UNIVERSITY OF BIRMINGHAM

EFFECTS OF NUTRIENT AND EXERCISE INTERACTIONS ON THE COMPONENTS OF ENERGY BALANCE.

By

ISRAEL A. PODESTÁ DONOSO, PT, MSc

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Abstract

Obesity and overweight are rising worldwide. One common strategy to manage body mass is to modify physical activity levels. However, in most cases, the effects of increasing physical activity or exercise are not reflected in body mass changes, possibly due to the trigger of changes in the components of energy balance to counteract the energy deficit. Metabolically, it has been suggested that carbohydrate availability/metabolism (e.g., glucostatic, glycogenostatic or hepatostatic theories) could play a role in modulating the changes in the components of energy balance as a signal of energy availability. The aim of this thesis was to investigate how, from a metabolic perspective, dietary carbohydrates and physical activity/exercise interact in the short term to modulate energy balance components (such as energy expenditure, energy intake, and appetite).

Chapter 3 explored the short-term effects of replacing exercise energy expenditure with a high carbohydrate/low fat (HCLF) or a low carbohydrate/High fat drink (LCHF) on energy balance components. After the exercise energy expenditure was replaced with two different drinks, participants were studied across four days, where appetite, energy intake, activity- and total energy expenditure were evaluated. This chapter showed two novel findings - an increased cumulative activity energy expenditure and a decreased appetite for the HCLF treatment compared with LCHF condition. Therefore, it showed that a nutritionally different energy replacement post-exercise could lead to divergent responses on energy balance and appetite components.

Following these observations, Chapter 4 investigated the effects of a carbohydrate-fed (FEDex) versus an overnight-fasted (FASTex) exercise bout on the components of energy

balance (i.e., energy intake, activity and total energy expenditure, appetite) and interstitial glucose metric across four days. Although no significant differences between the two conditions were observed, the study expanded the previously published data time frame, providing a more comprehensive understanding of acute and long-term energy balance studies. The findings suggest that both approaches could be used interchangeably as exercise strategies for body mass management.

Finally, Chapter 5 investigated the relationship between the habitual diet of individuals, considering both nutrients and energy intake, and their physical activity energy expenditure (AEE). The study was conducted using data from the UK Biobank cohort, where a Random intercept model was fitted from 52,643 participants. The study tested the predictability of two models - one including carbohydrate and energy intake, and the other including only carbohydrate intake - on the AEE levels. Although both models predicted around the same percentage of the variance (~2%) for AEE, the model that only included carbohydrates presented a better goodness of fit/complexity ratio according to the Akaike Index Criterion (AIC). These findings showed that habitual carbohydrate intake weakly but significantly predicts AEE levels in the UK Biobank cohort, suggesting that the effects of carbohydrates are not only due to their energy content but also their metabolic effects.

Overall, this thesis has provided new data suggesting that carbohydrate availability/metabolism around exercise/physical activity can affect AEE and appetite differently. These results fit some features of previous theories (e.g., glucostatic, glycogenostatic, and hepatostatic), although the observed responses are weaker than previously thought.

Dedication

To my son Benjamín, my wife Soledad, and my parents, Claudia, Herminia, and Álvaro.

Acknowledgements

First, I would like to thank my beloved wife for all her support and understanding over the past four years. Her constant encouragement has motivated me to be better. Without her and our son Benjamin, nothing would have been possible.

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Fourth, I would like to express my gratitude to my supervisors, Gareth and Andy. Their mentorship, patience, and expertise have been invaluable in shaping my growth as a scientist. I am grateful for the opportunity to work with them and for their continuous support.

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Student: Dr. Einstein, aren’t these the same questions as in last year's exam?

Dr. Einstein: Yes, but this year the answers are different.

— **Albert Einstein**

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COVID-19 statement:

The COVID-19 pandemic had a significant impact on the amount of work included in this thesis. Due to restrictions, there was no access to facilities or participants for 18 months during the four-year PhD process. As a result, a third experimental chapter had to be replaced with an observational study that was conducted using the UK Biobank.

