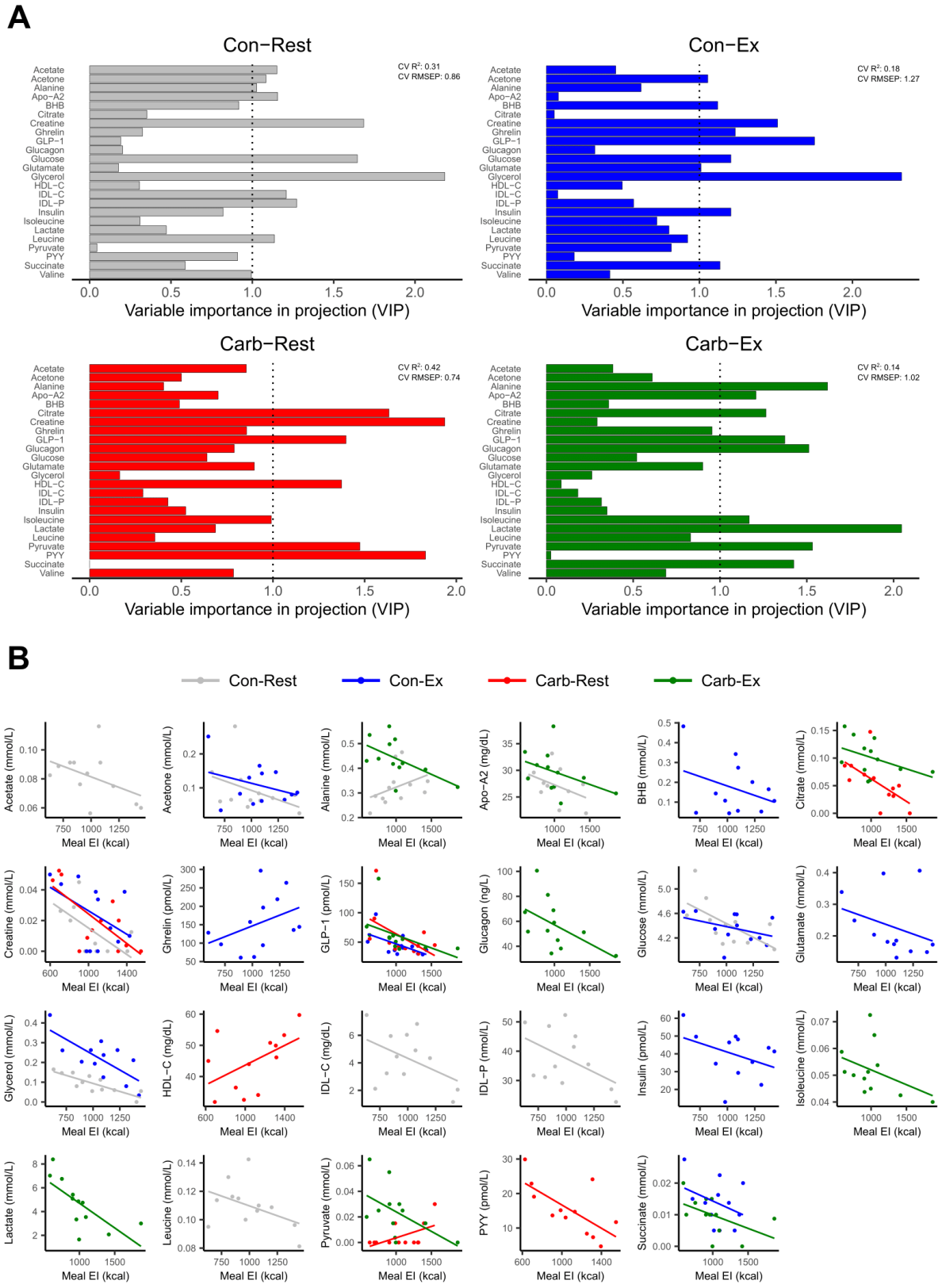


Figure 4.8: Prediction of ad libitum energy intake from preceding metabolic environment. (A) Variable importance in projection scores from partial least squares regression models for each study intervention. Only outcomes measured with a significant main effect of carbohydrate and/or exercise, and/or a significant interaction effect were included in the analyses. (B) Simple univariable linear regression plots for outcomes with a VIP score >1. Root mean square error of prediction (RMSEP) and explained variance (R^2) calculated via leave-one-out cross-validation (CV). EI, energy intake.

dietary carbohydrate and exercise generate independent or interactive effects on gastrointestinal and pancreatic hormones associated with appetite regulation. Utilising targeted and untargeted metabolomic analyses, we also demonstrate that dietary carbohydrate and exercise generate distinct metabolic phenotypes, leading to the identification of novel putative mediators of exercise-induced changes in appetite and energy intake.

GLP-1, PYY, and ghrelin have well-recognised roles as key regulators of appetite and energy intake[45]. Our study reveals that dietary carbohydrate and exercise independently increase GLP-1 and PYY levels, and decrease ghrelin concentrations. Consequently, both carbohydrate and exercise create a hormonal milieu conducive to appetite and energy intake suppression. Despite the pattern of response for gastrointestinal hormones across study conditions being reflected in subjective appetite responses, this was not observed for meal energy intake, suggesting other factors may contribute to the observed between-condition differences in energy intake.

In accord with previous research[46], exercise without carbohydrate ingestion (which can also be regarded as 'fasted exercise') resulted in the lowest acute energy balance. The long-term implications of this finding are nevertheless unclear due to the inherent trade-off between internal and ecological validity. For example, the time at which participants ate was fixed and therefore any effect of eating latency (possibly as the result of gastrointestinal hormone modulation) on subsequent energy intake was unknown. Further work is thus needed to investigate if the lower acute energy balance when exercise is performed in the fasted state translates into greater weight loss with fasted exercise training.

We also provide, for the first time, extensive characterisation of the acute lipoprotein response to exercise, showing a transient increase in IDL-P, IDL-C, and Apo-A2 concentrations following acute exercise. Although chronic changes in fasting levels of these lipoprotein parameters are associated with modified cardiovascular disease risk[47,48], the increase observed with acute exercise is likely a physiological response to the increased energy demands imposed by exercise, and thus represents an increase in energy mobilisation. Furthermore, the characterisation of the acute lipoprotein response to exercise may also facilitate the understanding of how chronic exercise modifies lipoprotein profiles and associated cardiovascular disease risk.

Exercise is often accompanied by a temporary suppression of appetite commonly referred to as exercise-induced anorexia[49]. Correlation network analyses resulted in the identification of key relationships between the plasma metabolome and exercise-induced appetite suppression. GLP-1, lactate, and acetate all displayed strong negative correlations with

appetite in both exercise interventions irrespective of carbohydrate intake. The relationships between GLP-1 and lactate and exercise-induced appetite suppression are expected and have been reported previously[5,50]. However, a relationship between appetite and acetate during exercise has not been previously identified. The intestine is the primary site of acetate production in the fasting and postprandial state[51], whereas skeletal muscle assumes this responsibility during exercise[52]. Despite limited human evidence, exogenous administration of acetate has been shown to suppress appetite in rodent models, acting via a central homeostatic mechanism[53]. Indeed, increasing circulating acetate concentrations through intravenous infusions to levels similar to that achieved during exercise has been shown to increase GLP-1 concentrations[54], suggesting that acetate may regulate exercise-induced appetite suppression by direct and indirect mechanisms.

Appetite and energy intake are related but distinct constructs. Therefore, components of the plasma metabolome that were implicated in appetite suppression could not be assumed to be implicated in energy intake suppression. Consequently, we used partial least squares regression analyses in order to identify potential metabolic predictors of energy intake. GLP-1 and succinate were the only two components of the plasma metabolome to be identified as important predictors of energy intake in both exercise study interventions. Again, the identification of GLP-1 is unsurprising, and highlights the importance of this hormone in the relationship between dietary carbohydrate, exercise, appetite and energy intake. Recent studies investigating GLP-1 receptor agonists and exercise training have reported greater weight-loss when using both interventions concurrently versus either intervention alone[55]. This suggests that therapeutics based on mediators of exercise-induced changes in appetite and energy intake may be highly efficacious with respect to weight-loss, particularly when employed as a co-intervention alongside exercise training. A possible role for circulating succinate has, however, not been previously reported. Pre-clinical evidence indicates that succinate administration can decrease energy intake via an upregulation of intestinal gluconeogenesis[56], and may also play a key role in skeletal muscle remodelling following exercise[57].

Like acetate, lactate and succinate are produced in substantial amounts by contracting skeletal muscle during exercise[57,58], suggesting that muscle-derived metabolites may be key regulators of energy intake in the acute period following exercise. Despite participants exercising at the same relative intensity, our data also highlight notable inter-individual differences in acetate, lactate, and succinate responses across exercise interventions (Figure 4.9A). Furthermore, acetate and lactate responses exhibited strong positive correlations between exercise interventions (Figure 4.9B), suggesting that participants truly vary in their

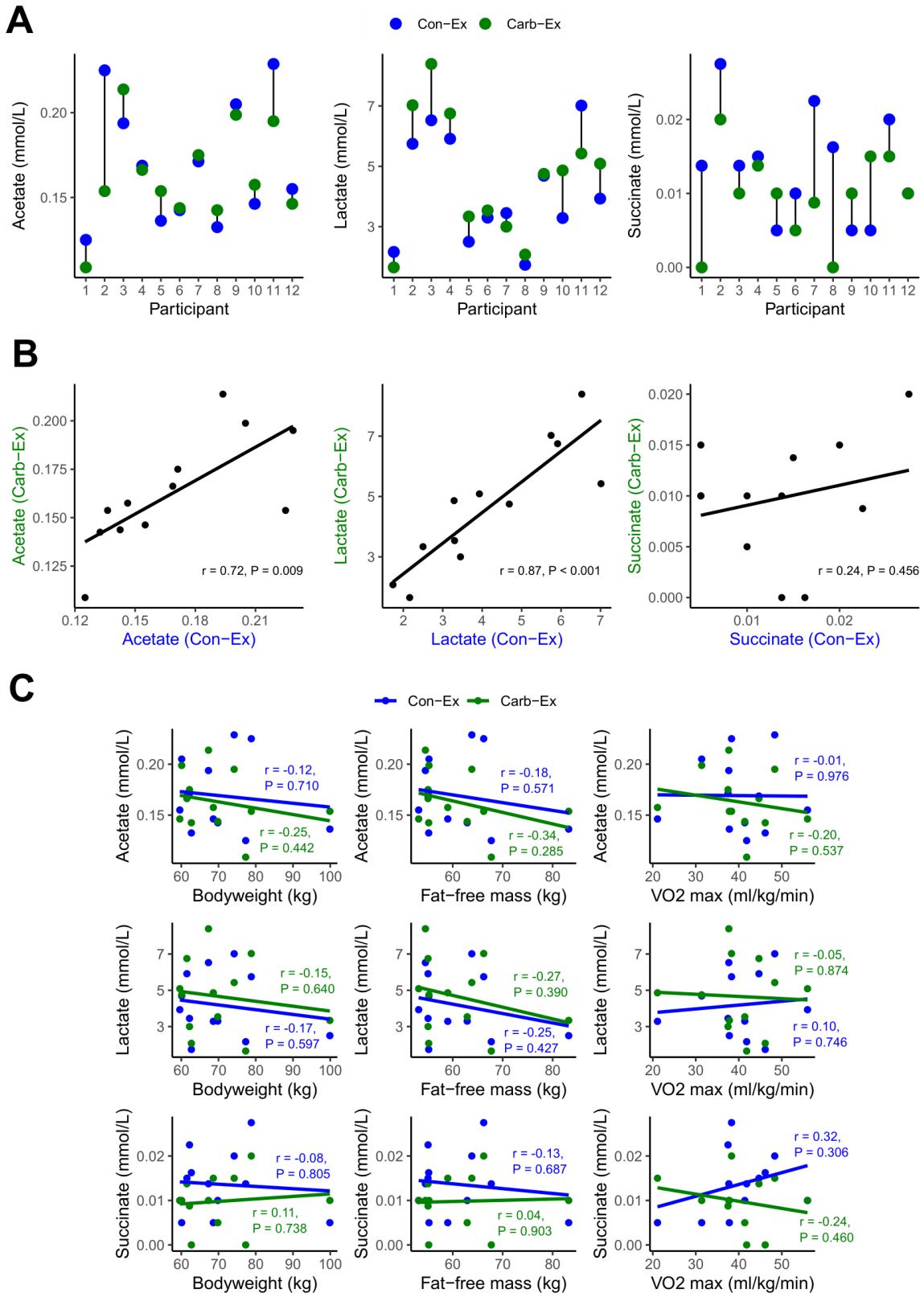


Figure 4.9: Inter-individual variation in muscle-derived metabolite responses during exercise interventions and their relationship with anthropometric and physiological characteristics. (A) Time-averaged AUC data from each participant for both exercise interventions. Length of black line represents difference between exercise interventions. (B) Correlation between Con-Ex and Carb-Ex time-averaged AUC data. (C) Correlation between muscle-derived metabolites (acetate, lactate, succinate) and anthropometric (bodyweight, fat-free mass) and physiological characteristics ($\dot{V}O_2$ max). r, Pearson's correlation coefficient.

capacity to produce muscle-derived metabolites with exercise. It has been recently demonstrated that appetite and energy intake responses to exercise also show substantial inter-individual variation[3,4]. Differences in the release of acetate, lactate, and succinate may therefore contribute to the variation in post-exercise appetite and energy intake responses, and consequently be a key therapeutic target for interventions aiming to augment exercise-induced weight loss. However, acetate, lactate, and succinate concentrations during exercise interventions were not related to bodyweight, fat-free mass, or $\dot{V}O_2$ max (Figure 4.9C), suggesting that increasing skeletal muscle mass or aerobic fitness is unlikely to increase the production of these metabolites. Alternative treatments (e.g. oral ingestion) may therefore be required if these metabolites are found to possess anorexigenic properties.

Previous chapters had identified glucagon as a potential mediator of exercise-induced changes in appetite and energy intake (Chapters 2 and 3). However, the results of the present study suggest that the increase in systemic glucagon concentrations that occur in response to acute exercise are not responsible for the concurrent suppression of appetite, or any changes in *ad libitum* energy intake in the immediate post-exercise period. Future work should look to confirm our findings before glucagon's role in exercise-induced changes in appetite and energy intake is dismissed.

The findings of our study must, however, be interpreted in the context of its limitations. This study only measured energy intake, and thus energy balance, in the immediate period following exercise completion. Therefore, any compensatory responses that may occur beyond this period would not be observed, and thus our findings cannot necessarily be applied to exercise training studies. Targeted metabolomic data was only available for select metabolites and thus the response of some metabolites (e.g. isobutyrate) that showed differential responses across study interventions in the untargeted data were not quantifiable. Our analysis may have therefore missed key metabolites involved in exercise induced changes in appetite and energy intake. The cohort used in the present study only included young lean males; the translation of our findings to other populations (such as individuals with obesity) cannot be assumed. For example, we observed increases in glucagon concentrations following carbohydrate ingestion, a response that may be only present in this demographic, and not in individuals with obesity and associated metabolic diseases[59]. Furthermore, the identification of metabolites involved in exercise-induced appetite and energy intake were not primary outcomes of our study, and thus out analyses and findings must be considered exploratory rather than confirmatory. Future investigations should attempt to manipulate succinate and acetate concentrations in the context of exercise, via the ingestion of oral agents that raise or lower their concentrations, to further elucidate their role in energy intake around exercise. Lastly, the carbohydrate and exercise dose selected for this study were chosen in

order to maximise gastrointestinal hormone response, and may therefore not reveal synergisms between carbohydrate and exercise that can only be observed at sub-maximal doses.

In conclusion, we show that dietary carbohydrate and exercise generate independent and interactive effects on various components of the plasma metabolome, resulting in the generation of distinct metabolic phenotypes. We also identify plasma acetate and succinate as novel putative regulators of exercise-induced appetite and energy intake suppression, but find no evidence to support the putative role of glucagon in exercise-induced changes in appetite and energy intake. Ultimately, the findings of our study may lead to the development of personalised exercise protocols that produce maximal generation of these metabolites, or oral agents that mimic this metabolic response.

4.5 References

1. Miller, W., Koceja, D. & Hamilton, E. A meta-analysis of the past 25 years of weight loss research using diet, exercise or diet plus exercise intervention. *Int. J. Obes.* **21**, 941–947 (1997).

2. Martin, C. K. *et al.* Effect of different doses of supervised exercise on food intake, metabolism, and non-exercise physical activity: The E-MECHANIC randomized controlled trial. *Am. J. Clin. Nutr.* **110**, 583–592 (2019).

3. Hopkins, M., Blundell, J. E. & King, N. A. Individual variability in compensatory eating following acute exercise in overweight and obese women. *Br. J. Sports Med.* **48**, 1472–1476 (2014).

4. Goltz, F. R. *et al.* Interindividual Responses of Appetite to Acute Exercise: A Replicated Crossover Study. *Med. Sci. Sports Exerc.* **50**, 758–768 (2018).

5. Martins, C., Morgan, L. M., Bloom, S. R. & Robertson, M. D. Effects of exercise on gut peptides, energy intake and appetite. *J. Endocrinol.* **193**, 251–258 (2007).

6. Contrepois, K. *et al.* Molecular Choreography of Acute Exercise. *Cell* **181**, 1112-1130.e16 (2020).

7. Vieira, A. F., Costa, R. R., Macedo, R. C. O., Coconcelli, L. & Kruel, L. F. M. Effects of aerobic exercise performed in fasted v. fed state on fat and carbohydrate metabolism in adults: a systematic review and meta-analysis. *Br. J. Nutr.* **116**, 1153–1164 (2016).

8. Frampton, J. *et al.* The Effect of a Single Bout of Continuous Aerobic Exercise on Glucose, Insulin and Glucagon Concentrations Compared to Resting Conditions in

Healthy Adults: A Systematic Review, Meta-Analysis and Meta-Regression. *Sport. Med.* (2021).

9. Edinburgh, R. M., Koumanov, F. & Gonzalez, J. T. Impact of pre-exercise feeding status on metabolic adaptations to endurance-type exercise training. *J. Physiol.* JP280748 (2021).

10. Hargreaves, M., Hawley, J. A. & Jeukendrup, A. Pre-exercise carbohydrate and fat ingestion: Effects on metabolism and performance. *J. Sports Sci.* **22**, 31–38 (2004).

11. Karhunen, L. J., Juvonen, K. R., Huotari, A., Purhonen, A. K. & Herzig, K. H. Effect of protein, fat, carbohydrate and fibre on gastrointestinal peptide release in humans. *Regul. Pept.* **149**, 70–78 (2008).

12. Ho, J. E. *et al.* Metabolite Profiles During Oral Glucose Challenge. *Diabetes* **62**, 2689–2698 (2013).

13. Dye, L. & Blundell, J. E. Menstrual cycle and appetite control: implications for weight regulation. *Hum. Reprod.* **12**, 1142–1151 (1997).

14. Brennan, I. M. *et al.* Effects of the phases of the menstrual cycle on gastric emptying, glycemia, plasma GLP-1 and insulin, and energy intake in healthy lean women. *Am. J. Physiol. Liver Physiol.* **297**, G602–G610 (2009).

15. Oosthuyse, T. & Bosch, A. N. The Effect of the Menstrual Cycle on Exercise Metabolism. *Sport. Med.* **40**, 207–227 (2010).

16. Meek, C. L., Lewis, H. B., Burling, K., Reimann, F. & Gribble, F. Expected values for gastrointestinal and pancreatic hormone concentrations in healthy volunteers in the fasting and postprandial state. *Ann. Clin. Biochem. Int. J. Lab. Med.* **58**, 108–116 (2021).

17. Ueda, S., Yoshikawa, T., Katsura, Y., Usui, T. & Fujimoto, S. Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. *J. Endocrinol.* **203**, 357–364 (2009).

18. Gonzalez, J. T., Veasey, R. C., Rumbold, P. L. S. & Stevenson, E. J. Breakfast and exercise contingently affect postprandial metabolism and energy balance in physically active males. *Br. J. Nutr.* **110**, 721–732 (2013).

19. Bachman, J. L., Deitrick, R. W. & Hillman, A. R. Exercising in the Fasted State Reduced 24-Hour Energy Intake in Active Male Adults. *J. Nutr. Metab.* **2016**, 1–7 (2016).

20. Edinburgh, R. M. *et al.* Skipping Breakfast Before Exercise Creates a More Negative 24-hour Energy Balance: A Randomized Controlled Trial in Healthy Physically Active Young Men. *J. Nutr.* **149**, 1326–1334 (2019).

21. Steinert, R. E., Frey, F., Tpfer, A., Drewe, J. & Beglinger, C. Effects of carbohydrate sugars and artificial sweeteners on appetite and the secretion of gastrointestinal satiety peptides. *Br. J. Nutr.* **105**, 1320–1328 (2011).

22. Adrian, T. E. *et al.* Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology* **89**, 1070–1077 (1985).

23. Kreymann, B., Ghatei, M. A., Williams, G. & Bloom, S. R. Glucagon-like peptide-1 7-36: A physiological incretin in man. *Lancet* **330**, 1300–1304 (1987).

24. Frayn, K. N. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J. Appl. Physiol.* **55**, 628–634 (1983).

25. Weir, J. B. de V. New methods for calculating metabolic rate with special reference to protein metabolism. *J. Physiol.* **109**, 1–9 (1949).

26. Dona, A. C. *et al.* Precision High-Throughput Proton NMR Spectroscopy of Human Urine, Serum, and Plasma for Large-Scale Metabolic Phenotyping. *Anal. Chem.* **86**, 9887–9894 (2014).

27. Jiménez, B. *et al.* Quantitative Lipoprotein Subclass and Low Molecular Weight Metabolite Analysis in Human Serum and Plasma by 1 H NMR Spectroscopy in a Multilaboratory Trial. *Anal. Chem.* **90**, 11962–11971 (2018).