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Thesis outline:

The present thesis investigated the short-term effects of dietary carbohydrates and physical activity/exercise interactions on energy balance components (such as energy expenditure, energy intake, and appetite). The thesis begins with Chapter 1, the General Introduction to the thesis. This section provides a narrative review of the potential short-term modulators of energy balance components with a special focus on carbohydrate availability and metabolism. Chapter 1 finished by providing the research gaps and the thesis aims. Chapter 2 describes the General Methods used in this thesis. The specific protocols used during the experimental chapters are described in the relevant sections. Chapter 3 is the first experimental chapter of the present thesis. It investigates the short-term effects of replacing exercise energy expenditure with a high carbohydrate/low fat (HCLF) or a Low carbohydrate/High fat drink (LCHF) on energy balance components. Chapter 4 explores the effects of a carbohydrate-fed (FEDex) versus an overnight-fasted (FASTex) exercise bout on the components of energy balance (i.e., energy intake, activity and total energy expenditure, appetite) and interstitial glucose metric across four days. Chapter 5 investigates the relationship between the habitual diet of individuals, considering both nutrients and energy intake, and their physical activity energy expenditure (AEE) in a sample of the UK Biobank cohort. Lastly, Chapter 6 summarises key findings from Chapters 3-5, discusses the implications of the research, and suggests areas for future research in light of relevant literature.

Abbreviations

AA amino acid

AEE activity energy expenditure

AT adaptive thermogenesis

BMI body mass index

CCK cholecystokinin

EB energy balance

ECW extracellular water

ECs Energy compensation responses

EE energy expenditure

EI energy intake

FFA free fatty acids

FFM fat-free mass

FM fat mass

GH growth hormone

GLP-1 glucagon-like peptide-1

Gluc-ox glucose oxidation rate

ICW intracellular water

Lip-ox lipid oxidation rate

MM skeletal muscle mass

NEAT non-exercise activity thermogenesis

NP natriuretic peptides

nREE non-resting energy expenditure

OM masses of high metabolically active organs

PNS parasympathetic nervous

Prot-ox protein oxidation rate

PP pancreatic polypeptide

PYY peptide YY,

RMR resting metabolic rate

rT3, reverse T3

SNS sympathetic nervous system activity

TEE total energy expenditure

TEI total energy intake,

TEF thermic effect of food

TSH thyroid-stimulating hormone

TV television

T3 triiodothyronine

T4 thyroxine

VO_2max maximal oxygen uptake

Chapter 1: General Introduction

1.1. Introduction

In the last five decades, the prevalence of overweight and obesity has tripled worldwide (Bentham et al., 2017; Di Cesare et al., 2016). According to the World Health Organisation, 1.9 billion people aged 18 years and above live with overweight or obesity (World Health Organization, 2016). The excess body mass is associated with a higher risk of morbidity and mortality. Moreover, it has been related to cardiovascular disease, type 2 diabetes, several types of cancer, non-alcoholic fatty liver disease, respiratory dysfunction, hormonal alterations, muscle-skeletal problems and premature death (Di Angelantonio et al., 2016; Heymsfield & Wadden, 2017; Jayedi et al., 2020; Khan et al., 2018; Kotsis et al., 2018; Mafort et al., 2016; Nyberg et al., 2018; Ortega et al., 2016; Renehan et al., 2008; Ye et al., 2018). A simplistic explanation for obesity would be a long-lasting positive energy balance due to the increased energy intake (EI) and decreased energy expenditure (EE) by the constant pressures of the obesogenic environment. However, the path to obesity is a long and complex process, with several causes like genetic, social pressures, environment, epigenetic, physiology, and behaviour interacting to promote overweight and obesity (Blüher, 2019; Bray et al., 2018; González-Muniesa et al., 2017).

The treatments for people living with overweight or obesity are focused on manipulating the energy-balance variables (EB), attempting to establish a negative-energy balance to lose body mass. The first line of treatment is intensive lifestyle modification programmes. Here, diet, exercise and psychological/behavioural interventions are the cornerstones. It is common for weight loss achieved through these treatments to reach its peak in the sixth month, resulting

in a loss of 5-10% of the initial body weight. However, their effectiveness tends to decrease in the twelfth month, with only 3-5% of the initial body weight being lost (Heymsfield & Wadden, 2017). Even if these results can be considered successful, it is necessary to weigh them against the high drop-out prevalence – close to 50% of the people leave their treatment before completion (Herring et al., 2014)– and biological variability (Bray et al., 2019; Dent et al., 2020), undermining the potential benefits. For the people who adhere to lifestyle treatments, another problem arises when they try to keep the weight off. Indeed, studies have shown that 80% of the body weight lost is regained within the next five years after completing lifestyle treatments (Hall & Kahan, 2018; Langeveld & Devries, 2015).

The next line of treatment is pharmacotherapy. It is utilised with people who could not lose weight with lifestyle treatments or those with BMI above 30 kg/m^2 or BMI > 27 kg/m^2 plus any cardiovascular comorbidity (Patel, 2015; Pilitsi et al., 2019). On average, pharmacotherapy reduces at least 5 to 10% of initial body weight (Khera et al., 2016) and as much as 15% (Wilding et al., 2021). Depending on the medicament of choice, the proportion of participants achieving at least 5% and at least 10% of weight loss varies between 44-75% and 20-54%, respectively (Khera et al., 2016). However, as obesity is a chronic relapsing condition, maintaining these results requires continuous efforts (Daneschvar et al., 2016; Tak & Lee, 2021; Wadden et al., 2021).

The last method in obesity management is surgery. This is recognised as the most effective therapy in the mid-long term for people with a BMI > 40 kg/m^2 or 35 kg/m^2 with any other disease (Bray et al., 2016). Gastric bypass and vertical sleeve gastrectomy are the most practised techniques, and they achieve a weight reduction of 20-35% of the initial body

weight at years 2 or 3 (Heymsfield & Wadden, 2017). However, despite being considered the most effective long-term treatment for obesity, close to 20-30% of the patients do not achieve the target body weight after surgery (Karlsson et al., 2007), and 20-25% of weight regain happens within ten years after the surgery (Kanerva et al., 2017; Sjöström et al., 2007). Also, it is important to mention that the current access to these interventions is still limited due to the high treatment costs.

Even though all these treatments can produce substantial weight loss, most patients regain body weight. This can be partly attributed to physiological, psychological, and environmental factors that resist weight loss. Such resistance may lead to energy compensation responses (ECs), which involve decreased energy expenditure and increased appetite/energy intake, undermining compliance, and long-term results. Therefore, this General Introduction uses the literature presented to develop previously unexplored areas that form the basis of the aims and subsequent experimental chapters within the thesis.

1.2. Energy compensation: metabolic and behavioural adaptation to weight loss

As is well known, the responsiveness to weight-loss interventions is variable. The results depend on the interactions between environmental, behavioural, genetic, and physiological factors. In this regard, when an energy deficit is imposed, several physiological processes are triggered to achieve energy balance and prevent energy shortage. These are known as energy compensations. Even though it is highly complex, it can be simplistically summarised as increased EI or decreased EE. Among all the factors affecting energy balance, the most predominant energy compensation is increased EI (J. L. Dorling et al., 2019). Quantitatively, it

has been estimated that the EI contributes nearly 70-75%, and EE 25% of the total energy compensations (Polidori et al., 2016; Stubbs et al., 2021).

During negative energy balance, the body increases hunger and reduces satiety to stimulate EI. However, an individual's energy intake is ultimately determined by their eating behaviour traits (self-control, disinhibition, and self-efficacy). Several theories have been proposed to explain the physiological signals behind the upregulated appetite levels. The body's substrate balances (i.e., fat, carbohydrate, and protein content) have been pointed out as a potential factor involved. On the other hand, irrespective of appetite origin, the studies that have targeted hunger, fullness, and satiety to favour weight loss have shown mixed results (Halford et al., 2018; Hansen, Andersen, et al., 2019; Hansen, Mead, et al., 2019). Nonetheless, it is essential to note that changes in appetite do not always translate directly into modifications of EI (Sadoul et al., 2014).

Regarding energy expenditure, most of the adaptations are involuntary and unconscious. For instance, the resting metabolic rate (RMR) and spontaneous physical activity decrease, and movement efficiency increase (Martin et al., 2011; Villablanca et al., 2015). Among those, one of the most well-characterised responses is the disproportionate reduction in RMR (Careau et al., 2021). Early in the weight loss process, the RMR denotes a rapid decline (observed within three days of energy restriction), usually beyond what would be expected after adjusting for body mass loss (the so-called adaptive thermogenesis (AT)). With further body weight loss, the RMR decreases proportionally to body mass changes. During weight loss maintenance, the RMR shrinkage persists and will only be recovered when fat mass levels are restored (Müller et al., 2016). Furthermore, data looking at changes in spontaneous physical

activity during weight loss, have shown mixed results, possibly linked to the variety and the low sensitivity of the methods utilised during free-living conditions to detect subtle changes in low-intensity physical activity (Silva et al., 2018; Washburn et al., 2014). Section 1.3 will provide a clear description of the weight loss phases for better comprehension.