28. Koller, M. robustlmm : An R Package for Robust Estimation of Linear Mixed-Effects Models. *J. Stat. Softw.* **75**, (2016).

29. Kuznetsova, A., Brockhoff, P. B. & Christensen, R. H. B. lmerTest Package: Tests in Linear Mixed Effects Models. *J. Stat. Softw.* **82**, (2017).

30. Lenth, R. V. emmeans: Estimated Marginal Means, aka Least-Squares Means. *R Packag. version 1.6.1.* (2021).

31. Slinker, B. K. The Statistics of Synergism. *J. Mol. Cell. Cardiol.* **30**, 723–731 (1998).

32. Dieterle, F., Ross, A., Schlotterbeck, G. & Senn, H. Probabilistic Quotient Normalization as Robust Method to Account for Dilution of Complex Biological Mixtures. Application in 1 H NMR Metabonomics. *Anal. Chem.* **78**, 4281–4290 (2006).

33. Posma, J. M. *et al.* Optimized Phenotypic Biomarker Discovery and Confounder Elimination via Covariate-Adjusted Projection to Latent Structures from Metabolic Spectroscopy Data. *J. Proteome Res.* **17**, 1586–1595 (2018).

34. Posma, J. M. *et al.* Subset Optimization by Reference Matching (STORM): An Optimized Statistical Approach for Recovery of Metabolic Biomarker Structural Information from 1 H NMR Spectra of Biofluids. *Anal. Chem.* **84**, 10694–10701 (2012).

35. Bakdash, J. Z. & Marusich, L. R. Repeated Measures Correlation. *Front. Psychol.* **8**, 1–13 (2017).

36. Benjamini, Y., Krieger, A. M. & Yekutieli, D. Adaptive linear step-up procedures that control the false discovery rate. *Biometrika* **93**, 491–507 (2006).

37. Gu, Z., Gu, L., Eils, R., Schlesner, M. & Brors, B. circlize implements and enhances circular visualization in R. *Bioinformatics* **30**, 2811–2812 (2014).

38. Campbell, M. J. & Swinscow, T. D. V. Correlation and regression. in *Statistics at Square One* (BMJ books, 1997).

39. Rohart, F., Gautier, B., Singh, A. & Lê Cao, K.-A. mixOmics: An R package for 'omics feature selection and multiple data integration. *PLOS Comput. Biol.* **13**, e1005752 (2017).

40. Julious, S. A. Sample size of 12 per group rule of thumb for a pilot study. *Pharm. Stat.* **4**, 287–291 (2005).

41. Wyatt, P. *et al.* Postprandial glycaemic dips predict appetite and energy intake in healthy individuals. *Nat. Metab.* **3**, 523–529 (2021).

42. Holt, G. M. *et al.* Systematic literature review shows that appetite rating does not predict energy intake. *Crit. Rev. Food Sci. Nutr.* **57**, 3577–3582 (2017).

43. King, J. A., Wasse, L. K. & Stensel, D. J. Acute exercise increases feeding latency in healthy normal weight young males but does not alter energy intake. *Appetite* **61**, 45–51 (2013).

44. Kuhn, M. & Johnson, K. *Applied Predictive Modeling*. *Applied Predictive Modeling* (Springer New York, 2013).

45. Steinert, R. E. *et al.* Ghrelin, CCK, GLP-1, and PYY(3–36): Secretory Controls and Physiological Roles in Eating and Glycemia in Health, Obesity, and After RYGB. *Physiol. Rev.* **97**, 411–463 (2017).

46. Frampton, J., Edinburgh, R. M., Ogden, H. B., Gonzalez, J. T. & Chambers, E. S. The acute effect of fasted exercise on energy intake , energy expenditure , subjective hunger and gastrointestinal hormone release compared to fed exercise in healthy individuals : a systematic review and network meta-analysis. *Int. J. Obes.* (2021).

47. Hodis, H. N. *et al.* Intermediate-Density Lipoproteins and Progression of Carotid Arterial Wall Intima-Media Thickness. *Circulation* **95**, 2022–2026 (1997).

48. Birjmohun, R. S. *et al.* Apolipoprotein A-II Is Inversely Associated With Risk of Future Coronary Artery Disease. *Circulation* **116**, 2029–2035 (2007).

49. King, N. A., Burley, V. J. & Blundell, J. E. Exercise-induced suppression of appetite: effects on food intake and implications for energy balance. *Eur. J. Clin. Nutr.* **48**, 715–24 (1994).

50. Vanderheyden, L. W., McKie, G. L., Howe, G. J. & Hazell, T. J. Greater lactate accumulation following an acute bout of high-intensity exercise in males suppresses acylated ghrelin and appetite postexercise. *J. Appl. Physiol.* **128**, 1321–1328 (2020).

51. Kirschner, S. K., ten Have, G. A. M., Engelen, M. P. K. J. & Deutz, N. E. P. Transorgan short-chain fatty acid fluxes in the fasted and postprandial state in the pig. *Am. J. Physiol. Metab.* **321**, E665–E673 (2021).

52. Van Hall, G., Sacchetti, M. & Rådegran, G. Whole body and leg acetate kinetics at rest, during exercise and recovery in humans. *J. Physiol.* **542**, 263–272 (2002).

53. Frost, G. *et al.* The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat. Commun.* **5**, 1–11 (2014).

54. Freeland, K. R. & Wolever, T. M. S. Acute effects of intravenous and rectal acetate on glucagon-like peptide-1, peptide YY, ghrelin, adiponectin and tumour necrosis factor-α. *Br. J. Nutr.* **103**, 460–466 (2010).

55. Lundgren, J. R. *et al.* Healthy Weight Loss Maintenance with Exercise, Liraglutide, or Both Combined. *N. Engl. J. Med.* **384**, 1719–1730 (2021).

56. Wang, K. *et al.* Parabacteroides distasonis Alleviates Obesity and Metabolic Dysfunctions via Production of Succinate and Secondary Bile Acids. *Cell Rep.* **26**, 222-235.e5 (2019).

57. Reddy, A. *et al.* pH-Gated Succinate Secretion Regulates Muscle Remodeling in Response to Exercise. *Cell* **183**, 62-75.e17 (2020).

58. Juel, C. & Halestrap, A. P. Lactate transport in skeletal muscle — role and regulation

of the monocarboxylate transporter. *J. Physiol.* **517**, 633–642 (1999).

59. Wagner, R. *et al.* Nonsuppressed Glucagon After Glucose Challenge as a Potential Predictor for Glucose Tolerance. *Diabetes* **66**, 1373–1379 (2017).

Chapter 5: General discussion (digestible carbohydrate and gastroenteropancreactic hormone release during exercise)

Exercise often produces modest long-term weight loss, especially when compared to dietary energy restriction[1]. The comparatively poor efficacy of exercise to induce weight loss is largely attributed to compensatory eating behaviours, rather than changes in energy expenditure[2]. Understanding the interaction between diet and exercise may therefore help to improve the efficacy of exercise as a weight loss tool.

A single bout of exercise is typically accompanied by a temporary suppression of appetite referred to as exercise-induced anorexia[3]. Acute exercise also modulates the release of gastrointestinal hormones involved in appetite-regulation, such as GLP-1, PYY, and acyl-ghrelin[4]. Consequently, these hormones are believed to be key regulators of exercise-induced anorexia[5]. Exercise can also induce changes in the pancreatic hormones insulin and glucagon[6], as well as induce large shifts in the plasma metabolome[7]; both of which have been implicated in appetite regulation[8,9]. However, the contribution of hormones involved in glucose homeostasis and the plasma metabolome in exercise-induced anorexia is largely unknown.

Humans typically spend the majority of their waking period in the postprandial state[10], in which dietary (digestible) carbohydrate is the primary energy-contributing macronutrient for most western societies[11]. Exercise is therefore commonly performed in the postprandial state. Indeed, individuals who regularly perform exercise also commonly ingest a source of digestible carbohydrate prior to exercise[12]. Digestible carbohydrate can modulate the concentrations of acyl-ghrelin, GLP-1, and PYY in the systemic circulation, as well as the hormones involved in glucose homeostasis and the plasma metabolome[13,14]. Therefore, the combination of dietary carbohydrate and exercise may produce interactive effects with regards to their effects on these hormones and the plasma metabolome, which could have important implications for appetite regulation in the immediate period following exercise completion.

The results from chapter 2 characterised the acute effect of exercise on glucose, insulin, glucagon concentrations via a systematic review and meta-analysis. These results showed that acute exercise decreased glucose and insulin in the fed state, and increased glucagon irrespective of metabolic state. As higher concentrations of glucose, insulin, and glucagon would be expected to be associated with suppressed appetite, these results suggested a potential role of glucagon in exercise-induced anorexia.

The findings from chapter 2 were followed up in chapter 3, again using a systematic review and meta-analysis, to investigate the effect of acute glucagon administration on appetite and

energy intake in humans. Despite a consistent effect being observed in pre-clinical models[15], the available evidence investigating the effect of acute glucagon administration on appetite and energy intake in humans was inconsistent, and therefore the contribution of raised systemic glucagon concentrations to exercise-induced anorexia remains unclear.

In chapter 4, the interactive effect of dietary carbohydrate and exercise on gastrointestinal and pancreatic hormones, the plasma metabolome, and its implications for appetite and energy intake were explored. Carbohydrate and exercise were found to independently and interactively modulate the hormonal milieu and plasma metabolome, resulting in the generation of distinct metabolic phenotypes. However, glucagon was not strongly associated with exercise-induced changes in appetite nor an important predictor of post-exercise energy intake. Instead GLP-1, acetate, lactate, and succinate were identified as putative mediators of exercise-induced suppression of appetite and energy intake, which could ultimately be targeted in the development of weight lost therapeutics.

Nevertheless, the studies conducted in chapters 2, 3, and 4 were all limited by their investigation of acute effects. The results observed in these studies may therefore not translate to long-term effects when exercise is performed chronically (e.g. multiple exercise sessions per week, for multiple weeks). Large-scale changes to the hormonal milieu and plasma metabolome in response to exercise are unlikely to occur during chronic exercise interventions. However, exercise training studies show a possible augmentation of post-exercise release of appetite-suppressing hormones[16], which may extend to other circulating factors. Future work should therefore explore the potential appetite regulating properties of acetate, lactate, and succinate, utilising pre-clinical and clinical investigations, while also looking into how the post-exercise response of these metabolites changes during chronic exercise interventions and if these are related to individual differences in appetite regulation and body weight. Despite no apparent role in exercise-induced changes in appetite and energy intake, further investigation is also needed in order to clarify the role of glucagon in appetite regulation in humans. Ideally, this work would use both physiological and supraphysiological doses, thereby permitting the identification of effects that are only observed at physiological and supraphysiological glucagon concentrations.

In summary, acute exercise is a potent stimulator of glucagon release into this systemic circulation, irrespective of whether exercise is performed in the fed or fasted state. The available evidence indicates that glucagon is however unlikely to play a role in exercise-induced suppression of appetite and energy intake, with its anorexic potential when administered pharmacologically remaining unclear. Dietary carbohydrate and exercise exhibit independent and antagonistic effects of multiple components of the plasma metabolome, with

changes in GLP-1, acetate, lactate, and succinate concentrations associated with changes in exercise induced suppression of appetite and/or energy intake. Future work should clarify the effect of glucagon in appetite regulation and energy balance in humans, and explore the role of acetate and succinate in exercise-induced changes in appetite and energy intake.

5.1 References

1. Miller, W., Koceja, D. & Hamilton, E. A meta-analysis of the past 25 years of weight loss research using diet, exercise or diet plus exercise intervention. *Int. J. Obes.* **21**, 941–947 (1997).

2. Martin, C. K. *et al.* Effect of different doses of supervised exercise on food intake, metabolism, and non-exercise physical activity: The E-MECHANIC randomized controlled trial. *Am. J. Clin. Nutr.* **110**, 583–592 (2019).

3. King, N. A., Burley, V. J. & Blundell, J. E. Exercise-induced suppression of appetite: effects on food intake and implications for energy balance. *Eur. J. Clin. Nutr.* **48**, 715–24 (1994).

4. Schubert, M. M., Sabapathy, S., Leveritt, M. & Desbrow, B. Acute Exercise and Hormones Related to Appetite Regulation: A Meta-Analysis. *Sport. Med.* **44**, 387–403 (2014).

5. Martins, C., Morgan, L. M., Bloom, S. R. & Robertson, M. D. Effects of exercise on gut peptides, energy intake and appetite. *J. Endocrinol.* **193**, 251–258 (2007).

6. Ueda, S. *et al.* Changes in gut hormone levels and negative energy balance during aerobic exercise in obese young males. *J. Endocrinol.* **201**, 151–159 (2009).

7. Schranner, D., Kastenmüller, G., Schönfelder, M., Römisch-Margl, W. & Wackerhage, H. Metabolite Concentration Changes in Humans After a Bout of Exercise: a Systematic Review of Exercise Metabolomics Studies. *Sport. Med. - Open* **6**, 11 (2020).

8. Geary, N. Glucagon and the Control of Appetite. in *Handbook of Experimental Pharmacology* (ed. Lefebvre, P.) vol. 123/III 223–238 (Springer-Verlag, 1996).

9. Austin, J. & Marks, D. Hormonal Regulators of Appetite. *Int. J. Pediatr. Endocrinol.* **2009**, 1–9 (2009).

10. Schrauwen-Hinderling, V. B. & Carpentier, A. C. Molecular imaging of postprandial metabolism. *J. Appl. Physiol.* **124**, 504–511 (2018).

11. Dehghan, M. *et al.* Associations of fats and carbohydrate intake with cardiovascular

disease and mortality in 18 countries from five continents (PURE): a prospective cohort study. *Lancet* **390**, 2050–2062 (2017).

12. Rothschild, J. A., Kilding, A. E. & Plews, D. J. Pre-Exercise Nutrition Habits and Beliefs of Endurance Athletes Vary by Sex, Competitive Level, and Diet. *J. Am. Coll. Nutr.* **0**, 1–12 (2020).

13. Karhunen, L. J., Juvonen, K. R., Huotari, A., Purhonen, A. K. & Herzig, K. H. Effect of protein, fat, carbohydrate and fibre on gastrointestinal peptide release in humans. *Regul. Pept.* **149**, 70–78 (2008).

14. Ho, J. E. *et al.* Metabolite Profiles During Oral Glucose Challenge. *Diabetes* **62**, 2689–2698 (2013).

15. Geary, N., Le Sauter, J. & Noh, U. Glucagon acts in the liver to control spontaneous meal size in rats. *Am. J. Physiol. Integr. Comp. Physiol.* **264**, R116–R122 (1993).

16. Ueda, S. *et al.* Effects of exercise training on gut hormone levels after a single bout of exercise in middle-aged Japanese women. *Springerplus* **2**, 83 (2013).

Chapter 6: Non-digestible carbohydrate, short-chain fatty acid production and skeletal muscle metabolism

6.1 Non-digestible carbohydrate and the gut microbiota

The gut microbiota constitutes the community of microbes present within the gastrointestinal tract. This microbial ecosystem plays an important role in host health, facilitating the metabolism of dietary nutrients, synthesizing micronutrients, and cofactors, modulating the mucosal immune system and influencing energy balance[1].

This is achieved in part by the ability of the gut microbiota to produce bioactive metabolites from ingested dietary material[2], for example, the production of short-chain fatty acids (SCFAs) from non-digestible carbohydrate sources such as dietary fibre[3]. Pre-clinical and clinical data demonstrate that SCFAs can modify host metabolism and are therefore hypothesised to play an important role in gut microbiota-host crosstalk[4].

Growing evidence indicates that changes in the gut microbiota away from a healthy phenotype, e.g. a reduction in taxa diversity and/or microbial gene richness, may play a role in the pathogenesis of cardiometabolic diseases, such as type 2 diabetes[5]. Decreased SCFA production is hypothesised to mediate - at least in part - this relationship between gut microbiota dysbiosis and cardiometabolic disease[5]. Understanding how SCFAs influence human metabolism may therefore be useful in the development of therapeutics aimed at the prevention, management and treatment of these diseases.

6.2 Short-chain fatty acids

The three primary SCFAs are acetate, propionate, and butyrate. Acetate, propionate and butyrate are produced in the approximate ratio 3:1:1 following the fermentation of non-digestible carbohydrate by resident gut microbes[3]. SCFAs are produced primarily in the colon, with concentrations showing little difference between the ascending (123 mmol/kg of luminal content), transverse (117 mmol/kg), descending (80 mmol/kg) and sigmoid colon (100 mmol/kg)[6]. In contrast, SCFA production in the ileum is relatively low (13 mmol/kg)[6]. Daily SCFA production is estimated to be ~100 mmol per 10g of dietary fibre consumed, and therefore individuals consuming a high-fibre diet can produce approximately 400-800 mmol of SCFAs per day[6].

Following their generation in the gut lumen, acetate, propionate and butyrate are almost entirely absorbed by colonocytes (only 5–15 mmol per day is excreted in the faeces) and subsequently metabolized or transported via the portal vein to the liver[7]. Interestingly, SCFAs produced in the distal colon may bypass hepatic metabolism and directly enter the systemic circulation via the internal iliac vein[8]. Butyrate is a key energy source for colonocytes, and

consequently concentrations in the portal vein, hepatic vein and systemic circulation are relatively low (Table 6.1). In contrast, acetate and propionate concentrations remain largely unaffected by colonocyte metabolism but are subject to substantial hepatic metabolism (Table 6.1). Circulating levels of acetate and propionate are therefore considerably lower relative than portal vein concentrations (Table 6.1).

Table 6.1: Portal (P), hepatic (H), arterial (A), and venous (V) short-chain fatty acid concentrations.

	Acetate (μmol·l^{-1})				**Propionate (μmol·l^{-1})**				**Butyrate (μmol·l^{-1})**			
	P	**H**	**A**	**V**	**P**	**H**	**A**	**V**	**P**	**H**	**A**	**V**
Cummings *et al.*[116]	258	115	-	70	88	21	-	5	29	12	-	4
Peters *et al.* [117]	128	-	-	67	34	-	-	4	18	-	-	U
Bloemen *et al.* [118]	263	220	173	-	30	7	4	-	30	12	8	-
van der Beek *et al.* [119]	182	71	126	-	30	1	2	-	15	1	1	-
Neis *et al.* [120]	41	24	22	-	25	3	1	-	21	3	1	-
$\overline{x}$	**174**	**108**	**107**	**69**	**41**	**8**	**2**	**5**	**23**	**7**	**3**	**4**

Investigations have measured the hepatic uptake and systemic availability of short chain fatty acids by comparing concentrations between the portal (P), hepatic (H), arterial (A) and venous (V) plasma in sudden death victims [116] and patients undergoing abdominal surgery [117–120]. The mean ($\bar{x}$) from the five investigations has been calculated. U, undetectable.

All three SCFAs are available as precursors for lipid or carbohydrate synthesis. Acetate and butyrate enter the tricarboxylic acid cycle as acetyl-CoA and are used as substrates for hepatic de novo lipogenesis. Propionate enters the tricarboxylic acid cycle as succinyl-CoA and is used as a precursor for hepatic gluconeogenesis[9,10]. SCFAs can therefore contribute up to 10% of total energy requirements in humans[11].

In addition to their role as an energy source, acetate, propionate and butyrate may also influence host metabolism via activation of the G-protein coupled receptors GPR41 (also known as free fatty acid receptor 3, FFAR3) and GPR43 (also known as free fatty acid receptor 2, FFAR2). Both these receptors are sensitive to SCFA concentrations but differ in their affinity for each SCFA. Propionate is the most potent agonist of both FFAR2 and FFAR3, with acetate being more active at the FFAR2 (vs FFAR3) and butyrate more active at the FFAR3 (vs FFAR2)[12]. Many of the effects of SCFAs on host metabolism are thought to be mediated by FFAR activation. This includes FFAR-dependent modulation of intracellular kinases (such as protein kinase B) and release of hormones (such as GLP-1, PYY and leptin) that are involved in glucose and lipid metabolism[7].

FFAR2 and FFAR3 are expressed in a wide variety of organs and tissues, including the liver, adipose, brain, gastrointestinal tract and skeletal muscle[13]; suggesting SCFAs possess diverse physiological functions within the human body. Skeletal muscle is arguably the largest organ in the body (~40% of total body mass) and plays a crucial role in whole-body energy metabolism[14]. Despite these characteristics, there is comparatively little research investigating the effect of SCFAs on skeletal muscle metabolism and the underlying mechanisms involved.

6.3 Skeletal muscle

The human body contains over 600 different skeletal muscles[15]. These muscles are highly vascularised, and unlike cardiac muscle, they are innervated by the somatic nervous system permitting voluntary contraction. Skeletal muscle is a highly plastic organ, being able to remodel itself in response to a variety of external stimuli (e.g. resistance exercise)[15]. Furthermore, skeletal muscle possesses an extraordinary regenerative capacity. Skeletal muscle function and structure can be fully restored in a matter of weeks following major tissue damage[15].

6.3.1 Skeletal muscle composition and structure

Skeletal muscle is primarily composed of water (75%) and protein (20%), with inorganic salts, minerals, lipids and carbohydrates making up the remaining 5%[14]. Skeletal muscle is a highly organized organ comprising bundles of myofibers (each bundle is called a skeletal muscle fascicle and can contain up to 150 myofibers[16]). Within each myofiber there are bundles of myofibrils (each myofiber is made of thousands of myofibrils). Each myofibril comprises bundles of myofilaments; thick myofilaments contain the protein myosin, and thin myofilaments contain the proteins actin, troponin, and tropomyosin.

Skeletal muscles, skeletal muscle fascicles and myofibers are each encapsulated by a layer of connective tissue referred to as the epimysium, perimysium, and endomysium, respectively. These connective structures account for up to 15% of skeletal muscle volume and act as a “skeletal muscle skeleton” that helps to regulate its activity[17].