The weight-reduced state's adaptations seem to continue even after a new energy balance is reached (Fothergill et al., 2016; Leibel et al., 1995; Leibel & Hirsch, 1984; Rosenbaum et al., 2008; Sumithran et al., 2011; Weyer et al., 2000). An example of this is Fothergill's research on the participants of the TV show "The Biggest Loser" (Fothergill et al., 2016). The study revealed that even six years after the intervention, the participants experienced a reduction of 499 ± 207 kcal/day in their RMR after adjusting for body mass. Notwithstanding, the participants with the largest RMR reductions had higher weight loss maintenance rates, possibly due to increased physical activity levels (Kerns et al., 2017). Reflecting that, even if physical activity can voluntarily increase EE, the long-lasting metabolic adaptations will require continuous active effort to sustain this effect (Kerns et al., 2017; Ostendorf et al., 2019). Conversely, recent perspectives propose that AT would be part of a normalisation attempt of energy homeostasis more than the body's intention to regain weight (DeBenedictis et al., 2020; Martins, Dutton, et al., 2020; Martins, Gower, et al., 2020).

In summary, with diet and/or exercise interventions, ECs reach an average of 30-40% of the energy deficit through changes in energy intake and expenditure (Doucet, McInis, & Mahmoodianfard, 2018). These compensations are not usually captured by short-term studies (up to 24 hours). Consequently, to obtain a complete picture, more extended follow-up periods may be needed (e.g., two to five days), as a lag of days has been reported before

the ECs take effect (Bray et al., 2008; Champagne et al., 2013a; Rocha et al., 2013). Section 1.3 will delve into how these changes work by elaborating on the physiological effects of weight loss and reduced body weight.

1.3. Physiological changes during weight loss and maintenance.

For changes in body weight, whether weight gain or loss, fat mass (FM) and fat-free mass (FFM) will contribute to energy partitioning. Dugdale and Payne denominated that proportion as the "p-ratio hypothesis" (Dugdale & Payne, 1977; Payne & Dugdale, 1977b, 1977a). They described how the protein stores would vary constantly with changes in energy balance. Afterwards, according to Webster's work (Webster et al., 1984), this proportion was established to be around 0.25 kg of FFM per kg body weight lost (also known as the "quarter rule"). However, Forbes and colleagues found different results (M. R. Brown et al., 1983; Forbes, 1987; Forbes et al., 1986). Forbes showed that FFM changes are more pronounced for low initial body-fat levels and vice versa. Consequently, he realised that this proportion varies individually and according to the direction and magnitude of the energy imbalance (Hall, 2007). In this regard, a more contemporary model by Thomas and colleagues, with a considerable sample size from the US national health and nutrition examination survey (NHANES), illustrates that for an average person, the contribution of FFM to every kg of weight lost is 0.32 and 0.37 kg for men and women, respectively (Thomas et al., 2010).

When an intervention establishes a negative energy balance, a variable amount of FM and FFM will be lost. As losing weight is not a linear process, the proportion of FM and FFM will vary over time depending on several physiological and behavioural adaptations. Within these factors, we can distinguish that some adaptations will be more predominant across weight

loss phases (Figure 1). Regarding the characteristics of each phase for weight loss, i.e., the hormonal milieu, substrates predominance, and the energy partitioning ratio, it is possible to split them in three. Due to the third phase only being observed during extreme conditions (e.g., famine or starvation), only the first two phases plus the weight-reduced state will be explained here —as they are most commonly observed during voluntary/therapeutic weight loss (Heymsfield et al., 2011; Müller et al., 2015, 2016).

The first phase typically lasts one to four weeks, during which the body loses a high proportion of FFM compared to FM. However, FFM loss is mainly water, proteins, and glycogen due to reduced insulin secretion. Although, it has been correlated to the early drop in RMR (Heinitz et al., 2020; Müller et al., 2015). Indeed, due to the reduced FM contribution, the energy content for each kilogram lost during this phase is around 4400 Kcal. The second weight loss phase follows, lasting up to 100-300 days. Here the contribution of FFM is reduced, and FM reaches 66% of the total body weight lost. Consequently, the energy content per kilogram of body weight changes, increasing to 5200-7100 Kcal. The AT remains constant in this phase without additional changes from phase one. However, RMR and the non-resting energy expenditure continue decreasing proportionally with body mass. Usually, the higher the starting fat levels, the longer each phase is. The changes in FM and FFM during phases one and two, have been identified as possible drivers of increased hunger and decreased satiation during weight loss (Dulloo, 2021; Dulloo et al., 2012).

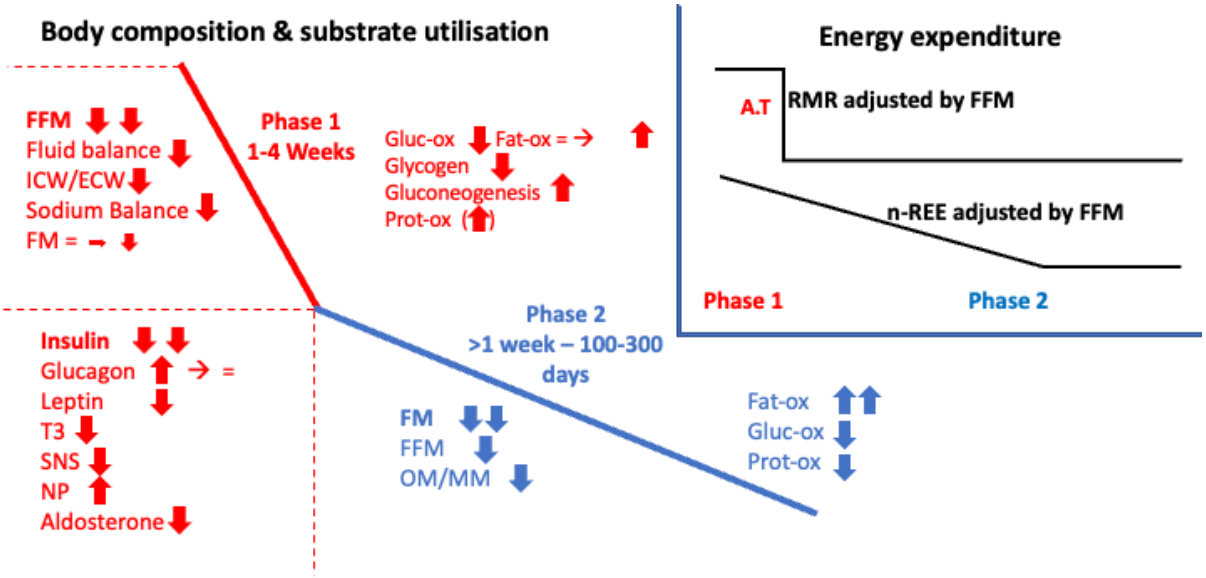


***Figure 1.1. Weight loss phases**. Overview of the metabolic adaptation that occurs during weight loss and maintenance of reduced body weight. When a person begins a caloric restriction (first week), their body goes through an adaptive thermogenesis (AT) phase, where the resting component of energy expenditure (RMR) decreases due to a depletion of hepatic glycogen stores caused by a decrease in insulin secretion. This leads to the mobilization of glycogen, which affects fluid balance and FFM. AT is an immediate response to negative energy balance and is part of the body's weight regulation mechanism. During phase 2 of weight loss, the body experiences a loss of FM in response to the negative energy balance, and this continues until a settling point is reached, where a new steady state is achieved. The graph inserted on the right shows that during phase 1, AT causes a decrease in the resting energy expenditure, which remains constant during further weight loss and successful maintenance of reduced body weight. On the other hand, the non-resting component of energy expenditure (nREE) decreases proportionally to weight loss. Early changes in nREE have not been thoroughly investigated, and data is only available after 3 weeks of semi-starvation. ECW (extracellular water), FFM (fat-free mass), FFA (free fatty acids), FM (fat mass), Gluc-ox (glucose oxidation rate) ICW (intracellular water), lip-ox (lipid oxidation rate), MM (skeletal muscle mass), NP (natriuretic peptides), OM (masses of high metabolically active organs), prot-ox (protein oxidation rate), SNS (sympathetic nervous system activity), and T3 (triiodothyronine). Adapted from Muller et al., 2016.*

For therapeutic weight loss interventions, after achieving the targeted weight reduction, weight maintenance follows. The weight-reduced state is characterised by decreased energy expenditure (resting and non-resting EE components), fat mass, leptin levels, T3 and T4 hormones, satiation, and sympathetic nervous system activity (Aronne et al., 2021). It also increases the reliance on carbohydrates for energy production, nutrient uptake and storage (to reach a positive fat balance), hunger levels, and muscle work efficiency. For differences between active weight loss and weight-reduced state, refer to Table 1.1

Table 1.1 Comparison of adaptations during weight loss and weight-reduced state.