The myofiber is considered as the ‘skeletal muscle cell’ and contains all three classical cell components: a cell membrane (sarcolemma), a cytoplasm (sarcoplasm) and a nucleus (myonucleus). However, unlike many other cells, myofibers are multinucleated and contain between 100-200 myonuclei. Myofibers are consequently considered the largest cell in the human body[17].

Myofibers are typically classified based on the myosin heavy chain (MHC) isoform that is predominantly expressed. In humans, type I myofibers predominantly express the MHC I isoform, type II myofibers predominantly express MHC IIA, and type IIX myofibers

predominantly express MHC IIX[18]. These myofiber classes possess different properties which are summarised in Table 6.2.

Table 6.2: Properties of skeletal muscle fibre types

	Slow-twitch	**Fast-twitch**		
Fibre type	type 1	type 2a	type 2x	type 2b
Speed of fatigue	slow	fast	fast	fast
Speed of contraction	slow	fast	fast	fast
Metabolic type	oxidative	oxidative	glycolytic	glycolytic
Representative myosin	MYH7	MYH2	MYH1	MYH4

Fibre types are presented from slowest to fastest (left to right). Adapted from Talbot and Maves[18].

6.3.2 Skeletal muscle metabolism

Skeletal muscle contraction is an energy intensive activity, requiring large amounts of ATP. Consequently, skeletal muscle plays a major role in energy homeostasis[19].

ATP for skeletal muscle contraction is primarily generated within skeletal muscle by two metabolic pathways: (i) glycolysis and (ii) oxidative phosphorylation[20]. Glycolysis involves the catabolism of glucose into pyruvate, which can then (depending on oxygen availability) be converted to acetyl-CoA and undergo oxidative phosphorylation via the tricarboxylic acid cycle (sufficient oxygen), or it can be converted to lactate (when there is insufficient oxygen)[21]. In contrast, oxidative metabolism involves the catabolism of both lipids and glucose into acetyl-CoA, which can then also enter the tricarboxylic acid cycle and subsequently undergo oxidative phosphorylation[21]. Skeletal muscle contraction in the form of physical activity (including exercise) causes an increased demand in ATP and a subsequent upregulation of these processes[20]. The contribution of glycolytic and oxidative metabolism to ATP generation is dependent on the intensity and duration of the activity; long, lower-intensity activities rely primarily on oxidative metabolism, whereas short, higher-intensity activities rely primarily on glycolytic metabolism[20,22].

Skeletal muscle acts as a major storage depot for glucose, lipids, and amino acids. Glucose is stored as glycogen within skeletal muscle, with skeletal muscle being the largest glycogen store in the human body (~500g)[23]. Skeletal muscle is also considered the primary site of insulin-stimulated glucose uptake[24]. Indeed, skeletal muscle contraction can induce translocation of GLUT4 (the primary glucose transporter in skeletal muscle) and thus increase glucose uptake[25]. Lipids are stored as triglycerides in lipid droplets within skeletal muscle (referred to as intramyocellular lipids)[26]. These lipid droplets can be stored just below the

sarcolemma (subsarcolemmal lipid droplets) and between myofibrils (intermyofibrillar lipid droplets)[27]. Intramyocellular lipid accounts for ~1-4% of total skeletal muscle fibre area depending on adiposity status[28]. Lastly, amino acids are stored as proteins within skeletal muscle, with ~75% of all body proteins stored in skeletal muscle[29]. These proteins can be mobilised via skeletal muscle proteolysis to provide gluconeogenic substrates under conditions of metabolic stress[29].

6.3.3 Skeletal muscle metabolism, insulin resistance and cardiometabolic disease pathogenesis

In healthy individuals, skeletal muscle can efficiently switch between glucose and lipid utilization when exposed to glucose abundance (increased insulin concentrations) and scarcity (decreased insulin concentrations); a characteristic referred to as 'metabolic flexibility'[30]. Conversely, individuals at risk of developing cardiometabolic disease (e.g. type 2 diabetes) display an attenuated capacity to be able to switch between glucose and lipid utilization in response to changes in insulin concentrations i.e. 'metabolic inflexibility'[30]. The degree of metabolic flexibility an individual possesses is therefore related to the degree to which skeletal muscle responds to insulin: metabolically flexible individuals possess a higher level of skeletal muscle insulin sensitivity (low insulin resistance), whereas metabolically inflexible individuals possess a lower level of skeletal muscle insulin sensitivity (high insulin resistance)[30].

Defects in skeletal muscle glucose and lipid metabolism have both been hypothesised to be major causes of skeletal muscle insulin resistance. With regards to glucose metabolism, a decreased rate of skeletal muscle glycogen synthesis - the primary pathway for non-oxidative glucose metabolism - is thought to be a significant contributor to this increase in insulin resistance[31]. This has been primarily attributed to a decrease in the activation of GLUT4[31].

Skeletal muscle insulin resistance is also associated with intramyocellular lipid accumulation. This lipid accumulation is believed to be due to decreased lipid oxidation and/or increased lipid uptake[32]. As mitochondria are the primary site of fatty acid oxidation, impaired mitochondrial function is hypothesised to be a key characteristic of intramyocellular lipid accumulation and thus skeletal muscle insulin resistance[33]. This is supported by observations that mitochondrial content, function, and oxidative capacity are decreased in individuals with insulin resistance, obesity and/or type 2 diabetes[34,35]. Increases in intramyocellular lipid content interfere with intramyocellular insulin signalling following insulin receptor activation, ultimately resulting in a reduction in GLUT4 activity and glucose uptake[31]. Dysregulation of intramyocellular lipid metabolism may therefore be a key feature of skeletal muscle insulin resistance.

Changes in protein metabolism may also be involved in the development of skeletal muscle insulin resistance. Chronically higher rates of protein degradation relative to protein synthesis would cause a reduction in skeletal muscle mass[36]. This would result in a reduction in tissue capable of storing glucose and lipids, thereby exacerbating the changes in metabolism implicated in the pathogenesis of skeletal muscle insulin resistance[37].

Unsurprisingly, skeletal muscle insulin resistance has been implicated in the development of several cardiometabolic diseases, including type 2 diabetes, the metabolic syndrome and non-alcoholic fatty liver disease (NAFLD)[38–40]. The decrease in glucose uptake and glycogen synthesis characteristic of skeletal muscle insulin resistance results in higher circulating levels of glucose (hyperglycaemia) and a consequent shift in glucose-derived energy towards hepatic de novo lipogenesis, promoting dyslipidaemia and NAFLD[38,40]. Therapeutic interventions that decrease intramyocellular lipid accumulation and increase GLUT4 activity may be therefore useful in the prevention and/or treatment of diseases associated with skeletal muscle insulin resistance.

6.4 Short-chain fatty acids and skeletal muscle metabolism

SCFAs have been shown to influence lipid, glucose, and protein metabolism in skeletal muscle tissues both *in vitro* and *in vivo*. This metabolic link between SCFAs and skeletal muscle has driven the development of the concept of a of a gut–muscle axis[41,42] and highlights the potential of SCFAs as a therapeutic agent in the treatment, prevention and management of skeletal muscle insulin resistance and associated comorbidities.

6.4.1 Lipid metabolism

SCFAs modulate several aspects of skeletal muscle lipid metabolism, including lipid uptake, oxidation and storage. *In vitro* studies have demonstrated that fatty acid oxidation increases by approximately 30% after butyrate administration (0.5 mM) in L6 myotubes (rat-derived terminally differentiated skeletal muscle cells)[53]. Similarly, fatty acid uptake increases by ~30% after 0.5 mM acetate treatment in this same cell line[43]. In keeping with the increase in fatty acid oxidation, L6 myotubes incubated with acetate and 0.6 μM palmitic acid (an agent used to induce an insulin-resistant state) for 24–48 hours exhibit a ~15–30% decrease in triglyceride accumulation relative to the level of triglyceride accumulation observed after treatment with palmitic acid alone[43].

Ex vivo, gastrocnemius muscle isolated from C57BL/6J mice supplemented with butyrate (5% (w/w)) after 13 weeks on a high-fat diet shows a 200% increase in fatty acid oxidation, measured as ^{14}C-labelled CO_2 production after administration of ^{14}C-labelled palmitic acid[53]. Complementary decreases in skeletal muscle lipid accumulation have also been reported *in vivo*. In C57BL/6J mice fed a high-fat diet, Hong et al.[54] have reported a decrease in skeletal

muscle triglyceride and cholesterol concentrations after 10 days of alternate-day supplementation with butyrate (oral gavage with 80 mg). Likewise, skeletal muscle triglyceride concentrations decrease in rabbits after 4 days of subcutaneous acetate injections (2 g per kg body weight per day)[55]. Long-term supplementation with butyrate (5% (w/w)) for 10 months also prevents intramuscular lipid accumulation in C57BL/6J mice fed a standard diet[56].

Changes in the skeletal muscle expression of enzymes integral to lipid metabolism tend to support these changes in lipid uptake, oxidation and storage. The expression of lipoprotein lipase, an enzyme that hydrolyses circulating triglycerides and provides free fatty acids to cells, has been reported to increase in rodents and rabbits after SCFA supplementation[54,55], though not all studies have found this result[57]. The expression of hormone-sensitive lipase, an enzyme responsible for the hydrolysis of intracellular fatty acids, appears to also be increased in SCFA-supplemented mice[54,58]. Acetyl-CoA carboxylase, an enzyme whose primary function is malonyl-CoA synthesis and the consequent promotion of fatty acid biosynthesis, has been reported to be downregulated by SCFA administration in C57BL/6J mice and L6 myotubes[43,58]. However, this change has not been observed in C2C12 myotubes (mouse-derived terminally differentiated skeletal muscle cells) 24 hours after SCFA administration[57].

In summary, the available evidence indicates that SCFAs increase fatty acid uptake and oxidation while preventing lipid accumulation in skeletal muscle.

6.4.2 Glucose metabolism

Skeletal muscle glucose uptake appears to be enhanced by SCFA administration. Acetate and propionate increase insulin-independent glucose uptake in L6 and C2C12 myotubes, respectively[43,44]. Furthermore, insulin-stimulated glucose uptake in these myotubes is increased by the coadministration of propionate, an effect partly mediated by the SCFA receptor GPR41[44].

Acetate has also been demonstrated to enhance skeletal muscle glycogen synthesis in various murine models. Acute oral administration of acetate in Sprague-Dawley rats after food deprivation or exhaustive exercise increases the rate of skeletal muscle glycogen synthesis but does not increase glycogen content above resting levels[45–47]. However, in KK-A(y) mice, a model of obesity and type 2 diabetes, feeding of a standard diet supplemented with acetate for 8 weeks increases skeletal muscle glycogen levels tenfold relative to those in mice consuming a standard diet alone[48]. Interestingly, SCFAs do not appear to affect glycogen synthase gene expression, protein expression or enzymatic activity[45,49]. SCFAs might possibly upregulate glycogenesis instead via the inhibition of glycolysis and the consequent accumulation of glucose 6-phosphate[45,47]. Indeed, rats given acetate display a decrease in the

fructose 1,6-bisphosphate/fructose 6-phosphate ratio[45,47] an indicator of phosphofructokinase 1 activity and thus of glycolysis[50].

The enhancement in glucose uptake and glycogen repletion is likely to be facilitated by the effects of SCFAs on the expression of GLUT4, which is the primary glucose transporter protein in skeletal muscle and thus plays a pivotal role in skeletal muscle glucose uptake and metabolism[51]. In OLETF rats (a model of non-insulin-dependent diabetes mellitus) injected with 5.2 mg acetate 5 days per week for 6 months, the skeletal muscle mRNA expression of GLUT4 has been found to be approximately twofold greater than that in controls[52]. In accordance with this finding, Maruta et al.[43] have reported increased GLUT4 mRNA and protein expression in L6 myotubes after acute treatment (10 min) with 0.5 mM acetate. The expression of Krüppel-like factor 15[52] and myocyte-specific enhancer factor 2A[43], two proteins involved in GLUT4 transcription, is also upregulated after acetate administration *in vivo* and i*n vitro*, respectively. However, this stimulatory effect on GLUT4 expression may not be exhibited by all SCFAs, because no changes in GLUT4 gene or protein expression have been reported in L6 myotubes after a 24-hour incubation with 5 mM butyrate[49].

6.4.3 Insulin sensitivity

Measures of whole-body insulin sensitivity improve after SCFA administration in various murine models[53,59]. Given that skeletal muscle is the primary site of insulin-stimulated glucose uptake[60], the observed improvements in whole-body insulin sensitivity may be partly attributable to SCFA-induced changes in skeletal muscle physiology.

Despite the observed effects of SCFAs on GLUT4 expression, few studies have specifically investigated the effects of SCFAs on the skeletal muscle insulin-signalling pathway. Butyrate administration has been shown to mitigate the insulin-resistant state induced by palmitate in L6 myotubes[49], as demonstrated by increased phosphorylation of protein kinase B and mitogen-activated protein kinase (the two major branches of intracellular insulin signalling) and elevated expression of insulin receptor substrate 1[49]. Insulin signalling appears to also be improved by butyrate *in vivo*: C57BL/6J mice fed a high-fat diet exhibit increased phosphorylation of protein kinase B and insulin-receptor substrate 1 when the diet is supplemented with butyrate[53].

These findings, coupled with the previously discussed effects of SCFAs on glucose uptake, GLUT4 expression and intramuscular lipid accumulation, strongly support an insulin-sensitizing role of SCFAs on skeletal muscle.

6.4.4 Protein Metabolism

There are currently no studies that have investigated the direct effects of SCFAs on protein metabolism in skeletal muscle. Nonetheless, changes in skeletal muscle mass and phenotype are ultimately brought about by changes in protein metabolism; for example, increased skeletal muscle accretion is the result of skeletal muscle protein synthesis exceeding muscle protein breakdown[36]. Changes in mass and phenotype can therefore be used to infer changes in protein metabolism within skeletal muscle.

6.4.4.1 Skeletal muscle mass

Several authors have reported data demonstrating the ability of SCFAs to influence skeletal muscle mass *in vivo*[53,56,61,62]. Initial data suggest that chronic supplementation with butyrate preserves skeletal muscle in rodents when incorporated into a high-fat diet at 5% (w/w)[53]. However, this study reported changes in skeletal muscle mass as a percentage of total body mass, a measurement confounded by the large increase in body mass observed in the control rodents. Kondo et al.[61] have detected no differences in absolute skeletal muscle mass or the skeletal muscle mass/body mass ratio in C57BL/6J mice fed a high-fat diet with versus without acetate supplementation. Similarly, Henagan et al.[62] have reported no significant difference in absolute skeletal muscle mass values between butyrate-supplemented mice and control mice on a high-fat diet.

When used to supplement a non-obesogenic diet over a 40-week period, butyrate has been reported to prevent skeletal muscle atrophy during ageing in mice[56]. Changes in skeletal muscle mass in that study are again expressed as a percentage of total body mass, and the values may consequently be confounded by differences in body mass between treatments. Nevertheless, the body mass did not appear to differ between groups, thus suggesting that butyrate may be capable of maintaining skeletal muscle mass during the ageing process. Jørgensen et al.[63] have also reported that infusion of an SCFA mixture containing acetate, butyrate and propionate into growing pigs increases nitrogen retention when the pigs are fed below the energy requirement for maximum weight gain. Similarly, Imoto and Namioka[64] have observed a dose–response relationship between the amount of acetate incorporated into the diet and nitrogen retention in growing pigs. SCFAs might not influence skeletal muscle mass under conditions of energy surplus but instead may be beneficial under conditions of metabolic stress or increased metabolic demand, such as those experienced during ageing, energy restriction or developmental growth. This notion is supported by recent *in vitro* and *in vivo* work by Lahiri et al.[65], which has demonstrated that an SCFA cocktail decreases dexamethasone-induced atrophy in C2C12 myotubes and increases skeletal muscle mass in germ-free mice lacking a gut microbiota.

6.4.4.2 Skeletal muscle phenotype

Growing evidence indicates that SCFA supplementation promotes an oxidative skeletal muscle phenotype (elevated oxidative capacity, mitochondrial content and proportion of type I fibres) in rodents fed a high-fat diet[53,58,62]. This effect has been demonstrated via increased expression of type I myosin heavy chain proteins after both butyrate[53,62] and acetate supplementation[58], and of key enzymes involved in beta oxidation, including carnitine palmitoyltransferase-1b[53,58], cytochrome c oxidase subunit I[53] and cytochrome c oxidase subunit IV[54]. The expression of myoglobin—a marker of oxidative type I fibres—has also been reported to be upregulated in vitro by SCFAs[43] and in vivo[52,53] after SCFA administration. However, other studies have reported no change in skeletal muscle fibres in mice fed a standard diet supplemented with butyrate[54,56]. The reason for this incongruence is unclear but may be related to the study durations and diets used. Hong et al.[54] have used a protocol in which butyrate was incorporated into diets for only 10 days, a time period possibly insufficient to induce a shift in fibre composition. By comparison, studies showing a shift in fibre composition have administered SCFAs for 8–16 weeks in the context of a high-fat diet[53,58,62]. Walsh et al.[56] have supplemented mice with butyrate for up to 26 months and observed an absence of effects on the skeletal muscle phenotype; however, the mice received a standard diet, whereas other studies have used a high-fat diet[53,58,62]. The effects of SCFAs on skeletal muscle fibre composition may therefore be context dependent; that is, SCFAs may preserve an oxidative phenotype in rodents consuming a high-fat diet but may confer no benefit in rodents consuming a standard diet.

6.5 Mechanisms of short-chain fatty acid-induced changes in skeletal muscle metabolism and function

6.5.1 Increased phosphorylation of AMP-activated protein kinase

AMP-activated protein kinase (AMPK) is a fundamental regulator of cellular metabolism that is activated by a decline in intracellular ATP production relative to increases in AMP or ADP generation[66]. After phosphorylation, AMPK promotes catabolic pathways that generate more ATP while simultaneously curbing anabolic processes[66].

Several studies have demonstrated the ability of SCFAs to induce the phosphorylation of AMPK in myotubes[53] and skeletal muscle[52–54,58], probably because of the ability of SCFAs to increase AMP concentrations and the AMP/ATP ratio within skeletal muscle tissue[52,67]. Importantly, AMPK activation can produce a metabolic milieu similar to that generated by SCFA administration. This milieu is characterized by an increase in fatty acid uptake and oxidation, glucose uptake and glycogenesis, and an inhibition of lipogenesis and glycolysis[66]. AMPK phosphorylation may therefore be a key mechanism through which SCFAs exert changes in skeletal muscle metabolism (Figure 6.1).

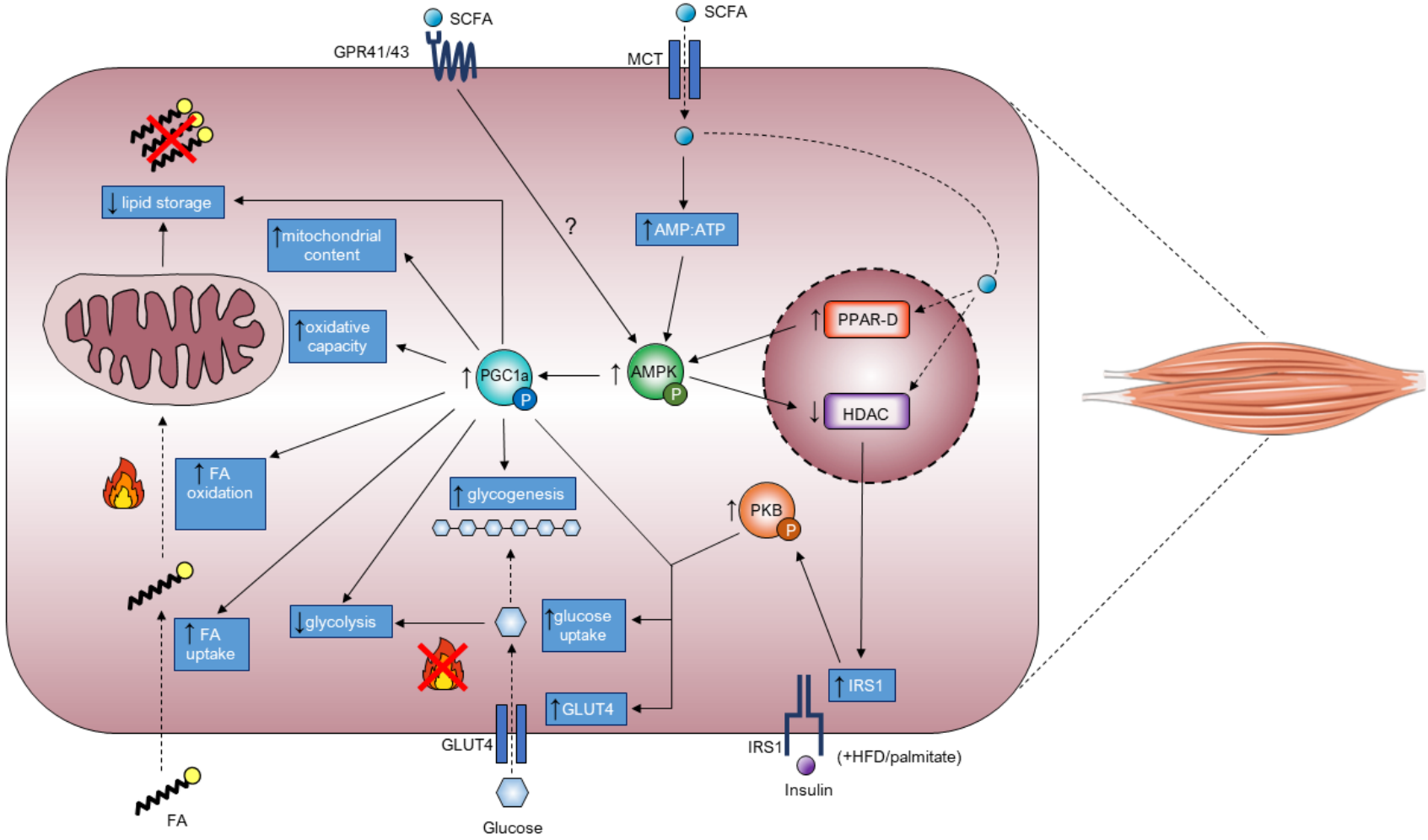


Figure 6.1: The effect of short chain fatty acids on metabolic signalling pathways in skeletal muscle. AMP, adenosine monophosphate; ATP, adenosine triphosphate; AMPK, AMP-activated protein kinase; FA, fatty acid; HDAC, histone deacetylase; HFD, high-fat diet; IRS1, insulin receptor substrate 1 MCT, monocarboxylate transporter; PGC1a, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPAR-D, peroxisome proliferator-activated receptor-delta; SCFA, short-chain fatty acid.