	Active weight loss	**Reduced body weight**
Prior metabolic state	Usual Body weight (EB)	Weight loss (negative EB)
Current metabolic state	Negative EB	EB
Energy expenditure	↓↓ RMR	↓ RMR
	↓ non resting EE	↓ non resting EE
	↑Muscle contraction efficiency	↑Muscle contraction efficiency
Neuroendocrine axes	↓↓ T3, T4, TSH. ↑rT3	↓ T3, T4, TSH. ↑rT3
	↓↓ Leptin/FM	↓ Leptin/FM
	↑ Cortisol	Normal [Cortisol]
	↑ GH	No change or small ↑ GH
Autonomic nervous system	↑↑ PNS ↓↓SNS tone	↑ PNS ↓ SNS tone
Energy intake/appetite	↑↑ Hunger ↓↓ Satiation	↑ Hunger ↓ Satiation

*EB, energy balance; EE, energy expenditure; FM, fat mass; GH, growth hormone; PNS, parasympathetic nervous system; RMR, resting metabolic rate; rT3, reverse T3; SNS, sympathetic nervous system; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone. Adapted from (*Aronne et al., 2021a)

In summary, the amount of energy required to change body weight varies depending on several factors such as biological sex (Millward et al., 2014), body weight status (normal, overweight, or obesity), and the weight loss stage. Additionally, the expression of the physiological and behavioural mechanisms linked to the ECs will vary depending on the extension and rate of weight loss during lifestyle interventions (Stubbs & Turicchi, 2021). These factors will be further explored in sections 1.4 and 1.5.

1.4. Energy balance: the reciprocal relationship between energy- intake and expenditure.

To fully grasp the concept of energy compensations, it is necessary to define energy balance and its regulation. The energy balance is a dynamic state where the energy needs are matched by energy intake over time, and any difference between them is expressed by changes in energy stores (i.e., energy intake - energy expenditure = energy stores) (See Figure 1.2). Some also differentiate between energy balance and substrates' balance, arguing that the latter is also important for regulating body weight and composition (Galgani & Ravussin, 2008; Müller, Geisler, Heymsfield, & Bosy-Westphal, 2018).