Downstream targets of AMPK in skeletal muscle are also phosphorylated after SCFA administration, including p38 mitogen-activated protein kinases (p38 MAPKs)[53] and peroxisome proliferator–activated receptor gamma coactivator 1α (PGC1α)[43,53,58]. AMPK and p38 MAPK both increase PGC1α phosphorylation in skeletal muscle[68,69], and the AMPK activation of PGC1α may be partly mediated by p38 MAPK25. PGC1α is a master regulator of mitochondrial biogenesis and function[70], and therefore its upregulation may play a crucial role in the promotion of an oxidative skeletal muscle phenotype observed after SCFA administration[71].

6.5.2 Increased expression of peroxisome proliferator-activated receptors

Peroxisome proliferator-activated receptors (PPARs) are a family of ligand-dependent nuclear receptors that act as transcription factors. Three PPAR isotypes have been identified: PPAR-α, PPAR-δ and PPAR-γ. All three isoforms are expressed in skeletal muscle and share structural and functional similarities[72].

Butyrate has been found to increase the expression of PPAR-δ in both L6 myotubes and skeletal muscle in C57BL/6J mice in vivo[53]. PPAR-δ, the most abundant isoform in skeletal muscle, has been implicated as a key regulator of lipid and glucose metabolism, as well as skeletal muscle fibre type[72,73]. Moreover, PPAR-δ activation has recently been shown to prolong running time in mice via the suppression of glycolysis and a consequent delay in hypoglycaemia onset[74]. These changes in metabolism, exercise capacity and skeletal muscle phenotype observed after SCFA administration may therefore be partly mediated by increased expression of PPAR-δ (Figure 6.1). A relationship between PPAR-δ and AMPK may also exist, as evidenced by increased AMPK activity after PPAR-δ agonist treatment, and the dependence of PPAR-δ induced glucose uptake on the presence of AMPK in primary human myotubes[73,75].

The effects of SCFAs on PPAR-α and PPAR-γ are less clear. These receptors are believed to be involved in skeletal muscle lipid metabolism and insulin sensitivity, respectively[76,77]. Henagan et al.[62] have reported a significant increase in skeletal muscle PPAR-γ expression in C57BL/6J mice supplemented with butyrate. However, den Besten et al.[57] have reported no effects of acetate, propionate and butyrate on PPAR-γ expression *in vitro* or *in vivo*. Hong et al.[54] have reported a significant increase in skeletal muscle PPAR-α mRNA expression but no effect on protein expression in mice supplemented with butyrate for 10 days.

6.5.3 Inhibition of histone deacetylases

Histone deacetylases (HDACs) - also known as lysine deacetylases—are a class of enzymes that regulate gene transcription via the removal of acetyl groups from lysine residues on histones (and other proteins), thus closing chromatin DNA to transcription factors[78]. These

activities directly oppose the function of histone acetyltransferases—enzymes that acetylate lysine residues on histones and thus open chromatin DNA to transcription factors[78]. Modulation of HDACs and histone acetyltransferases can therefore influence protein expression via chromatin remodelling.

HDACs have been associated with numerous aspects of skeletal muscle metabolism and function. For example, HDAC1 and HDAC4 promote skeletal muscle atrophy in response to disuse/nutrient deprivation[79] and denervation[80], respectively. HDAC4 and HDAC5 regulate myoblast differentiation[81], and HDAC3 suppresses mitochondrial biogenesis[82]. HDAC4 and HDAC5 may also modulate skeletal muscle glucose and lipid oxidation[83].

The three primary SCFAs—acetate, propionate and butyrate—have been identified as HDAC inhibitors in various tissues and cell lines[84–86]. The HDAC-inhibitory activity of butyrate specifically in skeletal muscle has been confirmed both *in vitro*[49] and *in vivo*[53,54,56]. Gao et al.[53] have reported a 50% decrease in HDAC activity in nuclear extracts of skeletal muscle samples from mice supplemented with butyrate. This response is associated with an increase in skeletal muscle fatty acid oxidation and the promotion of an oxidative skeletal muscle phenotype. Similarly, butyrate decreases expression of HDAC1 and increases acetylation of histone H3 Lys 9 in mice[54]. In contrast, HDAC4 expression in mice does not appear to be affected by butyrate supplementation[56]. Butyrate increases H3 acetylation at both histone H3 Lys 9 and Lys 14 residues in L6 myotubes throughout the differentiation process, and induces hyperacetylation of histone H3 in fully differentiated L6 myotubes[49]. The acetylation of histone H3 after butyrate supplementation is associated with increased mRNA and protein expression of insulin receptor substrate 1[49], thus suggesting that SCFA-induced improvements in insulin sensitivity may be partly mediated via HDAC inhibition (Figure 6.1). To our knowledge, no data are available regarding the HDAC-inhibitory potential of either acetate or propionate in skeletal muscle tissue.

Interestingly, SCFA-induced modulation of HDACs may be partly mediated through activation of AMPK. AMPK can influence HDACs and histone acetyltransferases either directly via phosphorylation, or indirectly by the production of acetyl CoA and NAD+, which act as substrates for both these enzyme classes[87]. Therefore, changes in skeletal muscle attributed to increased AMPK phosphorylation might possibly be achieved via modulation of HDACs, as has already been demonstrated in primary human myotubes, in which AMPK phosphorylation of HDAC5 leads to its export from the nuclei, thus increasing GLUT4 expression[88].

6.6 Indirect effects of short-chain fatty acids on skeletal muscle metabolism

6.6.1 Blood flow

Research conducted in anesthetized dogs has demonstrated the vasodilatory properties of acetate in skeletal muscle[89,90]. Infusion of acetate decreases vascular resistance and produces vasodilation in a dose-dependent manner, an effect that appears to be partly mediated by an increase in tissue adenosine content[89,90]. This increase in blood flow observed with SCFA administration may also augment the rate of nutrient delivery to skeletal muscle tissue and therefore play a role in the SCFA-induced upregulation of glucose and fatty acid uptake.

6.6.2 Hormonal responses to short-chain fatty acids

SCFAs can trigger the release of hormones from several organ sites into the systemic circulation (Figure 6.2). These hormones therefore have access to skeletal muscle tissue and largely include peptide or protein hormones involved in appetite regulation and glucose homeostasis, such as glucagon-like peptide-1 (GLP-1), insulin and leptin[7,91]. Importantly, these hormones have well-established effects on skeletal muscle metabolism. In skeletal muscle, GLP-1 can increase the recruitment of microvasculature, glucose uptake, metabolism and glycogen synthesis[92–96]. Insulin is a key regulator of skeletal muscle protein balance, glycogenesis and lipolysis, as well as amino acid, glucose and fatty acid uptake[97,98]. Leptin has also been shown to increase skeletal muscle lipid and glucose oxidation, possibly via an AMPK-dependent mechanism[99].

Beyond appetite regulation, SCFA supplementation can restore systemic levels of insulin-like growth factor 1 (IGF-1) in antibiotic-treated mice[100]. IGF-1 is synthesized by various tissues (including the liver and skeletal muscle) and is widely acknowledged as a major governor of skeletal muscle growth, differentiation and regeneration[101]. However, local production of IGF-1 within skeletal muscle (rather than systemic IGF-1) is believed to be a key regulator of skeletal muscle anabolism[102]. Systemic IGF-1 concentrations may instead play a greater role in skeletal muscle metabolism and insulin sensitivity[103].

6.6.3 Anti-inflammatory properties of short-chain fatty acids

SCFAs also regulate systemic inflammation. Acetate, propionate and butyrate all display anti-inflammatory properties, likely mediated by GPR42/GPR43 activation or HDAC inhibition[104,105]. Increased production of inflammatory cytokines has been implicated as a key driver of skeletal muscle wasting, insulin resistance and altered lipid metabolism[106,107]. Increased production of SCFAs may therefore dampen the inflammatory response and prevent or mitigate its negative effects on skeletal muscle.

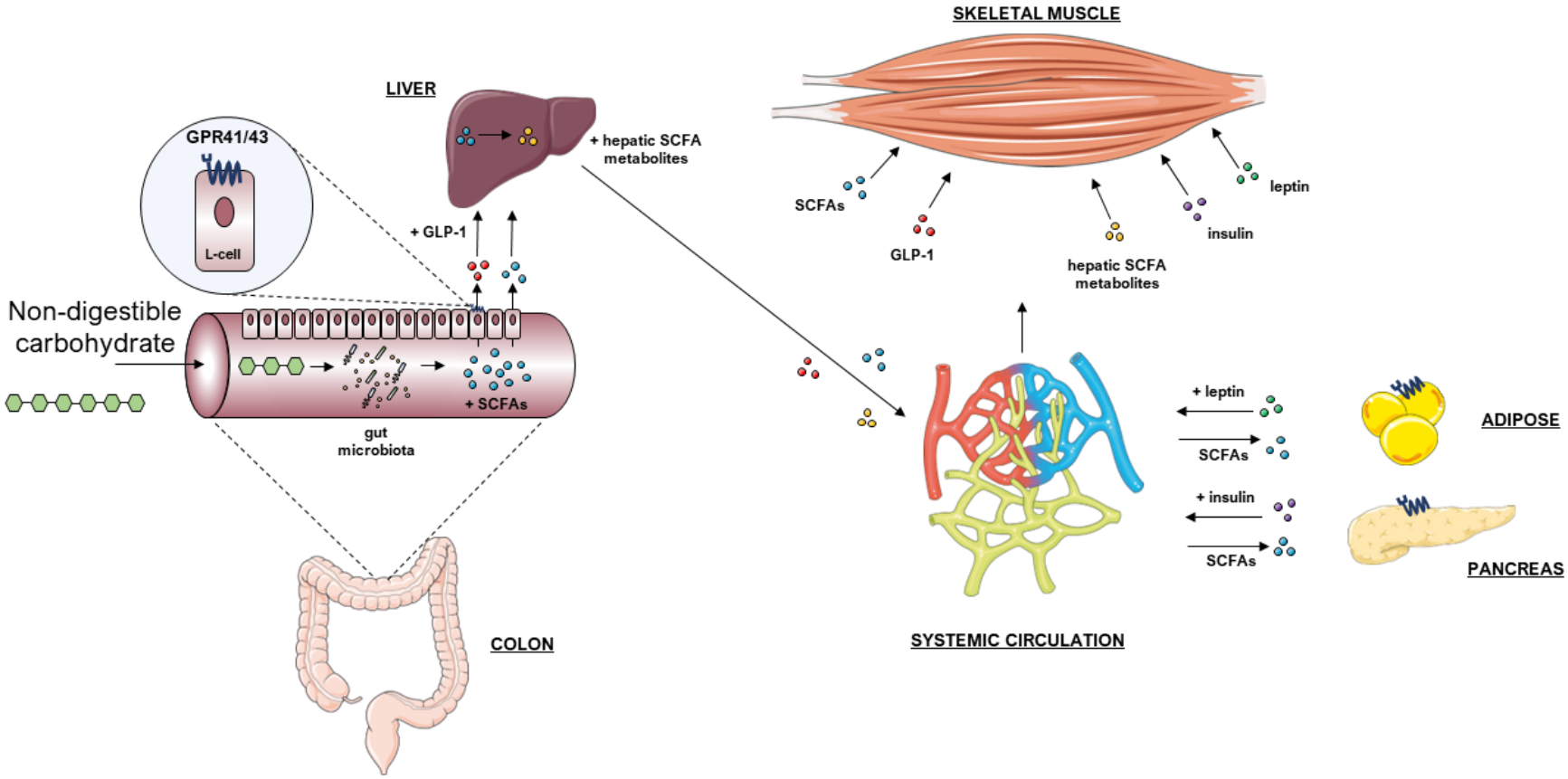


Figure 6.2: The contribution of short chain fatty acids to the gut-muscle axis. Non-digestible carbohydrate is fermented by the gut microbiota to produce short chain fatty acids (SCFAs). SCFAs trigger the release of GLP-1 (colon), leptin (adipose) and insulin (pancreas). These hormones, alongside SCFAs, enter the systemic circulation and subsequently interact with skeletal muscle to influence lipid, carbohydrate and protein metabolism. GLP-1, glucagon-like peptide 1; SCFA, short-chain fatty acid.

6.7 Pitfalls of rodent myoblast models

To date, most *in vitro* research investigating the effects of SCFAs on skeletal muscle has used immortalized cell lines from rodents, primarily C2C12 myoblasts from mice[44,57,108] and L6 myoblasts from rats[43,49,53]. Although these models are useful, they raise the issue of interspecies differences between rodents and humans. For example, inflammatory responses and gene expression in rodent myoblasts significantly differ from those in human myoblasts[109]. A human equivalent of these cell lines, LHCN-M2 myoblasts, has been available since 2007[110] and, together with primary human skeletal muscle cells obtained from biopsy, could be used to investigate the potential translational relevance of the effects observed in rodent models. This possibility is particularly relevant given the absence of human data on the influence of SCFAs on skeletal muscle.

6.8 Absence of acetate research in humans

Systemic concentrations of acetate are considerably higher than those of either propionate or butyrate (Table 6.1). Acetate is thus regarded as the principal SCFA metabolized by skeletal muscle tissue. Despite this observation, there is a clear absence of data investigating the effects of acetate on skeletal muscle metabolism, particularly in humans, probably because acetate is a well-known end product of hepatic ethanol metabolism[111], and ethanol ingestion is associated with skeletal muscle dysregulation[112]. However, the negative effects of ethanol ingestion on skeletal muscle appear to be mediated by either the direct action of ethanol itself[113] or its metabolite acetaldehyde[114]. Given the natural bioavailability of acetate to skeletal muscle (Table 6.1), and the established safety of (sodium) acetate administration[115], future research investigating the effects of acetate on skeletal muscle is therefore a logical next step.

6.9 Synopsis and general aims

Non-digestible carbohydrate (which mostly comprises dietary fibre) escapes host digestion and is available for fermentation by the resident gut microbiota. Fermentation of non-digestible carbohydrate results in the production of bioactive metabolites, including the SCFAs acetate, propionate, and butyrate. These SCFAs can enter the systemic circulation and influence numerous organs and tissues, including skeletal muscle.

Preclinical data has shown that SCFAs can have beneficial effects on glucose, lipid and protein metabolism, which may translate into improvements in insulin sensitivity, skeletal muscle mass and function. However, there is very limited data on the effect of non-digestible carbohydrate and SCFAs on skeletal muscle metabolism and function in humans. In particular, there is limited clinical research investigating the impact of acetate, the most abundant SCFA in the systemic circulation, on glucose and protein metabolism.

6.9.1 General aims:

Chapter 7:

- To explore the relationship between dietary fibre and skeletal muscle mass and function in a population at risk of skeletal muscle atrophy

An additional aim of this PhD research project was to investigate the effect of acute acetate supplementation on glucose and protein metabolism in humans. However, due to the COVID-19 pandemic, this project was not completed within the timeline of the PhD and is presently ongoing. This is discussed further in Chapter 8.

6.10 References

1. O'Connor, E. M. The role of gut microbiota in nutritional status. *Curr. Opin. Clin. Nutr. Metab. Care* **16**, 509–516 (2013).
2. Blaak, E. E. *et al.* Short chain fatty acids in human gut and metabolic health. *Benef. Microbes* **11**, 411–455 (2020).
3. Byrne, C. S., Chambers, E. S., Morrison, D. J. & Frost, G. The role of short chain fatty acids in appetite regulation and energy homeostasis. *Int. J. Obes.* **39**, 1331–1338 (2015).
4. Morrison, D. J. & Preston, T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes* **7**, 189–200 (2016).
5. Fan, Y. & Pedersen, O. Gut microbiota in human metabolic health and disease. *Nat. Rev. Microbiol.* (2020).
6. Canfora, E. E., Jocken, J. W. & Blaak, E. E. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat. Rev. Endocrinol.* **11**, 577–591 (2015).
7. den Besten, G. *et al.* The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J. Lipid Res.* **54**, 2325–2340 (2013).
8. van der Beek, C. M., Dejong, C. H. C., Troost, F. J., Masclee, A. A. M. & Lenaerts, K. Role of short-chain fatty acids in colonic inflammation, carcinogenesis, and mucosal protection and healing. *Nutr. Rev.* **75**, 286–305 (2017).
9. den Besten, G. *et al.* Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. *AJP Gastrointest. Liver Physiol.* **305**, G900–G910 (2013).
10. Boets, E. *et al.* Systemic availability and metabolism of colonic-derived short-chain

fatty acids in healthy subjects: a stable isotope study. *J. Physiol.* **595**, 541–555 (2017).

11. Bergman, E. N. Energy Contributions of Volatile Fatty Acids From the Gastrointestinal Tract in Various Species. *Physiol. Rev.* **70**, 567–583 (1990).

12. Le Poul, E. *et al.* Functional Characterization of Human Receptors for Short Chain Fatty Acids and Their Role in Polymorphonuclear Cell Activation. *J. Biol. Chem.* **278**, 25481–25489 (2003).

13. Mishra, S. P., Karunakar, P., Taraphder, S. & Yadav, H. Free Fatty Acid Receptors 2 and 3 as Microbial Metabolite Sensors to Shape Host Health: Pharmacophysiological View. *Biomedicines* **8**, 154 (2020).

14. Frontera, W. R. & Ochala, J. Skeletal Muscle: A Brief Review of Structure and Function. *Behav. Genet.* **45**, 183–195 (2015).

15. Dumont, N. A., Bentzinger, C. F., Sincennes, M. C. & Rudnicki, M. A. Satellite cells and skeletal muscle regeneration. *Compr. Physiol.* **5**, 1027–1059 (2015).

16. Bertorini, T. E. *Neuromuscular Case Studies*. (Elsevier Health Sciences, 2008).

17. Cretoiu, D. *et al.* Myofibers. in *Advances in Experimental Medicine and Biology* 23–46 (2018).

18. Talbot, J. & Maves, L. Skeletal muscle fiber type: using insights from muscle developmental biology to dissect targets for susceptibility and resistance to muscle disease. *Wiley Interdiscip. Rev. Dev. Biol.* **5**, 518–534 (2016).

19. Mukund, K. & Subramaniam, S. Skeletal muscle: A review of molecular structure and function, in health and disease. *Wiley Interdiscip. Rev. Syst. Biol. Med.* 1–46 (2019).

20. Hargreaves, M. & Spriet, L. L. Skeletal muscle energy metabolism during exercise. *Nat. Metab.* **2**, 817–828 (2020).

21. Frayn, K. *Metabolic Regulation*. (Wiley-Blackwell, 2010).

22. van Loon, L. J. C., Greenhaff, P. L., Constantin-Teodosiu, D., Saris, W. H. M. & Wagenmakers, A. J. M. The effects of increasing exercise intensity on muscle fuel utilisation in humans. *J. Physiol.* **536**, 295–304 (2001).

23. Jensen, J., Rustad, P. I., Kolnes, A. J. & Lai, Y.-C. The Role of Skeletal Muscle Glycogen Breakdown for Regulation of Insulin Sensitivity by Exercise. *Front. Physiol.* **2**, 1–11 (2011).

24. DeFronzo, R. A. *et al.* The Effect of Insulin on the Disposal of Intravenous Glucose: Results from Indirect Calorimetry and Hepatic and Femoral Venous Catheterization. *Diabetes* **30**, 1000–1007 (1981).

25. Lauritzen, H. P. M. M. Insulin- and Contraction-Induced Glucose Transporter 4 Traffic in Muscle. *Exerc. Sport Sci. Rev.* **41**, 77–86 (2013).

26. Li, X. *et al.* Skeletal Muscle Lipid Droplets and the Athlete's Paradox. *Cells* **8**, 249 (2019).

27. Koh, H.-C. E., Nielsen, J., Saltin, B., Holmberg, H.-C. & Ørtenblad, N. Pronounced limb and fibre type differences in subcellular lipid droplet content and distribution in elite skiers before and after exhaustive exercise. *J. Physiol.* **595**, 5781–5795 (2017).

28. Corcoran, M. P., Lamon-Fava, S. & Fielding, R. A. Skeletal muscle lipid deposition and insulin resistance: effect of dietary fatty acids and exercise. *Am. J. Clin. Nutr.* **85**, 662–77 (2007).

29. Argilés, J. M., Campos, N., Lopez-Pedrosa, J. M., Rueda, R. & Rodriguez-Mañas, L. Skeletal Muscle Regulates Metabolism via Interorgan Crosstalk: Roles in Health and Disease. *J. Am. Med. Dir. Assoc.* **17**, 789–796 (2016).

30. Goodpaster, B. H. & Sparks, L. M. Metabolic Flexibility in Health and Disease. *Cell Metab.* **25**, 1027–1036 (2017).

31. Petersen, K. F. & Shulman, G. I. Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. *Am. J. Cardiol.* **90**, 11–18 (2002).

32. Turcotte, L. P. & Fisher, J. S. Skeletal Muscle Insulin Resistance: Roles of Fatty Acid Metabolism and Exercise. *Phys. Ther.* **88**, 1279–1296 (2008).

33. Martins, A. R. *et al.* Mechanisms underlying skeletal muscle insulin resistance induced by fatty acids: importance of the mitochondrial function. *Lipids Health Dis.* **11**, 30 (2012).

34. Holloway, G. P. *et al.* Skeletal muscle mitochondrial FAT/CD36 content and palmitate oxidation are not decreased in obese women. *Am. J. Physiol. Metab.* **292**, E1782–E1789 (2007).

35. Schrauwen-Hinderling, V. B. *et al.* Impaired in vivo mitochondrial function but similar intramyocellular lipid content in patients with type 2 diabetes mellitus and BMI-matched control subjects. *Diabetologia* **50**, 113–120 (2007).

36. Tipton, K. D. & Wolfe, R. R. Exercise, protein metabolism, and muscle growth. *Int. J.*

Sport Nutr. Exerc. Metab. **11**, 109–32 (2001).

37. Cleasby, M. E., Jamieson, P. M. & Atherton, P. J. Insulin resistance and sarcopenia: mechanistic links between common co-morbidities. *J. Endocrinol.* **229**, R67–R81 (2016).

38. Petersen, K. F. *et al.* The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. *Proc. Natl. Acad. Sci.* **104**, 12587–12594 (2007).

39. DeFronzo, R. A. & Tripathy, D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care* **32 Suppl 2**, (2009).

40. Jornayvaz, F. R., Samuel, V. T. & Shulman, G. I. The Role of Muscle Insulin Resistance in the Pathogenesis of Atherogenic Dyslipidemia and Nonalcoholic Fatty Liver Disease Associated with the Metabolic Syndrome. *Annu. Rev. Nutr.* **30**, 273–290 (2010).

41. Ticinesi, A. *et al.* Aging gut microbiota at the cross-road between nutrition, physical frailty, and sarcopenia: Is there a gut–muscle axis? *Nutrients* **9**, 1–20 (2017).

42. Grosicki, G. J., Fielding, R. A. & Lustgarten, M. S. Gut Microbiota Contribute to Age-Related Changes in Skeletal Muscle Size, Composition, and Function: Biological Basis for a Gut-Muscle Axis. *Calcif. Tissue Int.* **102**, 433–442 (2018).

43. Maruta, H. *et al.* Activation of AMP-activated protein kinase and stimulation of energy metabolism by acetic acid in L6 myotube cells. *PLoS One* **11**, 1–19 (2016).

44. Han, J. H. *et al.* The effects of propionate and valerate on insulin responsiveness for glucose uptake in 3T3-L1 adipocytes and C2C12 myotubes via G protein-coupled receptor 41. *PLoS One* **9**, 1–9 (2014).

45. Fushimi, T. *et al.* Acetic Acid Feeding Enhances Glycogen Repletion in Liver and Skeletal Muscle of Rats. *J. Nutr.* **131**, 1973–1977 (2001).

46. Nakao, C. *et al.* Effect of acetate on glycogen replenishment in liver and skeletal muscles after exhaustive swimming in rats. *Scand. J. Med. Sci. Sport.* **11**, 33–37 (2001).

47. Fushimi, T. & Sato, Y. Effect of acetic acid feeding on the circadian changes in glycogen and metabolites of glucose and lipid in liver and skeletal muscle of rats. *Br. J. Nutr.* **94**, 714 (2005).

48. Sakakibara, S., Yamauchi, T., Oshima, Y., Tsukamoto, Y. & Kadowaki, T. Acetic acid

activates hepatic AMPK and reduces hyperglycemia in diabetic KK-A(y) mice. *Biochem. Biophys. Res. Commun.* **344**, 597–604 (2006).

49. Chriett, S., Zerzaihi, O., Vidal, H. & Pirola, L. The histone deacetylase inhibitor sodium butyrate improves insulin signalling in palmitate-induced insulin resistance in L6 rat muscle cells through epigenetically-mediated up-regulation of Irs1. *Mol. Cell. Endocrinol.* **439**, 224–232 (2017).

50. Wakelam, M. J. & Pette, D. The control of glucose 1,6-bisphosphate by developmental state and hormonal stimulation in cultured muscle tissue. *Biochem. J.* **204**, 765–769 (1982).

51. Huang, S. & Czech, M. P. The GLUT4 Glucose Transporter. *Cell Metab.* **5**, 237–252 (2007).

52. Yamashita, H. *et al.* Effects of acetate on lipid metabolism in muscles and adipose tissues of type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *Biosci. Biotechnol. Biochem.* **73**, 570–6 (2009).

53. Gao, Z. *et al.* Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* **58**, 1509–17 (2009).

54. Hong, J. *et al.* Butyrate alleviates high fat diet-induced obesity through activation of adiponectin-mediated pathway and stimulation of mitochondrial function in the skeletal muscle of mice. *Oncotarget* **7**, 56071–56082 (2016).

55. Liu, L., Fu, C. & Li, F. Acetate Affects the Process of Lipid Metabolism in Rabbit Liver, Skeletal Muscle and Adipose Tissue. *Animals* **9**, 799 (2019).

56. Walsh, M. E. *et al.* The histone deacetylase inhibitor butyrate improves metabolism and reduces muscle atrophy during aging. *Aging Cell* **14**, 957–970 (2015).

57. den Besten, G. *et al.* Short-Chain Fatty Acids Protect Against High-Fat Diet–Induced Obesity via a PPARγ-Dependent Switch From Lipogenesis to Fat Oxidation. *Diabetes* **64**, 2398–2408 (2015).

58. Pan, J. H. *et al.* Acetic acid enhances endurance capacity of exercise-trained mice by increasing skeletal muscle oxidative properties. *Biosci. Biotechnol. Biochem.* **79**, 1535–1541 (2015).

59. Gonzalez, A. *et al.* Sodium butyrate ameliorates insulin resistance and renal failure in CKD rats by modulating intestinal permeability and mucin expression. *Nephrol. Dial. Transplant* **34**, 783–794 (2019).

60. Koistinen, H. A. & Zierath, J. R. Regulation of glucose transport in human skeletal muscle. *Ann. Med.* **34**, 410–418 (2002).

61. Kondo, T., Kishi, M., Fushimi, T. & Kaga, T. Acetic acid upregulates the expression of genes for fatty acid oxidation enzymes in liver to suppress body fat accumulation. *J. Agric. Food Chem.* **57**, 5982–5986 (2009).

62. Henagan, T. M. *et al.* Sodium butyrate epigenetically modulates high-fat diet-induced skeletal muscle mitochondrial adaptation, obesity and insulin resistance through nucleosome positioning. *Br. J. Pharmacol.* **172**, 2782–2798 (2015).

63. Jørgensen, H., Larsen, T., Zhao, X.-Q. & Eggum, B. O. The energy value of short-chain fatty acids infused into the caecum of pigs. *Br. J. Nutr.* **77**, 745–756 (1997).

64. Imoto, S. & Namioka, S. Nutritive Value of Acetate in Growing Pigs. *J. Anim. Sci.* **56**, 858–866 (1983).

65. Lahiri, S. *et al.* The gut microbiota influences skeletal muscle mass and function in mice. *Sci. Transl. Med.* **11**, eaan5662 (2019).

66. Mihaylova, M. M. & Shaw, R. J. The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat. Cell Biol.* **13**, 1016–1023 (2011).

67. Yan, H. *et al.* Gut microbiota can transfer fiber characteristics and lipid metabolic profiles of skeletal muscle from pigs to germ-free mice. *Sci. Rep.* **6**, 1–12 (2016).

68. Akimoto, T. *et al.* Exercise Stimulates Pgc-1a Transcription in Skeletal Muscle through Activation of the p38 MAPK Pathway. *J. Biol. Chem.* **280**, 19587–19593 (2005).

69. Jäger, S., Handschin, C., St Pierre, J. & Spiegelman, B. M. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1a. *Proc. Natl. Acad. Sci.* **104**, 12017–12022 (2007).

70. Fernandez-marcos, P. J. & Auwerx, J. Regulation of PGC-1 a , a nodal regulator of mitochondrial biogenesis. *Am. J. Clin. Nutr.* **93**, 884–890 (2011).

71. Conley, K. E. Mitochondria to motion: Optimizing oxidative phosphorylation to improve exercise performance. *J. Exp. Biol.* **219**, 243–249 (2016).

72. Ehrenborg, E. W. A. & Krook, A. Regulation of Skeletal Muscle Physiology and Metabolism by Peroxisome Proliferator-Activated Receptor Delta. *Pharmacol. Rev.* **61**, 373–393 (2009).

73. Kramer, D. K. *et al.* Direct Activation of Glucose Transport in Primary Human Myotubes After Activation of Peroxisome Proliferator-Activated Receptor. *Diabetes* **54**, 1157–1163 (2005).

74. Fan, W. *et al.* PPARδ Promotes Running Endurance by Preserving Glucose. *Cell Metab.* **25**, 1186-1193.e4 (2017).

75. Krämer, D. K. *et al.* Role of AMP Kinase and PPAR Delta in the Regulation of Lipid and Glucose Metabolism in Human Skeletal Muscle. *J. Biol. Chem.* **282**, 19313–19320 (2007).

76. Finck, B. N. *et al.* A potential link between muscle peroxisome proliferator- activated receptor alpha signaling and obesity-related diabetes. *Cell Metab.* **1**, 133–144 (2005).

77. Amin, R. H., Mathews, S. T., Camp, H. S., Ding, L. & Leff, T. Selective activation of PPAR gamma in skeletal muscle induces endogenous production of adiponectin and protects mice from diet-induced insulin resistance. *Am. J. Physiol. - Endocrinol. Metab.* **298**, E28-37 (2010).

78. Walsh, M. E. & Remmen, H. Van. Emerging roles for histone deacetylases in age-related muscle atrophy. *Nutr. Heal. Aging* **4**, 17–30 (2016).

79. Beharry, A. W. *et al.* HDAC1 activates FoxO and is both sufficient and required for skeletal muscle atrophy. *J. Cell Sci.* **127**, 1441–1453 (2014).

80. Bongers, K. S. *et al.* Skeletal muscle denervation causes skeletal muscle atrophy through a pathway that involves both Gadd45a and HDAC4. *Am. J. Physiol. Metab.* **305**, E907–E915 (2013).

81. Lu, J., Mckinsey, T. A., Nicol, R. L. & Olson, E. N. Signal-dependent activation of the MEF2 transcription factor by dissociation from histone deacetylases. *Proc. Natl. Acad. Sci.* **97**, 4070–4075 (2000).

82. Galmozzi, A. *et al.* Inhibition of Class I Histone Deacetylases Unveils a Mitochondrial Signature and Enhances Oxidative Metabolism in Skeletal Muscle and Adipose Tissue. *Diabetes* **62**, 732–742 (2013).

83. McGee, S. L. & Hargreaves, M. Histone modifications and skeletal muscle metabolic gene expression. *Clin. Exp. Pharmacol. Physiol.* 392–396 (2010).

84. Davie, J. R. Inhibition of Histone Deacetylase Activity by Butyrate. *J. Nutr.* **133**, 2485–2493 (2003).

85. Waldecker, M., Kautenburger, T., Daumann, H., Busch, C. & Schrenk, D. Inhibition of histone-deacetylase activity by short-chain fatty acids and some polyphenol metabolites formed in the colon. *J. Nutr. Biochem.* **19**, 587–593 (2008).

86. Soliman, M. L. & Rosenberger, T. A. Acetate supplementation increases brain histone acetylation and inhibits histone deacetylase activity and expression. *Mol. Cell. Biochem.* **352**, 173–180 (2011).

87. Salminen, A., Kauppinen, A. & Kaarniranta, K. AMPK/Snf1 signaling regulates histone acetylation: Impact on gene expression and epigenetic functions. *Cell. Signal.* **28**, 887–895 (2016).

88. McGee, S. L. *et al.* AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. *Diabetes* **57**, 860–7 (2008).

89. Steffen, R. P., McKenzie, J. E. & Haddy, F. J. The possible role of acetate in exercise hyperemia in dog skeletal muscle. *Eur. J. Physiol.* **392**, 315–21 (1982).

90. Steffen, R. P., McKenzie, J. E., Bockman, E. L. & Haddy, F. J. Changes in dog gracilis muscle adenosine during exercise and acetate infusion. *Am. J. Physiol. Circ. Physiol.* **244**, H387–H395 (1983).

91. Lin, H. V. *et al.* Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. *PLoS One* **7**, 1–9 (2012).

92. Villanueva-Peñacarrillo, M. L., Alcántara, A. I., Clemente, F., Delgado, E. & Valverde, I. Potent glycogenic effect of GLP-1(7-36)amide in rat skeletal muscle. *Diabetologia* **37**, 1163–1166 (1994).

93. Villanueva-Peñacarrillo, M. L. *et al.* Regulatory Peptides Characteristic of GLP-1 effects on glucose metabolism in human skeletal muscle from obese patients. *Regul. Pept.* **168**, 39–44 (2011).

94. Green, C. J., Henriksen, T. I., Pedersen, B. K. & Solomon, T. P. J. Glucagon Like Peptide-1-Induced Glucose Metabolism in Differentiated Human Muscle Satellite Cells Is Attenuated by Hyperglycemia. *PLoS One* **7**, e44284 (2012).

95. Chai, W., Zhang, X., Barrett, E. J. & Liu, Z. Glucagon-Like Peptide 1 Recruits Muscle Microvasculature and Improves Insulin ' s Metabolic Action in the Presence of Insulin Resistance. *Diabetes* **63**, 2788–2799 (2014).

96. Subaran, S. C. *et al.* GLP-1 at physiological concentrations recruits skeletal and

cardiac muscle microvasculature in healthy humans. *Clin. Sci.* **127**, 163–170 (2014).

97. Dimitriadis, G., Mitrou, P., Lambadiari, V., Maratou, E. & Raptis, S. A. Insulin effects in muscle and adipose tissue. *Diabetes Res. Clin. Pract.* **93**, S52–S59 (2011).

98. Abdulla, H., Smith, K., Atherton, P. J. & Idris, I. Role of insulin in the regulation of human skeletal muscle protein synthesis and breakdown: a systematic review and meta-analysis. *Diabetologia* **59**, 44–55 (2016).

99. Ceddia, R. B. Direct metabolic regulation in skeletal muscle and fat tissue by leptin: Implications for glucose and fatty acids homeostasis. *Int. J. Obes.* **29**, 1175–1183 (2005).

100. Yan, J. *et al.* Gut microbiota induce IGF-1 and promote bone formation and growth. *Proc. Natl. Acad. Sci.* **113**, E7554–E7563 (2016).

101. Scicchitano, B. M., Rizzuto, E. & Musarò, A. Counteracting muscle wasting in aging and neuromuscular diseases: the critical role of IGF-1. *Aging (Albany. NY).* **1**, 451–457 (2009).

102. Barclay, R. D., Burd, N. A., Tyler, C., Tillin, N. A. & Mackenzie, R. W. The Role of the IGF-1 Signaling Cascade in Muscle Protein Synthesis and Anabolic Resistance in Aging Skeletal Muscle. *Front. Nutr.* **6**, 1–9 (2019).

103. Thankamony, A. *et al.* Low Circulating Levels of IGF-1 in Healthy Adults Are Associated With Reduced β-Cell Function, Increased Intramyocellular Lipid, and Enhanced Fat Utilization During Fasting. *J. Clin. Endocrinol. Metab.* **99**, 2198–2207 (2014).

104. McLoughlin, R. F., Berthon, B. S., Jensen, M. E., Baines, K. J. & Wood, L. G. Short-chain fatty acids, prebiotics, synbiotics, and systemic inflammation: a systematic review and meta-analysis. *Am. J. Clin. Nutr.* **10**, ajcn156265 (2017).

105. Li, M. *et al.* Pro- and anti-inflammatory effects of short chain fatty acids on immune and endothelial cells. *Eur. J. Pharmacol.* **831**, 52–59 (2018).

106. Londhe, P. & Guttrudge, D. C. Inflammation induced loss of skeletal muscle. *Bone* **80**, 131–142 (2017).

107. Wu, H. & Ballantyne, C. M. Skeletal muscle inflammation and insulin resistance in obesity. *J. Clin. Invest.* **127**, 43–54 (2017).

108. Murakami, Y. *et al.* Supplemental epilactose prevents metabolic disorders through uncoupling protein-1 induction in the skeletal muscle of mice fed high-fat diets. *Br. J.*

Nutr. **114**, 1774–1783 (2015).

109. Bareja, A. *et al.* Human and mouse skeletal muscle stem cells: Convergent and divergent mechanisms of myogenesis. *PLoS One* **9**, (2014).

110. Zhu, C. *et al.* Cellular senescence in human myoblasts is overcome by human telomerase reverse transcriptase and cyclin- dependent kinase 4 : consequences in aging muscle and therapeutic strategies for muscular dystrophies. *Aging Cell* **6**, 515–523 (2007).

111. Lundquist, F., Tygstrup, N., Winkler, K., Mellemgaard, K. & Munck-Petersen, S. Ethanol Metabolism and Production Of Free Acetate in the Human Liver. *J. Clin. Invest.* **41**, 955–961 (1962).

112. Steiner, J. L. & Lang, C. H. Dysregulation of skeletal muscle protein metabolism by alcohol. *Am. J. Physiol. - Endocrinol. Metab.* **308**, E699–E712 (2015).

113. Lang, C. H. *et al.* Alcohol intoxication impairs phosphorylation of S6K1 and S6 in skeletal muscle independently of ethanol metabolism. *Alcohol. Clin. Exp. Res.* **28**, 1758–1767 (2004).

114. Preedy, V. R., Keating, J. W. & Peters, T. J. the Acute Effects of Ethanol and Acetaldehyde on Rates of Protein Synthesis in Type I and Type Ii Fibre-Rich Skeletal Muscles of the Rat. *Alcohol Alcohol.* **27**, 241–251 (1992).

115. Veech, R. L. & Gitomer, W. L. The medical and metabolic consequences of administration of sodium acetate. *Adv. Enzyme Regul.* **27**, 313–343 (1988).

116. Cummings, J. H., Pomare, E. W., Branch, H. W. J., Naylor, C. P. E. & Macfarlane, T. Short chain fatty acids in human large intestine , portal , hepatic and venous blood. *Gut* **28**, 1221–1227 (1987).

117. Peters, S. G., Pomare, E. W. & Fisher, C. A. Portal and peripheral blood short chain fatty acid concentrations after caecal lactulose instillation at surgery. *Gut* **33**, 1249–1252 (1992).

118. Bloemen, J. G. *et al.* Short chain fatty acids exchange across the gut and liver in humans measured at surgery q. *Clin. Nutr.* **28**, 657–661 (2009).

119. van der Beek, C. M. *et al.* Hepatic Uptake of Rectally Administered Butyrate Prevents an Increase in Systemic Butyrate Concentrations in Humans. *J. Nutr.* **145**, 2019–2024 (2015).

120. Neis, E. P. *et al.* Distal versus proximal intestinal short-chain fatty acid release in

man. *Gut* **68**, 764–765 (2019).

Chapter 7: Higher dietary fibre intake is associated with increased skeletal muscle mass, strength, and improved glucose homeostasis in adults aged 40 years and older: A cross-sectional analysis using NHANES 2011-2018.

7.1 Introduction

Increased dietary fibre intake is associated with a reduction in cardiometabolic disease risk and all-cause mortality[1]. This relationship is likely mediated in part by the effect of dietary fibre on decreasing body mass[2]. However, human intervention studies demonstrate that increasing dietary fibre intake can lower adiposity without changing body mass[3,4], indicating that increased dietary fibre intake can also raise lean body mass. The improvements in cardiometabolic outcomes in these studies are typically attributed to a decrease in total and regional fat mass, whilst the impact of raised lean body mass is often neglected.

Skeletal muscle is the largest and most malleable component of lean body mass (accounting for ~50% of lean body mass[5]) and hence changes in lean body mass can be primarily attributed to skeletal muscle mass. The maintenance of skeletal muscle is fundamental to locomotion, energy homeostasis and overall quality of life[6,7]. Skeletal muscle is of particular importance to cardiometabolic disease risk, acting as the primary site of insulin-stimulated glucose uptake in the human body[8]. Indeed, skeletal muscle insulin resistance has been proposed as the principal defect in type 2 diabetes[9]. Skeletal muscle mass and associated strength, however, begin to decline after the fifth decade of life[10,11], with adults losing ~20% of their skeletal muscle mass between the ages of 40-80 years[12], this directly contributes to the metabolic dysregulation and functional impairments observed in the elderly population. Consequently, strategies that promote or protect skeletal muscle mass in middle-age are needed to help maintain functional independence and cardiometabolic health in later life.

In most countries there is a substantial 'dietary fibre-gap' between reported intakes in adult populations and the amount recommended by national dietary guidelines and health institutes. For example, in the U.S. mean intake is approximately 17 g/day and the recommended intake of 14g/1000 kcal (~28-34 g/day) is met by <10% adults[13,14]. However, the relationship between dietary fibre intake and skeletal muscle mass, strength and associated glycaemic parameters in adults at increased risk of skeletal muscle atrophy is currently unknown. Furthermore, any relationship could be mediated by increased production of SCFAs as discussed in Chapter 6.

We therefore used nationally representative U.S. population data from 2011 to 2018 to investigate associations between dietary fibre intake and body mass components, glucose homeostasis, and skeletal muscle strength in adults aged 40 years and older. We

hypothesised that a higher intake of dietary fibre would be associated with improved body composition (increased lean body mass *and* decreased fat mass). Furthermore, we hypothesised that this difference in body composition would be paralleled by an enhancement in glucose homeostasis and skeletal muscle strength with increasing dietary fibre intake. The findings from this study will help to inform whether interventions to raise dietary fibre intake are a worthwhile avenue for the prevention of age-associated declines in skeletal muscle mass and strength.

7.2 Methods

This manuscript was written in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement for cross-sectional studies[15].