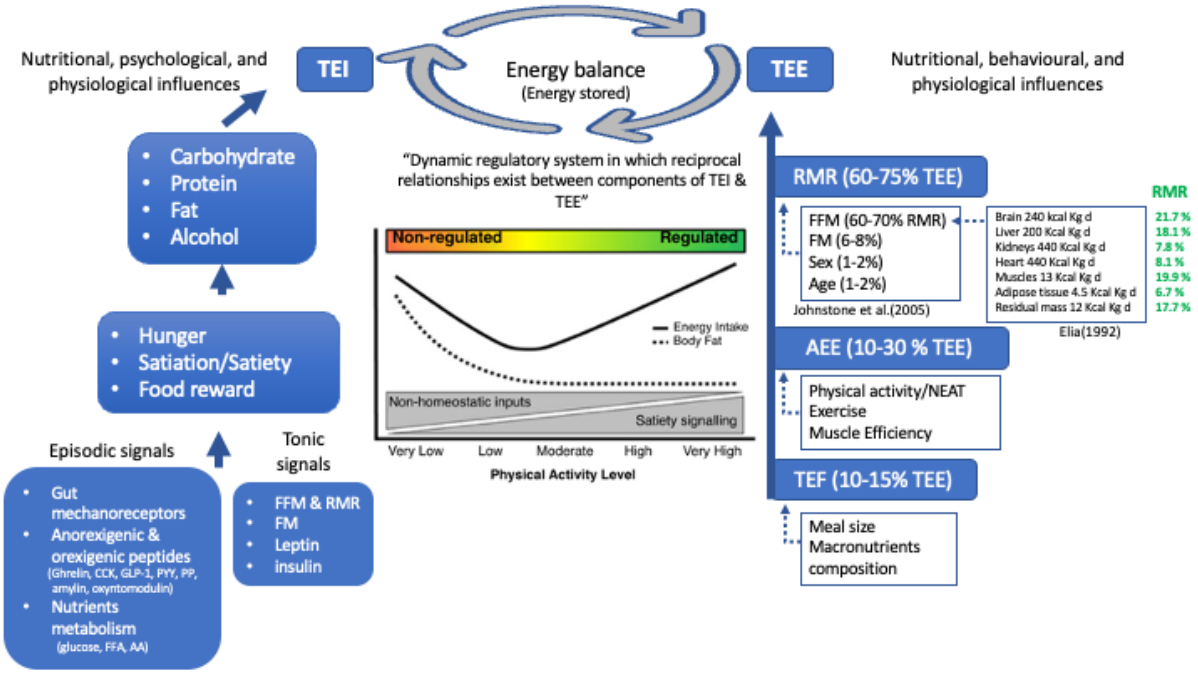


Figure 1.2. Overview of how energy balance is regulated*. The regulation of appetite can be better understood within the context of energy balance, which involves the balance between energy intake and energy expenditure. In this context, food intake does not only vary in response to energy homeostasis. Indeed, non-homeostatic factors such as food reward also modulate eating behaviour. Appetite regulation mechanisms involve excitatory and inhibitory feedback signals reflecting episodic (acute) and tonic (long-term) energy availability. Tonic mechanisms play a significant role in the regulation of appetite by providing a stable link between metabolic requirements, stored energy, and energy intake on a day-to-day basis. The inhibitory action of leptin has traditionally been considered the main feedback signal involved in appetite regulation. However, it is now acknowledged that metabolically active tissues also provide an enduring eating signal. Episodic signals (e.g., CCK, GLP-1, PYY, Ghrelin), on the other hand, respond to the presence or absence of nutrients in the gastrointestinal tract and are known to influence subjective appetite (i.e., hunger, satiety, satiation) and food preferences (eating timing, and type/amount of food). The following abbreviations were used: TEI = total energy intake, TEE = total energy expenditure, NEAT = non-exercise activity thermogenesis, CCK = cholecystokinin, PP = pancreatic polypeptide, PYY = peptide YY, GLP-1 = glucagon-like peptide-1, FFA = free-fatty acid, AA = amino acid, FFM = fat-free mass, FM = fat mass, AEE = activity energy expenditure, and TEF = thermic effect of food. Adapted from* Casanova et al., 2019

The first part of the energy balance equation is energy intake (EI). The control of EI is determined by homeostatic and non-homeostatic factors. The homeostatic control is mainly achieved by the physiological effects of episodic and tonic signals on appetite (J. E. Blundell et al., 2020). The short-term EI regulation (i.e., within a day) is subjected to psychophysiological factors that will modify appetite levels. Daily appetite depends mainly on food characteristics related to digestion (mechanical receptors, macronutrient metabolism, and gut peptides), hedonic components (liking and wanting), and circadian regulation (J. E. Blundell et al., 2020). These factors produce changes in the episodic signals (anorexigenic: GLP-1, CCK, PYY; orexigenic: ghrelin), affecting satiation, satiety, reward, and appetite (Carreiro et al., 2016). In this line, the active form of ghrelin (i.e., the acylated form) is the only known orexigenic hormone. Its influences on appetite/food intake, are mediated by increasing gastric emptying rate and decreasing the vagal-mediated satiation. Ghrelin displays a secretion pattern that peak before meals and decreases after eating depending on the caloric and macronutrient content (Yanagi et al., 2018), where carbohydrate intake induces a stronger suppressive effect compared with fats (Monteleone et al., 2003).

On the other side, long-term EI regulation is associated with FM and FFM. During energy balance, the influence of both compartments on EI is mediated by RMR (Hopkins et al., 2016), although FM plays a minor role. However, during an energy deficit, FM and FFM would directly increase appetite levels (Stubbs et al., 2018). Here, a reduction in leptin, which is a hormone generated by adipocytes in relation with the quantity of stored body fat, has been observed to lead to a decline in EE and an elevation in appetite, due to its impact on the central nervous system (Rosenbaum & Leibel, 2014).

The impact of EE on EI is not solely driven by RMR, but also by physical activity energy

expenditure (Edholm et al., 1955, 1970; Hopkins, Duarte, et al., 2019; Mayer et al., 1956; Piaggi et al., 2015). Research has shown that individuals who engage in consistent physical activity tend to display a spontaneous increase in their EI, which aligns with their EE, reducing fat mass accrual (Ando et al., 2019; Beaulieu et al., 2016a; Burton et al., 2010; Hägele et al., 2019a; Holliday & Blannin, 2014; Hume et al., 2016; Melby et al., 2017, 2019; Nas et al., 2020; Paris et al., 2016; Shook et al., 2015).