7.2.1 Study design

This study used publicly available data from the National Health and Nutrition Examination Survey (NHANES). NHANES is a continual cross-sectional survey designed to evaluate the health and nutritional status of the civilian, non-institutionalised population of the United States of America. NHANES employs a complex, multistage, probability sampling design with oversampling of specified population subgroups to increase the reliability and precision of estimates. Participants completed in-home interviews, physical examinations (including the collection of blood and urine samples), and dietary interviews. Comprehensive descriptions of methodology and data collection are provided elsewhere[16]. NHANES was conducted in line with the Declaration of Helsinki and approved by the National Center for Health Statistics Ethics Review Board. Informed consent was obtained from all participants prior to involvement.

7.2.2 Study participants

This study involved participants aged ≥40 years from four consecutive survey cycles: 2011-2012, 2013-2014, 2015-2016 and 2017-2018 (each survey cycle represents an independent sample from the population). A cut-off age of ≥40 years old was chosen based on evidence that loss of skeletal muscle mass may begin to accelerate after this age[10,11].

7.2.3 Dietary intake

NHANES uses in-person 24-hour dietary recall interviews using the United States Department of Agriculture (USDA) Automated Multiple-Pass Method to quantify food and beverage intake. Nutrient intakes are then calculated from food and beverage data using the USDA Food and Nutrient Database for Dietary Studies[17,18]. Energy intake (kcal), dietary fibre, alcohol, and macronutrient intake (grams) per day were then determined. The accuracy of this method has been repeatedly assessed and shown to produce estimates within 10% of true intake[19,20].

7.2.4 Outcomes

Outcomes included in this study were body mass, body mass index (BMI), total lean mass (excluding bone mineral content), appendicular lean mass (excluding bone mineral content), total fat, trunk fat, fasting glucose, fasting insulin, insulin resistance as calculated by the updated homeostasis model assessment (HOMA2-IR), and combined grip strength. However, several outcomes were only measured in specific survey cycles and subsamples. Analyses were therefore performed using three distinct datasets:

7.2.4.1 Body mass components dataset

Body mass (kg) and BMI (kg/m^2) were measured for all survey cycles (2011-2018) and collected in the mobile examination center.

For all survey cycles (2011-2018), total lean mass (g), total fat (g) and trunk fat (g) were measured using in eligible participants. Appendicular lean mass (g), a well-recognised proxy for skeletal muscle mass[21], was calculated by summing the lean mass (excluding bone mineral content) of the right and left leg, and the right and left arm as measured by DEXA. All variables measured by DEXA were expressed relative to body mass (g per kg of body mass; g/kg BM) to account for the influence of body mass on differences in these outcomes. Participants older than 59 years were not eligible for DEXA measurements and therefore the age range of all body mass components dataset variables (including body mass and BMI) was limited to 40-59 years.

7.2.4.2 Glucose homeostasis dataset

For all survey cycles (2011-2018), fasting measures of glucose (mmol/L) and insulin (pmol/L) were taken in a subsample of eligible participants. Fasting glucose and insulin concentrations were then used to calculate HOMA2-IR[22] (arbitrary units; AU).

7.2.4.3 Skeletal muscle strength dataset

For survey cycles 2011-2012 and 2013-2014 only, skeletal muscle strength was measured through a grip test using a handgrip dynamometer in eligible participants. Grip strength is a widely used objective measure of global skeletal muscle strength that predicts functional impairment and all-cause mortality[23]. Combined grip strength (kg) was calculated by summing the largest reading from the right and left hand, and expressed relative to body mass (kg/kg BM).

7.2.5 Covariates

Covariates included in this study were sex, age, ethnicity, social economic status, smoking status, sedentary activity, total daily energy intake, total alcohol intake, and the percentage of energy contributed by fat, carbohydrate and protein to total daily energy intake. All covariates

were assumed to confound the relationship between dietary fibre intake and outcome variables.

Age (years), sex (male, female), ethnicity, socioeconomic status, and smoking status (smoker, non-smoker) were self-reported during in-home interviews. Ethnic groups included Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, and other. Social economic status was classified using the ratio of family income to poverty (PIR), with participants being categorized as low (PIR $\leqslant$1.3), middle (PIR >1.3 to $\leqslant$3.5) or high (PIR >3.5) socioeconomic status. Sedentary activity (minutes) was calculated from the physical activity questionnaire and was preferred over other measures of physical activity due to its high response rate. Total daily energy intake (kcal) and total daily alcohol intake (grams) were derived from the 24-hour dietary recall (first day). The percentage of energy contributed by fat (%), carbohydrate (%) and protein (%) to total daily energy intake was calculated using the amount of each macronutrient consumed (g) derived from the 24-hour dietary recall (first day). This amount was multiplied by its energy content (4 kcal/g for carbohydrate and protein, 9kcal/g for fat) and then divided by total daily energy intake to provide a percentage.

7.2.6 Statistical analysis

All statistical procedures were performed in Stata 16 (StataCorp, USA) and accounted for the complex survey design used in the NHANES (stratification and clustering). Taylor series linearization methods were used for variance estimation.

Four- (2011-2014) and eight-year sample weights (2011-2018) were generated by combining two-year sample weights for each survey cycle as previously described[24]. Sample weights were applied to all analyses to produce nationally representative estimates. Eight-year dietary day one sample weights were used for the body mass components dataset, eight-year fasting subsample weights were used for the glucose homeostasis dataset, and four-year dietary day one sample weights were used for the skeletal muscle strength dataset.

7.2.6.1 Multiple imputation of missing data

As recommended in the NHANES analytic guidelines[24], outcome variables for which >10% of data are missing for eligible participants require adjustment prior to analysis. Consequently, multiple imputation with chained equations was used to impute missing values for total lean mass, right leg lean mass, left leg lean mass, right arm lean mass, left arm lean mass, total fat, and trunk fat. Full details of the imputation model are provided in Appendix 7.1.

7.2.6.2 Participant eligibility

For the body mass components dataset, participants who were not eligible for DEXA measurements or did not undertake the 24-hour dietary recall interviews (day 1) were

excluded from analysis. Participants with missing values for body mass components outcomes (body mass, BMI, trunk fat, total fat, total lean mass, appendicular lean mass), dietary fibre intake, and/or covariates were, however, not excluded, as multiple imputed values were used. This resulted in 6454 eligible participants for the body mass components dataset (Appendix 7.2).

Within the glucose homeostasis dataset, participants with missing data for fasting glucose, fasting insulin, HOMA2-IR, dietary fibre intake, or covariates were excluded from analysis. Similarly, participants with missing data for combined grip strength, dietary fibre intake, or covariates were excluded from analysis within the skeletal muscle strength dataset. This resulted in 5032 and 5326 eligible participants for the glucose homeostasis and skeletal muscle strength datasets, respectively (Appendix 7.2).

7.2.6.3 Association of dietary fibre intake with outcomes

Simple and multiple linear regression analyses were used to examine the association between dietary fibre intake (treated as continuous variable) and all outcome variables. Model 1 was an unadjusted model. Model 2 adjusted for socio-demographic and behavioural variables: sex, age, ethnicity, socioeconomic status, smoking status, and sedentary activity. Model 3 adjusted for socio-demographic, behavioural, and dietary variables: sex, age, ethnicity, socioeconomic status, smoking status, sedentary activity, total energy intake, total alcohol intake, percent energy from protein, percent energy from carbohydrate, and percent energy from fat. The results from Model 3 are presented as the main results. Restricted cubic splines were used to model non-linear relationships between dietary fibre intake and outcomes, with three knots placed at the 10^{th}, 50^{th} and 90^{th} percentiles[25].

Results from regression analyses are presented as regression coefficients (β) and corresponding 95% confidence intervals (CI), P values, and coefficients of determination (R^2). Significant differences were defined as $P < 0.05$.

7.3 Results

Population-weighted means of socio-demographic and behavioural characteristics for each dataset are presented in Table 7.1. Mean dietary fibre intake was ≈ 17g/day for all datasets.

7.3.1 Dietary fibre intake and body mass components

Dietary fibre intake showed a significant negative association with body mass and BMI (Table 7.2). Assuming linearity, each 5g increase in daily dietary fibre intake was associated with a decrease of 0.98 kg (95% CI, -1.39 to -0.55 kg) and 0.38 kg/m^2 (95% CI, -0.52 to -0.24 kg/m^2) in body mass and BMI, respectively.

Table 7.1: Population-weighted socio-demographic and behavioural characteristics of the glucose homeostasis, body mass components, and skeletal muscle function datasets.

Characteristics	Body mass components dataset[a] N=6454	Glucose homeostasis dataset[b] N=5032	Skeletal muscle strength dataset[c] N=5326
Sex (%)			
Male	48.3 (0.9)	47.9 (0.6)	48.0 (0.9)
Female	51.7 (0.9)	52.1 (0.6)	52.0 (0.9)
Age (years)	49.9 (0.1)	58.0 (0.3)	57.7 (0.2)
	[40 – 59]	[40 – 80[d]]	[40 – 80[d]]
Ethnicity (%)			
Mexican American	8.5 (1.0)	6.1 (0.7)	5.5 (1.0)
Other Hispanic	6.1 (0.7)	5.0 (0.6)	4.5 (0.7)
Non-Hispanic White	64.3 (1.9)	72.0 (1.7)	73.1 (2.3)
Non-Hispanic Black	11.8 (1.0)	9.7 (0.9)	10.3 (1.4)
Other	9.4 (0.6)	7.3 (0.5)	6.6 (0.6)
Socioeconomic status (%)			
Low	20.2 (1.3)	18.3 (1.2)	19.5 (1.8)
Middle	32.7 (1.3)	35.6 (1.2)	34.1 (1.4)
High	47.1 (1.8)	46.1 (1.8)	46.4 (2.4)
Sedentary activity (mins/day)	389.2 (5.0)	391.2 (4.5)	407.3 (4.9)
Total daily energy intake (kcal)	2180 (18)	2126 (16)	2077 (18)
Energy contribution from carbohydrate (%)	47.3 (0.3)	47.4 (0.3)	48.1 (0.3)
Energy contribution from fat (%)	34.7 (0.2)	35.2 (0.2)	34.0 (0.2)
Energy contribution from protein (%)	15.8 (0.1)	15.8 (0.1)	15.9 (0.1)
Fibre (g)	17.4 (0.2)	17.2 (0.2)	17.6 (0.3)

Data are %N (SE) or mean (SE) [range]. Weights used: [a]eight-year dietary day one sample weights (2011-2018); [b]eight-year fasting subsample weights (2011-2018); [c]four-year dietary day one sample (2011-2014); [d]individuals aged over 80 years were top-coded as 80 years.

Dietary fibre intake showed a significant positive association with relative total lean mass and relative appendicular lean mass (Table 7.2). Assuming linearity, each 5g increase in daily dietary fibre intake was associated with an increase of 3.44 g/kg BM (95% CI, 2.40 to 4.47 g/kg BM) and 1.69 g/kg BM (95% CI, 1.13 to 2.25 g/kg BM) in relative total lean mass and relative appendicular lean mass, respectively.

Dietary fibre intake showed a significant negative association with relative total fat and relative trunk fat (Table 7.2). Assuming linearity, each 5g increase in daily dietary fibre intake was

Table 7.2: Simple and multiple linear regression analyses of dietary fibre intake (g/day) and all outcomes.

Outcomes	Model 1[a]			Model 2[b]			Model 3[c]		
	β (95% CI)	P value	R^2	β (95% CI)	P value	R^2	β (95% CI)	P value	R^2
Body mass (kg)	0.05 (-0.01, 0.11)	0.093	<0.01	-0.04 (-0.10, 0.01)	0.127	0.16	-0.20 (-0.28, -0.11)	<0.001	0.18
BMI (kg/m^2)	-0.03 (-0.05, -0.01)	0.002	<0.01	-0.03 (-0.05, -0.01)	0.002	0.07	-0.08 (-0.10, -0.05)	<0.001	0.09
Relative total lean mass (g/kg BM)	1.46 (1.20, 1.71)	<0.001	0.04	0.59 (0.44, 0.73)	<0.001	0.56	0.69 (0.48, 0.89)	<0.001	0.57
Relative appendicular lean mass (g/kg BM)	0.84 (0.70, 0.97)	<0.001	0.04	0.33 (0.25, 0.40)	<0.001	0.62	0.34 (0.23, 0.45)	<0.001	0.63
Relative total fat (g/kg BM)	-1.46 (-1.73, -1.19)	<0.001	0.04	-0.59 (-0.74, -0.44)	<0.001	0.55	-0.68 (-0.89, -0.47)	<0.001	0.56
Relative trunk fat (g/kg BM)	-0.67 (-0.80, -0.55)	<0.001	0.03	-0.44 (-0.54, -0.33)	<0.001	0.28	-0.48 (-0.63, -0.33)	<0.001	0.29
Fasting glucose (mmol/L)	-0.00 (-0.01, 0.01)	0.934	<0.01	-0.00 (-0.01, 0.00)	0.143	0.03	-0.01 (-0.02, -0.00)	0.017	0.04
Fasting insulin (pmol/L)	0.02 (-0.20, 0.25)	0.847	<0.01	-0.14 (-0.37, 0.09)	0.249	0.03	-0.71 (-1.01, -0.41)	<0.001	0.05
HOMA2-IR (AU)	0.00 (-0.00, 0.01)	0.862	<0.01	-0.00 (-0.01, 0.00)	0.228	0.03	-0.02 (-0.02, -0.01)	<0.001	0.05
Relative combined grip strength (kg/kg BM)	0.004 (0.003, 0.005)	<0.001	0.03	0.002 (0.001, 0.003)	<0.001	0.39	0.002 (0.001, 0.003)	<0.001	0.40

[a]Unadjusted model. [b]Adjusted for gender, age, ethnicity, socioeconomic status, smoking status, and sedentary activity. [c]Adjusted for gender, age, ethnicity, socioeconomic status, smoking status, sedentary activity, total energy intake, total alcohol intake, percent energy from protein, percent energy from carbohydrate, and percent energy from fat. AU, arbitrary units; BMI, body mass index; HOMA2-IR, updated homeostasis model assessment - insulin resistance.

associated with a decrease of 3.40 g/kg BM (95% CI, -4.45 to -2.36 g/kg BM) and 2.40 g/kg BM (95% CI, -3.14 to -1.67 g/kg BM) in relative total fat and relative trunk fat, respectively.

7.3.2 Dietary fibre intake and glucose homeostasis

Dietary fibre intake showed a significant negative association with fasting glucose, fasting insulin, and HOMA2-IR (Table 7.2). Assuming linearity, each 5g increase in dietary fibre intake was associated with a decrease of 0.04 mmol/L (95% CI, -0.08 to -0.01 mmol/L), 3.55 pmol/L (95% CI, -5.03 to -2.07 pmol/L), and 0.08 AU (95% CI, -0.12 to -0.05 AU) in fasting glucose, fasting insulin, and HOMA2-IR, respectively.

7.3.3 Dietary fibre intake and skeletal muscle strength

Dietary fibre intake showed a significant positive association with relative combined grip strength (Table 7.2). Assuming linearity, each 5g increase in daily dietary fibre intake was associated with an increase of 0.012 kg/kg BM (95% CI, 0.006 to 0.018 kg/kg BM) in relative combined grip strength.

7.3.4 Dose-response relationships

Dose-response relationships between dietary fibre intake and all outcomes are shown in Figure 7.1 and 7.2. Outcomes were also analysed across levels of dietary guideline adherence (meeting recommended dietary fibre intake vs not meeting recommended dietary fibre intake), producing comparable findings to the main results (Appendix 7.3).

7.4 Discussion

The aim of the present analysis was to examine the relationship between dietary fibre intake and body mass components, glucose homeostasis, and skeletal muscle strength in adults aged 40 years and older. Using nationally representative data of the US population from the NHANES, we show that higher dietary fibre intakes in adults at increased risk of skeletal muscle mass loss are associated with an increase in relative total lean mass, relative appendicular lean mass, and relative combined grip strength. We also demonstrate that higher dietary fibre intakes are associated with a lower body mass, BMI, fasting glucose, fasting insulin, HOMA2-IR, relative fat mass, and relative trunk fat mass. To our knowledge, the association between dietary fibre and skeletal muscle mass and function has not been previously investigated in a cohort of this size and scope. Previous research in this area has used relatively small sample sizes, a narrower age range of study participants (65-79 years), and did not adjust for important covariates (e.g. socioeconomic status, physical activity level, smoking status, and alcohol intake)[26]. The findings of the present study indicate that the divergent changes in the lean and fat components of body mass associated with higher dietary fibre intakes are also allied to improvements in glucose homeostasis and skeletal muscle strength. Consequently, interventions that aim to increase dietary fibre intake, via

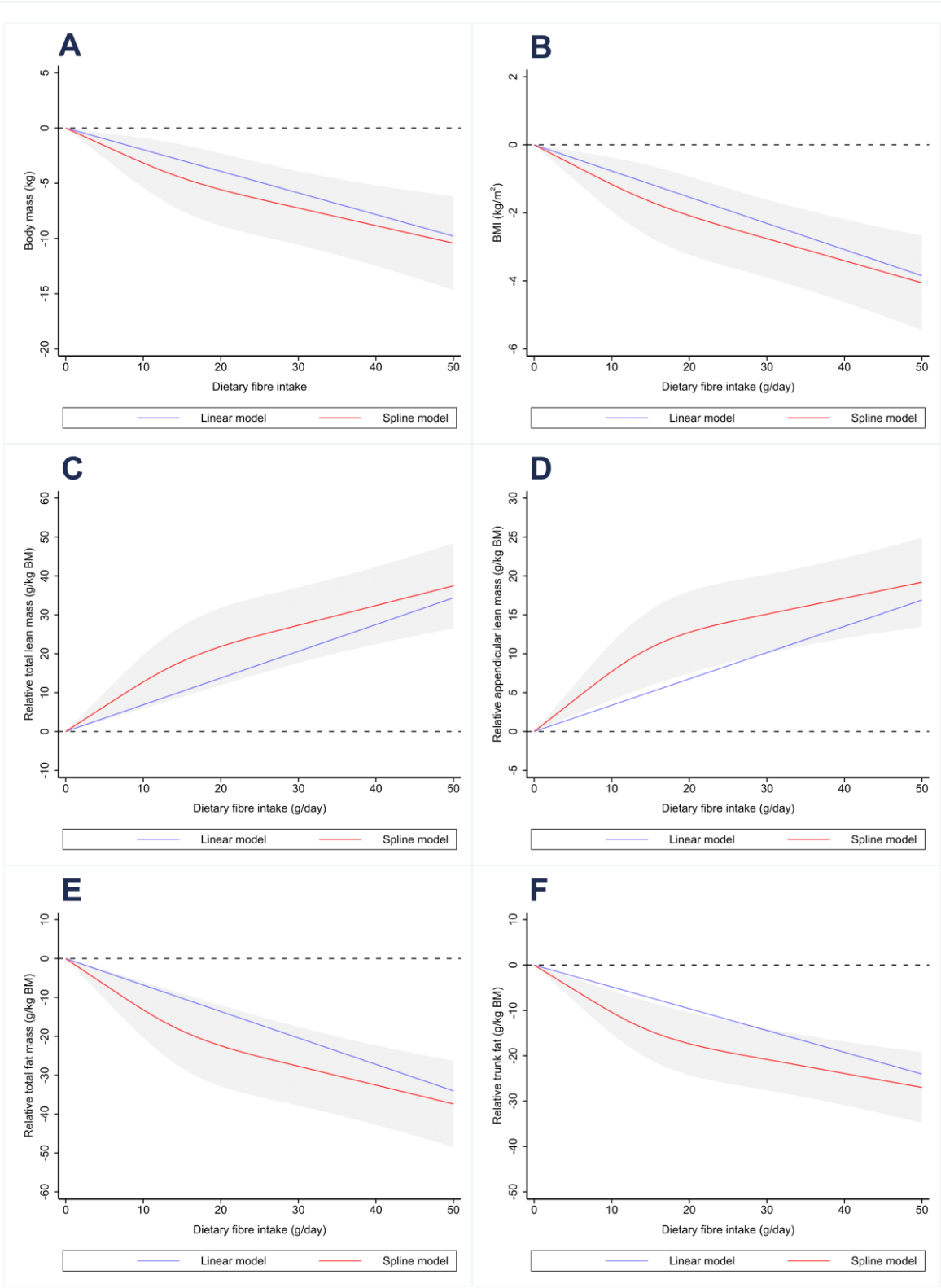


Figure 7.1: Dose-response relationship between dietary fibre intake and (A) body mass, (B) BMI, (C) relative total lean mass, (D) relative appendicular lean mass, (E) relative total fat, and (F) relative trunk fat. Values represent difference in predicted response in reference to a dietary fibre intake of zero. Red and blue solid lines represent linear and restricted cubic spline models, respectively. Black dotted line indicates no change from a dietary fibre intake of zero. Linear and spline models were adjusted for sex, age, ethnicity, socioeconomic status, smoking status, sedentary activity, total energy intake, total alcohol intake, percent energy from protein, percent energy from carbohydrate, and percent energy from fat. Grey shaded area represents 95% confidence interval from restricted cubic spline model predictions.

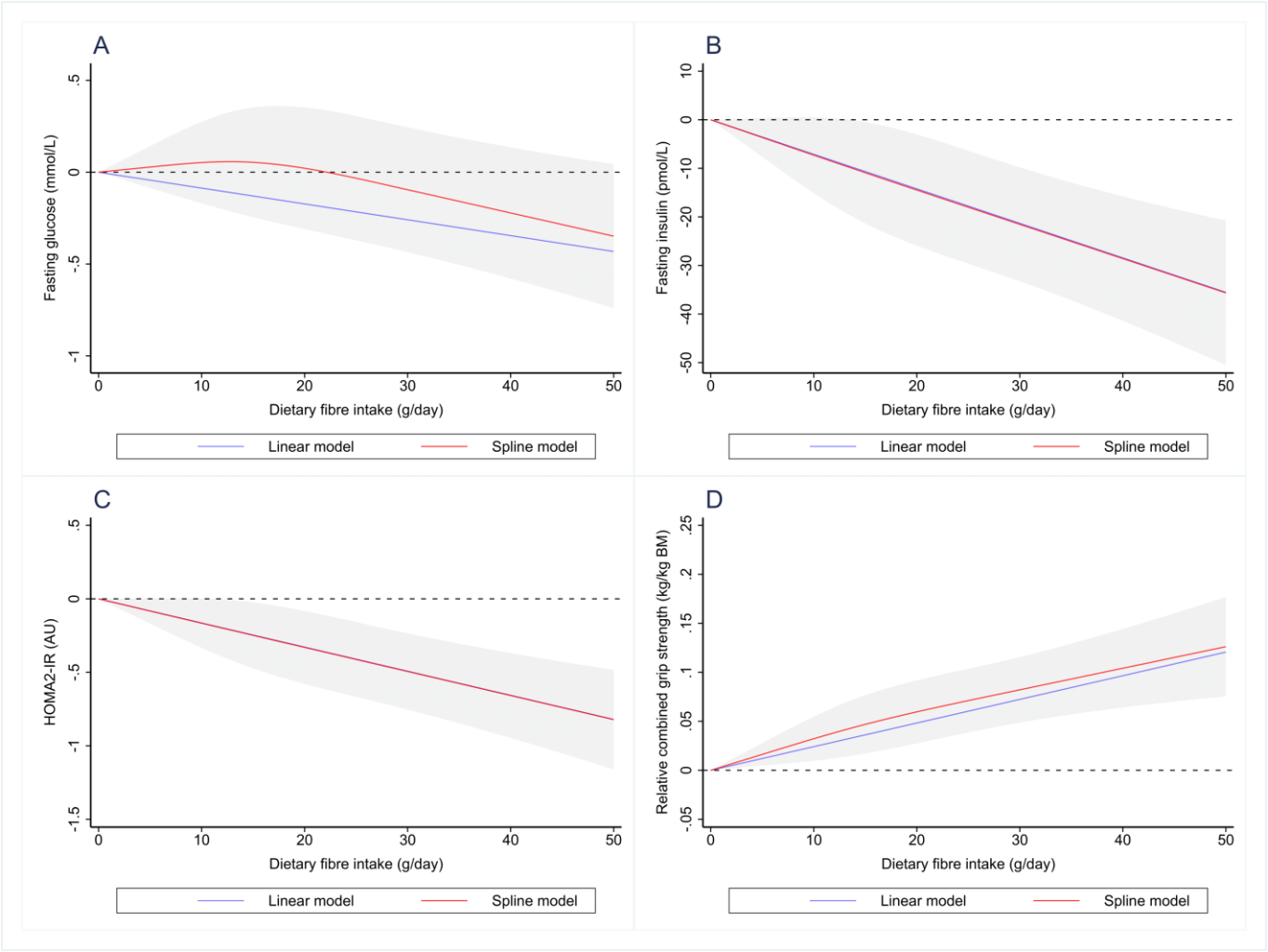


Figure 7.2: Dose-response relationship between dietary fibre intake and (A) fasting glucose, (B) fasting insulin, (C) HOMA2-IR, and (D) relative combined grip strength. Values represent difference in predicted response in reference to a dietary fibre intake of zero. Red and blue solid lines represent linear and restricted cubic spline models, respectively. Black dotted line indicates no change from a dietary fibre intake of zero. Linear and spline models were adjusted for sex, age, ethnicity, socioeconomic status, smoking status, sedentary activity, total energy intake, total alcohol intake, percent energy from protein, percent energy from carbohydrate, and percent energy from fat. Grey shaded area represents 95% confidence interval from restricted cubic spline model predictions.

supplementation or increased consumption of high-fibre foods, may be a viable strategy to prevent age-associated declines in lean body mass and associated strength.

The finding that a higher dietary fibre intake is associated with a lower body mass and BMI is consistent with prior observational, prospective, and interventional studies[1]. Assuming a linear relationship between dietary fibre intake and body mass, our analysis suggests that the average US citizen (dietary fibre intake of ~15g/day) would be ~2 to ~4kg heavier than a similar individual (matched by covariates included in model 3) who met the recommended dietary fibre intake (~30g/day). The effect of dietary fibre on fat mass observed in the present analysis is also supported by previous research investigating this relationship[27]. Furthermore, increased total fat and trunk fat are associated with insulin resistance[28], and therefore lower total fat and trunk fat likely contribute to the relationship between higher dietary fibre intakes and lower fasting glucose, fasting insulin and HOMA2-IR.

Alongside its favourable effects on body mass and fat mass, higher dietary fibre intakes are associated with an increase in relative total lean mass and relative appendicular lean mass. This is congruent with the limited available evidence in humans showing fibre supplementation can modify lean body mass [3,4,29]. Indeed, diets which have shown promise in preventing the age-related decline in skeletal muscle mass are typically characterised by a high intake of fibre-rich foods such as fruits, vegetables, and wholegrains[30]. The increase in relative appendicular lean mass (a proxy for skeletal muscle mass) with increasing dietary fibre intake likely contributes to the positive relationship between dietary fibre intake and improvements in glucose homeostasis outcomes (alongside the concurrent reduction in relative fat mass) as skeletal muscle is the primary site of glucose storage[31]. However, due to the scarcity of research in this area, the mechanisms responsible for the putative relationship between dietary fibre and skeletal muscle mass have been little explored or discussed. As dietary fibre exhibits pleiotropic effects on the human body, it may influence skeletal muscle mass via multiple avenues. For example, dietary fibre supplementation has been shown to decrease insulin resistance[32,33] (in agreement with the findings of the present analysis) and concentrations of pro-inflammatory cytokines[34,35]; two factors that increase muscle protein loss[36,37] and are implicated in the age-related decline in skeletal muscle mass and function [38].

Dietary fibre intake also has a major impact on the composition and metabolic activity of the gut microbiome. For example, higher dietary fibre intake raises the abundance of saccharolytic gut bacteria and production of short-chain fatty acids (SCFAs)[39,40], which have recently been proposed as regulators of skeletal muscle mass, metabolism, and function (this is extensively reviewed in Chapter 6)[41]. SCFAs are also implicated in energy balance and glucose

homeostasis[42], and may therefore contribute to the relationship between higher dietary fibre intakes and lower body mass, BMI, fasting glucose, fasting insulin, and HOMA2-IR.

The positive association between dietary fibre intake and relative combined grip strength is likely explained by the positive association between dietary fibre intake and relative appendicular lean mass, considering the strong linear relationship between skeletal muscle mass and strength[43]. This is in line with previous work showing elderly individuals in the highest tertile of skeletal muscle mass had significantly higher relative handgrip strength and physical function test scores than individuals in the lowest tertile[26], and that chronic fibre supplementation (comprising inulin and fructooligosaccharides) could significantly improve hand grip strength in this same population[44]. Collectively, this would suggest that dietary strategies that impact skeletal muscle mass likely have downstream effects on skeletal muscle strength and function, and may therefore have important implications for the maintenance of skeletal muscle mass and function during the aging process.

Dose-response curves suggest that most outcomes display an approximate linear relationship with dietary fibre intake and display no sign of a plateau over the range of dietary fibre intake explored. Despite some outcomes in the body mass components datasets showing a possible decrease in the strength of the relationship when dietary fibre intakes exceed 20 g/day, the data presented in the current analysis suggests that increasing dietary fibre intake above the current population intake (and towards recommended levels[45]) would likely be associated with improvements in body composition, glucose homeostasis, and strength in adults aged 40 years and older.

Nevertheless, the present analysis has several limitations. Firstly, this analysis only includes non-institutionalised participants from the US, and therefore it is unknown whether the relationship between dietary fibre and skeletal muscle mass and function are present in other populations or countries. The findings from this analysis were also partially limited by the age restrictions imposed on the DEXA measurements, resulting in total lean mass, appendicular lean mass, total fat, and trunk fat only being measured in individuals aged up to 59 years old. This may be problematic if the effect of dietary fibre on body composition is mediated by the gut microbiota, as reports have consistently identified age-related alterations to the composition and metabolic activity of the faecal microbiota[46,47]. However, previous research has shown that dietary fibre intake is a major factor influencing the composition of the gut microbiota in the elderly and correlates with decreased incidence of frailty[48]. Furthermore, the response of the gut microbiota to dietary fibre ingestion was shown to be comparable between middle-age (30-50 years) and older (≥70 years) adults[49]. Thus, any association between dietary fibre and body composition produced by the gut microbiota is still likely to be present

in adults aged >60 years. A further limitation of this analysis was the approach employed for dietary assessment. Dietary fibre intake and other diet-related variables were calculated using 24-hour dietary recall; a method prone to misreporting[50] and possibly not representative of an individual's typical diet. Additionally, specific classes (e.g. soluble vs insoluble fibre) or sources (e.g. vegetable vs wholegrain) of dietary fibre may be more efficacious than others for particular applications[45], but the method of dietary assessment employed did not differentiate between these. Lastly, inherent to all cross-sectional data analysis, a causal relationship between dependent and independent outcomes cannot be established.

In summary, higher dietary fibre intakes are associated with a lower body mass and an improvement in body composition (characterised by a higher ratio of lean body mass to fat mass) in adults aged 40 years and older. Moreover, the improvements in body composition with higher dietary fibre intakes are allied with improvements in glucose homeostasis and skeletal muscle strength. Future research should look to evaluate the therapeutic potential of increasing dietary fibre intake (via diet modification and/or supplementation) on skeletal muscle and associated outcomes, with a focus on the preservation of skeletal muscle mass in adults aged 40 years and older.

7.5 References

1. Reynolds, A. *et al.* Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet* **393**, 434–445 (2019).

2. Wanders, A. J. *et al.* Effects of dietary fibre on subjective appetite, energy intake and body weight: a systematic review of randomized controlled trials. *Obes. Rev.* **12**, 724–739 (2011).

3. Bouche, C. *et al.* Five-Week, Low-Glycemic Index Diet Decreases Total Fat Mass and Improves Plasma Lipid Profile in Moderately Overweight Nondiabetic Men. *Diabetes Care* **25**, 822–828 (2002).

4. Pal, S., Ho, S., Gahler, R. J. & Wood, S. Effect on body weight and composition in overweight/obese Australian adults over 12 months consumption of two different types of fibre supplementation in a randomized trial. *Nutr. Metab. (Lond).* **13**, 82 (2016).

5. Buckinx, F. *et al.* Pitfalls in the measurement of muscle mass: a need for a reference standard. *J. Cachexia. Sarcopenia Muscle* **9**, 269–278 (2018).

6. Baskin, K. K., Winders, B. R. & Olson, E. N. Muscle as a "Mediator" of Systemic Metabolism. *Cell Metab.* **21**, 237–248 (2015).

7. Argilés, J. M., Campos, N., Lopez-Pedrosa, J. M., Rueda, R. & Rodriguez-Mañas, L. Skeletal Muscle Regulates Metabolism via Interorgan Crosstalk: Roles in Health and Disease. *J. Am. Med. Dir. Assoc.* **17**, 789–796 (2016).

8. DeFronzo, R. A. *et al.* The Effect of Insulin on the Disposal of Intravenous Glucose: Results from Indirect Calorimetry and Hepatic and Femoral Venous Catheterization. *Diabetes* **30**, 1000–1007 (1981).

9. DeFronzo, R. A. & Tripathy, D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care* **32 Suppl 2**, (2009).

10. Deschenes, M. R. Effects of Aging on Muscle Fibre Type and Size. *Sport. Med.* **34**, 809–824 (2004).

11. Keller, K. & Engelhardt, M. Strength and muscle mass loss with aging process. Age and strength loss. *Muscles. Ligaments Tendons J.* **3**, 346–50 (2013).

12. Mitchell, W. K. *et al.* Sarcopenia, Dynapenia, and the Impact of Advancing Age on Human Skeletal Muscle Size and Strength; a Quantitative Review. *Front. Physiol.* **3**, 1–18 (2012).

13. US Department of Agriculture & Agricultural Research Service. Nutrient Intakes from Food and Beverages: Mean Amounts Consumed per Individual, by Gender and Age, What We Eat in America, NHANES 2017-2018. (2020).

14. U.S. Department of Agriculture and U.S. Department of Health and Human Services. Dietary Guidelines for Americans 2020-2025. https://dietaryguidelines.gov (2020).

15. von Elm, E. *et al.* The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* **370**, 1453–1457 (2007).

16. Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey. https://www.cdc.gov/nchs/nhanes/index.htm.

17. Raper, N., Perloff, B., Ingwersen, L., Steinfeldt, L. & Anand, J. An overview of USDA's Dietary Intake Data System. *J. Food Compos. Anal.* **17**, 545–555 (2004).

18. U.S. Department of Agriculture, A. R. S. USDA Food and Nutrient Database for Dietary Studies. http://www.ars.usda.gov/nea/bhnrc/fsrg.

19. Conway, J. M., Ingwersen, L. A. & Moshfegh, A. J. Accuracy of dietary recall using the USDA five-step multiple-pass method in men: An observational validation study. *J. Am. Diet. Assoc.* **104**, 595–603 (2004).

20. Blanton, C. A., Moshfegh, A. J., Baer, D. J. & Kretsch, M. J. The USDA Automated Multiple-Pass Method Accurately Estimates Group Total Energy and Nutrient Intake. *J. Nutr.* **136**, 2594–2599 (2006).

21. Kim, J., Wang, Z., Heymsfield, S. B., Baumgartner, R. N. & Gallagher, D. Total-body skeletal muscle mass: estimation by a new dual-energy X-ray absorptiometry method. *Am. J. Clin. Nutr.* **76**, 378–383 (2002).

22. University of Oxford Diabetes Trial Unit. HOMA2 Calculator. https://www.dtu.ox.ac.uk/homacalculator/.

23. Norman, K., Stobäus, N., Gonzalez, M. C., Schulzke, J.-D. & Pirlich, M. Hand grip strength: Outcome predictor and marker of nutritional status. *Clin. Nutr.* **30**, 135–142 (2011).

24. Centers for Disease Control and Prevention. NHANES Survey Methods and Analytic Guidelines.

25. Harrell, F. E. *Regression Modeling Strategies*. (Springer-Verlag, 2001).

26. Montiel-Rojas, D. *et al.* Dietary Fibre May Mitigate Sarcopenia Risk: Findings from the NU-AGE Cohort of Older European Adults. *Nutrients* **12**, 1075 (2020).

27. Slavin, J. L. Dietary fiber and body weight. *Nutrition* **21**, 411–418 (2005).

28. Abate, N., Garg, A., Peshock, R. M., Stray-Gundersen, J. & Grundy, S. M. Relationships of generalized and regional adiposity to insulin sensitivity in men. *J. Clin. Invest.* **96**, 88–98 (1995).

29. Robertson, M. D., Bickerton, A. S., Dennis, A. L., Vidal, H. & Frayn, K. N. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. *Am. J. Clin. Nutr.* **82**, 559–567 (2005).

30. Bloom, I., Shand, C., Cooper, C., Robinson, S. & Baird, J. Diet Quality and Sarcopenia in Older Adults: A Systematic Review. *Nutrients* **10**, 308 (2018).

31. Berg, J. M., Tymoczko, J. L. & Stryer, L. Glycogen Metabolism. in *Biochemistry* (W. H. Freeman, 2002).

32. Li, S. *et al.* NUTRIOSE dietary fiber supplementation improves insulin resistance and determinants of metabolic syndrome in overweight men: a double-blind, randomized, placebo-controlled study. *Appl. Physiol. Nutr. Metab.* **35**, 773–782 (2010).

33. Solà, R. *et al.* Soluble fibre (Plantago ovata husk) reduces plasma low-density

lipoprotein (LDL) cholesterol, triglycerides, insulin, oxidised LDL and systolic blood pressure in hypercholesterolaemic patients: A randomised trial. *Atherosclerosis* **211**, 630–637 (2010).

34. Aliasgharzadeh, A., Dehghan, P., Gargari, B. P. & Asghari-Jafarabadi, M. Resistant dextrin, as a prebiotic, improves insulin resistance and inflammation in women with type 2 diabetes: a randomised controlled clinical trial. *Br. J. Nutr.* **113**, 321–330 (2015).

35. Dehghan, P., Gargari, B. P., Jafar-Abadi, M. A. & Aliasgharzadeh, A. Inulin controls inflammation and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized-controlled clinical trial. *Int. J. Food Sci. Nutr.* **65**, 117–123 (2014).

36. Wilkes, E. A. *et al.* Blunting of insulin inhibition of proteolysis in legs of older subjects may contribute to age-related sarcopenia. *Am. J. Clin. Nutr.* **90**, 1343–1350 (2009).

37. Bach, E. *et al.* Direct Effects of TNF- on Local Fuel Metabolism and Cytokine Levels in the Placebo-Controlled, Bilaterally Infused Human Leg: Increased Insulin Sensitivity, Increased Net Protein Breakdown, and Increased IL-6 Release. *Diabetes* **62**, 4023–4029 (2013).

38. Tieland, M., Trouwborst, I. & Clark, B. C. Skeletal muscle performance and ageing. *J. Cachexia. Sarcopenia Muscle* **9**, 3–19 (2018).

39. David, L. A. *et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559–563 (2014).

40. So, D. *et al.* Dietary fiber intervention on gut microbiota composition in healthy adults: a systematic review and meta-analysis. *Am. J. Clin. Nutr.* **107**, 965–983 (2018).

41. Frampton, J., Murphy, K. G., Frost, G. & Chambers, E. S. Short-chain fatty acids as potential regulators of skeletal muscle metabolism and function. *Nat. Metab.* **2**, 840–848 (2020).

42. Canfora, E. E., Jocken, J. W. & Blaak, E. E. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat. Rev. Endocrinol.* **11**, 577–591 (2015).

43. Chen, L., Nelson, D. R., Zhao, Y., Cui, Z. & Johnston, J. A. Relationship between muscle mass and muscle strength, and the impact of comorbidities: a population-based, cross-sectional study of older adults in the United States. *BMC Geriatr.* **13**, 74 (2013).

44. Buigues, C. *et al.* Effect of a Prebiotic Formulation on Frailty Syndrome: A

Randomized, Double-Blind Clinical Trial. *Int. J. Mol. Sci.* **17**, 932 (2016).

45. Stephen, A. M. *et al.* Dietary fibre in Europe: current state of knowledge on definitions, sources, recommendations, intakes and relationships to health. *Nutr. Res. Rev.* **30**, 149–190 (2017).

46. Woodmansey, E. J., McMurdo, M. E. T., Macfarlane, G. T. & Macfarlane, S. Comparison of Compositions and Metabolic Activities of Fecal Microbiotas in Young Adults and in Antibiotic-Treated and Non-Antibiotic-Treated Elderly Subjects. *Appl. Environ. Microbiol.* **70**, 6113–6122 (2004).

47. Salazar, N. *et al.* Age-Associated Changes in Gut Microbiota and Dietary Components Related with the Immune System in Adulthood and Old Age: A Cross-Sectional Study. *Nutrients* **11**, 1765 (2019).

48. Claesson, M. J. *et al.* Gut microbiota composition correlates with diet and health in the elderly. *Nature* **488**, 178–184 (2012).

49. Alfa, M. J. *et al.* A randomized trial to determine the impact of a digestion resistant starch composition on the gut microbiome in older and mid-age adults. *Clin. Nutr.* **37**, 797–807 (2018).

50. Poslusna, K., Ruprich, J., de Vries, J. H. M., Jakubikova, M. & van't Veer, P. Misreporting of energy and micronutrient intake estimated by food records and 24 hour recalls, control and adjustment methods in practice. *Br. J. Nutr.* **101**, S73–S85 (2009).

51. Schenker, N. *et al.* Multiple imputation of missing dual-energy X-ray absorptiometry data in the National Health and Nutrition Examination Survey. *Stat. Med.* **30**, 260–276 (2011).

52. Morris, T. P., White, I. R. & Royston, P. Tuning multiple imputation by predictive mean matching and local residual draws. *BMC Med. Res. Methodol.* **14**, 75 (2014).

53. White, I. R., Royston, P. & Wood, A. M. Multiple imputation using chained equations: Issues and guidance for practice. *Stat. Med.* **30**, 377–399 (2011).

54. Rubin, D. B. *Multiple Imputation for Nonresponse in Surveys.* (Wiley, 1987).

55. Harel, O. The estimation of R2 and adjusted R2 in incomplete data sets using multiple imputation. *J. Appl. Stat.* **36**, 1109–1118 (2009).

Appendix 7.1: Multiple imputation procedure

Multiple imputation procedures were based on previous work by Schenker *et al.*[51]. Predictor variables included demographic and socioeconomic variables, body measurements, nutrient intakes, blood tests, health indicators, survey release cycle, sampling weights, strata, and primary sampling units (Table S7.1).

Prior to imputation, four sex (male, female) by age (40-49, 50-59 years) groups were created. The imputation procedure was then performed separately within each sex-by-age group due to concerns that the distribution of variables included in the model, their availability, and reasons for missingness vary by sex and age.

Multiple imputation with chained equations was used to impute missing values. Normally distributed continuous variables were modelled using linear regression, non-normally distributed continuous and ordinal variables were modelled using predictive mean matching (sampling from a pool of 10 donors[52]), and binary variables were modelled using logistic regression. Data were assumed to be missing at random.

Based on the recommendations of White et al.[53] that the number of imputations should be at least equal to the proportion of incomplete cases (average percentage of missing cases in imputed variables = 21%), 25 imputed datasets were created. Data were only imputed for non-pregnant participants aged ≥40 and ≤59 years that were eligible for dual-energy X-ray absorptiometry measurements and undertook the 24-hour dietary recall interviews (day 1). Multiply imputed values for covariates were included in subsequent analyses. All estimates from imputed datasets were pooled using Rubin's combination rules[54]. The coefficient of determination (R2) was calculated using the Fisher Z-transformation as recommended by Harel[55].

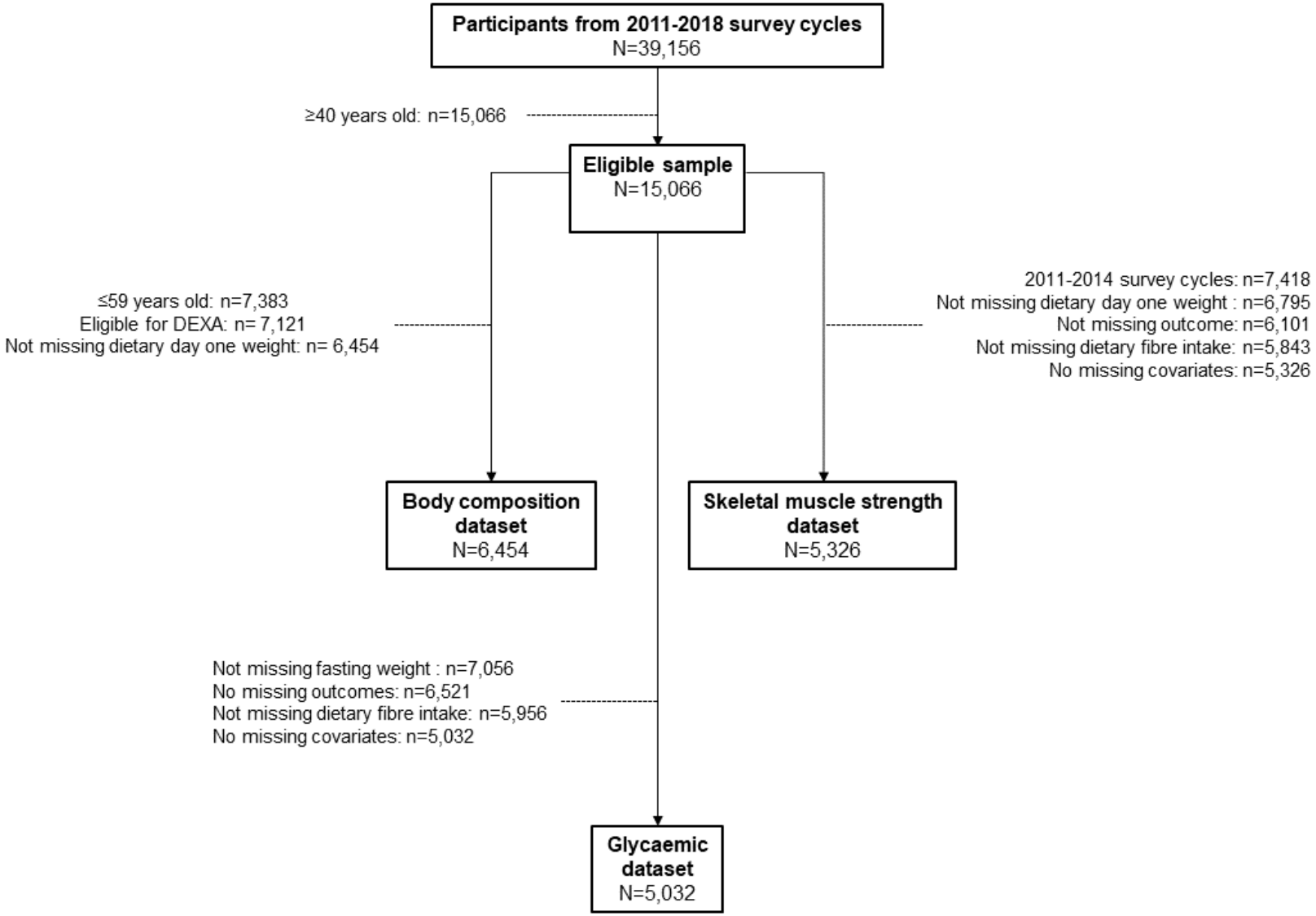
Table S7.1: Variables included in the multiple imputation model

Variable (units)	NHANES variable label[a]	% missing	Model
age (years)	ridageyr	0.00	-
annual household income ($)	indhhin2	6.38	PMM
arm circumference (cm)	bmxarmc	3.28	regression
body mass index (kg/m^2)	bmxbmi	1.05	PMM
body mass (kg)	bmxwt	0.92	PMM
bone mineral content (g)	dxdtobmc	22.43	regression
bone mineral density (g/cm^2)	dxdtobmd	22.43	regression
calcium supplements (mg)	dsqtcalc	63.01	PMM
daily alcohol (g)	dr1talco	7.22	PMM
daily carbohydrate intake (g)	dr1tcarb	7.19	PMM
daily fat intake (g)	dr1ttfat	7.19	PMM
daily fibre intake (g)	dr1tfibe	7.19	PMM
daily protein intake (g)	dr1tprot	7.19	PMM
daily total energy intake (kcal)	dr1tkcal	7.19	PMM
data release cycle	sddsrvyr	0.00	-
diastolic blood pressure (mmHg)	N/A[b]	3.51	PMM
education level	dmdeduc2	0.03	PMM
ethnicity	ridreth1	0.00	-
fasting glucose (mmol/L)	lbdglusi	53.51	PMM
fasting insulin (pmol/L)	lbdinsi	54.72	PMM
full sample 8 year dietary day 1 sample weight	N/A[c]	0.00	-
full sample 8 year MEC exam weight	N/A[c]	0.00	-
general health condition	hsd010	9.26	PMM
height (cm)	bmxht	0.91	regression
HOMA2-B (AU)	N/A[d]	54.80	PMM
HOMA2-IR (AU)	N/A[d]	54.77	PMM
HOMA2-S (AU)	N/A[d]	54.80	PMM
LDL-cholesterol (mmol/L)	lbdldlsi	56.07	PMM
left arm lean excl BMC (g)	dxdlale	15.19	regression
left leg lean excl BMC (g)	dxdllle	16.71	regression
past 30 day milk product consumption	dbq197	0.00	-
primary sampling units	sdmvpsu	0.00	-
ratio of family income to poverty	indfmpir	9.49	regression
right arm lean excl BMC (g)	dxdrale	15.92	regression
right leg lean excl BMC (g)	dxdrlle	17.08	regression
sampling strata	sdmvstra	0.00	-
sedentary activity (mins)	pad680	0.60	regression
smoking status	N/A[e]	0.01	logistic regression
systolic blood pressure (mmHg)	N/A[f]	3.51	PMM
testosterone (ng/dL)	lbxtst	28.31	PMM
total cholesterol (mmol/L)	lbdtcsi	5.23	PMM
total fat (g)	dxdtofat	22.65	PMM
total lean mass excluding BMC (g)	dxdtole	21.21	regression

triglyceride (mmol/L)	lbdtrsi	55.15	PMM
trunk fat (g)	dxxtrfat	18.10	PMM
vitamin D (D2 + D3) supplements (mcg)	dsqtvd	64.45	PMM
waist circumference (cm)	bmxwaist	4.20	regression
Average % missing:		**20.59**	

[a]refers to the label used in the NHANES database (https://wwwn.cdc.gov/Nchs/Nhanes/search/default.aspx). [b]calculated as an average of bpxdi1, bpxdi2, bpxdi3, and bpxdi4. [C]calculated using 2-year weights (see main text). [d]calculated using the HOMA2 calculator (see main text). [e]created using smq020 and smq040 (https://wwwn.cdc.gov/nchs/nhanes/tutorials/Module1.aspx; see 'Skip Patterns in NHANES Data'). f [b]calculated as an average of bpxsy1, bpxsy2, bpxsy3, and bpxsy4 as % missing was calculated from the number of missing values for participants that were eligible for dual-energy X-ray absorptiometry measurements and 24-hour dietary recall interviews (day 1), and aged between 40 and 59 years old (inclusive). Average % missing was calculated from variables that were imputed (i.e., % missing > 0). AU, arbitrary units; BMC, bone mineral content; HOMA2-B, updated homeostasis model assessment - beta cell function; HOMA2-IR, updated homeostasis model assessment - insulin resistance; HOMA2-S, updated homeostasis model assessment - insulin sensitivity; LDL, low-density lipoprotein; MEC, mobile examination center; N/A, not available; NHANES, national health and nutrition examination survey; PMM, predictive mean matching.

Appendix 7.2: Study inclusion flow chart.



Appendix 7.3: Dietary fibre intake guideline analysis

Participants were divided into two groups: (i) participants meeting the USDA (14g dietary fibre per 1000kcal), and (ii) participants not meeting the USDA guidelines. This grouping was based on dietary fibre intake as estimated by the 24-hour dietary recall (day 1).

Simple and multiple linear regression analyses were used to examine the association between dietary fibre intake guideline adherence and outcome variables. Model 1 was an unadjusted model. Model 2 was adjusted for gender, age, ethnicity, socioeconomic status, smoking status, and sedentary activity. Model 3 was adjusted for gender, age, ethnicity, socioeconomic status, smoking status, sedentary activity, total energy intake, total alcohol intake, percent energy from protein, percent energy from carbohydrate, and percent energy from fat. Results from analyses are presented by dataset in Table S7.2.

Table S7.2: Simple and multiple linear regression analyses of dietary fibre intake guideline adherence and all outcomes

Outcomes	Model 1[a]			Model 2[b]			Model 3[c]		
	β (95% CI)	P value	R^2	β (95% CI)	P value	R^2	β (95% CI)	P value	R^2
Body mass (kg)	-7.13 (-9.42, -4.84)	<0.001	0.01	-4.71 (-6.84, -2.59)	<0.001	0.16	-3.57 (-5.86, -1.27)	0.003	0.18
BMI (kg/m^2)	-1.71 (-2.43, -0.99)	<0.001	0.01	-1.75 (-2.48, -1.02)	<0.001	0.08	-1.53 (-2.35, -0.72)	<0.001	0.09
Relative total lean mass (g/kg BM)	-3.69 (-12.20, 4.82)	0.389	<0.01	10.01 (4.15, 15.88)	0.001	0.56	10.99 (4.58, 17.40)	0.001	0.57
Relative appendicular lean mass (g/kg BM)	-4.10 (-9.24, 1.04)	0.116	<0.01	4.37 (0.83, 7.92)	0.017	0.62	4.80 (0.99, 8.61)	0.014	0.62
Relative total fat (g/kg BM)	4.03 (-4.70, 12.76)	0.359	<0.01	-9.91 (-16.02, -3.81)	0.002	0.55	-10.72 (-17.40, -4.03)	0.002	0.56
Relative trunk fat (g/kg BM)	-3.41 (-8.10, 1.28)	0.151	<0.01	-9.84 (-14.52, -5.17)	<0.001	0.28	-9.85 (-15.00, -4.70)	<0.001	0.29
Fasting glucose (mmol/L)	-0.20 (-0.36, -0.05)	0.013	<0.01	-0.27 (-0.42, -0.11)	0.001	0.03	-0.27 (-0.43, -0.10)	0.002	0.04
Fasting insulin (pmol/L)	-8.09 (-15.52, -0.65)	0.033	<0.01	-10.08 (-18.21, -1.96)	0.016	0.03	-9.88 (-18.12, -1.65)	0.019	0.04
HOMA2-IR (AU)	-0.21 (-0.37, -0.04)	0.014	<0.01	-0.25 (-0.43, -0.07)	0.007	0.03	-0.25 (-0.43, -0.07)	0.008	0.04
Relative combined grip strength (kg/kg BM)	-0.001 (-0.028, 0.025)	0.922	<0.01	0.029 (0.003, 0.055)	0.028	0.39	0.033 (0.007, 0.058)	0.013	0.40

[a]Unadjusted model. [b]Adjusted for gender, age, ethnicity, socioeconomic status, smoking status, and sedentary activity. [c]Adjusted for gender, age, ethnicity, socioeconomic status, smoking status, sedentary activity, total energy intake, total alcohol intake, percent energy from protein, percent energy from carbohydrate, and percent energy from fat. AU, arbitrary units; BMI, body mass index; HOMA2-IR, updated homeostasis model assessment - insulin resistance.

Chapter 8: General discussion (non-digestible carbohydrate, short-chain fatty acid production and skeletal muscle metabolism)

Non-digestible carbohydrate (which mostly comprises dietary fibre) escapes digestion and is subsequently available for fermentation by colonic bacteria, resulting in the production of metabolites such as short-chain fatty acids (SCFAs)[1]. These SCFAs can enter the systematic circulation and interact with multiple organ sites including skeletal muscle[2]. Pre-clinical evidence demonstrates that SCFAs may induce positive effects on skeletal muscle metabolism and function[3].

The results from chapter 7 demonstrated that increased intake of dietary fibre is associated with higher skeletal muscle mass and function, as well improvements in whole-body insulin sensitivity in humans. Furthermore, this relationship was observed in a population at risk of skeletal muscle atrophy. Despite this study being observational in nature and therefore unable to elucidate causality and any underlying mechanism, the data are in line with the hypothesis that increasing SCFA production via dietary fibre intake can exert beneficial changes in skeletal muscle metabolism and function[4–7].

In order to build upon these findings, future work should consist of interventional studies that use fermentable fibres (to raise SCFA concentrations, e.g. inulin[8]) or SCFAs directly to investigate their impact on skeletal muscle metabolism and function in humans. These studies should largely focus on the role of acetate in skeletal muscle metabolism, as acetate is found in higher concentrations relative to both propionate and butyrate, and is thus considered the principal SCFA metabolised by skeletal muscle[3].

Our initial intention was to conduct and complete a clinical study that investigated the effect of sodium acetate on glucose and protein metabolism in the fed and fasted state, as well as in a young (18 -30 years) and old (≥ 65 years) cohort (Appendix 8.1). However, due to the COVID-19 pandemic and consequent national lockdowns, we were unable to complete this study prior to submission of this thesis. In addition to this clinical study, we intended to investigate the mechanisms by which acetate may mitigate the negative effects of chronic levels of pro-inflammatory cytokines on skeletal muscle using *in vitro* models. This study planned to use immortalised human myoblasts (LHCH-M2[9]) to explore how sodium acetate prevents tumour necrosis factor alpha-induced apoptosis[10]. Again, due to the COVID-19 pandemic, this study was not able to be completed. Preliminary work did, however, involve the development of tumour necrosis factor alpha model that produced a dose dependent reduction in myoblast proliferation (Figure 8.1A), as well as a proof-of-principle study showing that sodium acetate protects against tumour necrosis factor alpha-induced inhibition of myoblast proliferation

(Figure 8.1B). The aim of follow-on studies is to explore the impact of sodium acetate on the main signalling pathways that control myoblast proliferation (Pax7, Myf5 and MyoD) in different inflammatory environments. Our group intends to complete this series of studies in the coming years to evaluate the putative role of acetate as an important regulator of skeletal muscle metabolism and function.

In summary, high dietary fibre intake is associated with improvements in skeletal muscle mass and function. However, clinical intervention studies are required to determine if this relationship is causal. Future work is also required to understand the mechanism underlying dietary fibre-induced changes in skeletal muscle mass and function (e.g. increased SCFA production).

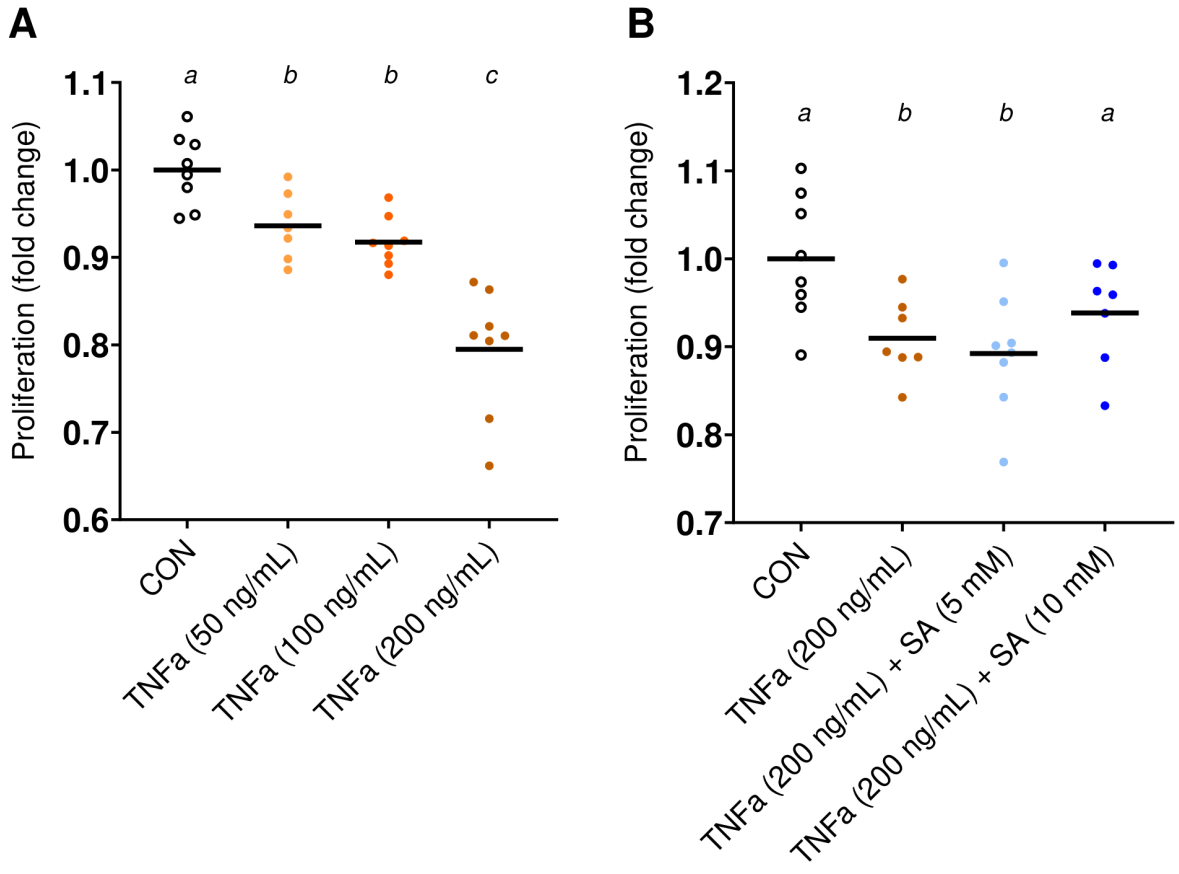


Figure 8.1: The effect of tumour necrosis factor alpha and sodium acetate on myoblast proliferation. (A) The effect of increasing doses of tumour necrosis factor alpha on myoblast proliferation. (B) The effect of sodium acetate on tumour necrosis factor alpha induced suppression of myoblast proliferation. Treatments with a different letter are significantly different from each other ($P < 0.05$). Con, control. SA, sodium acetate. TNFa, tumour necrosis factor alpha.

8.1 References

1. den Besten, G. *et al.* The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J. Lipid Res.* **54**, 2325–2340 (2013).

2. Tremaroli, V. & Bäckhed, F. Functional interactions between the gut microbiota and host metabolism. *Nature* **489**, 242–249 (2012).

3. Frampton, J., Murphy, K. G., Frost, G. & Chambers, E. S. Short-chain fatty acids as potential regulators of skeletal muscle metabolism and function. *Nat. Metab.* **2**, 840–848 (2020).

4. Ubachs, J. *et al.* Gut microbiota and short-chain fatty acid alterations in cachectic cancer patients. *J. Cachexia. Sarcopenia Muscle* **12**, 2007–2021 (2021).

5. Pötgens, S. A. *et al.* Multi-compartment metabolomics and metagenomics reveal major hepatic and intestinal disturbances in cancer cachectic mice. *J. Cachexia. Sarcopenia Muscle* **12**, 456–475 (2021).

6. Chen, L. *et al.* Probiotic supplementation attenuates age-related sarcopenia via the gut–muscle axis in SAMP8 mice. *J. Cachexia. Sarcopenia Muscle* **13**, 515–531 (2022).

7. Lv, W. *et al.* Human gut microbiome impacts skeletal muscle mass via gut microbial synthesis of the short-chain fatty acid butyrate among healthy menopausal women. *J. Cachexia. Sarcopenia Muscle* jcsm.12788 (2021) doi:10.1002/jcsm.12788.

8. van der Beek, C. M. *et al.* The prebiotic inulin improves substrate metabolism and promotes short-chain fatty acid production in overweight to obese men. *Metabolism.* **87**, 25–35 (2018).

9. Zhu, C. *et al.* Cellular senescence in human myoblasts is overcome by human telomerase reverse transcriptase and cyclin- dependent kinase 4 : consequences in aging muscle and therapeutic strategies for muscular dystrophies. *Aging Cell* **6**, 515–523 (2007).

10. Stewart, C. E. H., Newcomb, P. V. & Holly, J. M. P. Multifaceted Roles of TNF-α in Myoblast Destruction: A Multitude of Signal Transduction Pathways. *J. Cell. Physiol.* **198**, 237–247 (2004).

Appendix 8.1: Research Ethics Committee (REC) and Health Research Authority (HRA) letters of study approval

Health Research Authority

London - South East Research Ethics Committee
Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0207 972 2503

24 October 2019

Dr Edward Chambers
Imperial College London,
7th Floor Commonwealth Building
Hammersmith Hospital,
Du Cane Road, London
W12 0NN

Dear Dr Chambers

Study title:	**The effect of sodium acetate on energy metabolism in humans**
REC reference:	**19/LO/1470**
Protocol number:	**1**
IRAS project ID:	**262300**

Thank you for your letter of 23rd September 2019, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered in correspondence by the Chair of the REC.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Dr Edward Chambers
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7th Floor Commonwealth Building
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W12 0NN

Email: hra.approval@nhs.net
HCRW.approvals@wales.nhs.uk

24 October 2019

Dear Dr Chambers

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**The effect of sodium acetate on energy metabolism in humans**
IRAS project ID:	**262300**
Protocol number:	**1**
REC reference:	**19/LO/1470**
Sponsor	**Imperial College London**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.