The appetite-related physiological signals interact with the neurocognitive (non-homeostatic) food-related cues (hedonics and eating-behaviour traits). Hedonics refers to beliefs and sensory appreciation of food. It is generally split into the "liking" and "wanting" components. The first reflects the degree of sensory pleasure, and the latter is the motivation to get a particular food. In turn, eating-behaviour traits are related to the level of dietary restraint, disinhibition, binge eating, and food cravings (Beaulieu et al., 2018). All these signals are incorporated by neurons in the hypothalamic area (Andermann & Lowell, 2017), modifying behaviour and starting the feeding process (Watts et al., 2022). It is worth noting that with the current obesogenic environment, the neurocognitive factors tend to override the homeostatic control of energy intake. For a more comprehensive understanding, please consult (Andermann & Lowell, 2017; Watts et al., 2022).

The second factor of the EB equation is energy expenditure (EE). The total energy expenditure (TEE) is composed mainly of three factors, RMR, AEE, and thermic effect of food (TEF). The RMR typically encompasses 60-75% of TEE, with FFM accounting for 70% of the RMR. The AEE is the second most significant contributor to TEE and the most variable. On average, it achieves 20-30% of TEE and rises to 50% in highly active people. The last component is TEF.

Usually, it varies between 10 to 15% of TEE, depending on the quantity and proportion of the different macronutrients within the diet (protein > carbohydrates > fats). In the last few decades, it has been suggested that TEE would have a ceiling. However, the literature shows ambiguous results.

As humans, we have a fine-tuned ability to keep our energy needs within balance. According to annual body mass trends, people increase their body mass by 0.5 kg *per* year. That reflects a mismatch of 0.27% between the energy needs and supply (Speakman et al., 2011a). This translates to an additional intake of approximately 9-18 kcal daily. Nevertheless, epidemiological data suggest that body mass would not change daily. Conversely, yearly body mass changes would follow a seasonal pattern (Turicchi, O'Driscoll, et al., 2020; Westerterp, 2020). The trends in body mass, alongside the information presented early in this introduction, indicate that our body has an asymmetrical energy balance control, responding more strongly to energy deficits than energy surpluses. This asymmetry is supported by extensive evidence that shows clear resistance to weight loss, but mixed results are shown for weight gain (Diaz et al., 1992; Levine et al., 1999; Lund, Gerhart-Hines, et al., 2020; Lund, Lund, et al., 2020; Murgatroyd et al., 1999; Ravussin et al., 2014, 2018; Sims et al., 1968; Stubbs et al., 2004). For a more in-depth understanding of the effects of overfeeding on energy balance, please refer to (Bray & Bouchard, 2020).

Consequently, several theoretical models have attempted to explain the circumstances and reasons behind the activation of body mass's defence system (i.e., energy compensation responses). The following section will explore the physiological cues involved in short-term regulation.

1.5. Short- to mid-term energy balance modulators.

Within the homeostatic control of body mass (or energy stores), the cues from substrate balances have been proposed to play a central role (Müller, Geisler, Heymsfield, & Bosy-Westphal, 2018). Indeed, the effect of fat, protein and carbohydrate stores has been tested, linking their utilisation with the subsequent need for restoration. Thus, several theories for each nutrient have been described: “Lipo-static” (Kennedy, 1953), “amino-static” (Mellinkoff et al., 1956), “protein-static” (Millward, 1995), “protein leverage” (Simpson & Raubenheimer, 2005), “gluco-static (Mayer, 1953) and glycogen-static”(Flatt J. P., 1987). Furthermore, other theories have changed the focus to temperature, energy, and gravity force (D. A. Booth, 1972; G. Booth, 1936; Ohlsson et al., 2018). Whilst no theory in isolation is likely to completely explain the regulation of energy balance, some emergent evidence and gaps in the literature implicate short-to-mid-term changes in carbohydrate availability in the modulation of ECs.

1.5.1. Carbohydrates and energy balance regulation.

In the past, several theories have suggested that carbohydrate balance could modulate energy balance. They based their assumptions on the limited quantity and tight regulation of carbohydrates within the body, compared to fat stores. Although the amount of blood glucose usually remains around 4-5 grams, despite variations in food intake or energy expenditure (Wasserman, 2009), glycogen stores can range from 13 to 125 g in the liver and 35 to 650 g in muscles (Gonzalez et al., 2019). In contrast, the fat stores, considering 75 kg, 15% of body fat man, will be around 10.3 kg. When compared, the energy stored as glucose/glycogen equals 1600-1800 kcal and fat mass 100,000-130,000 kcal. Therefore, during short-term

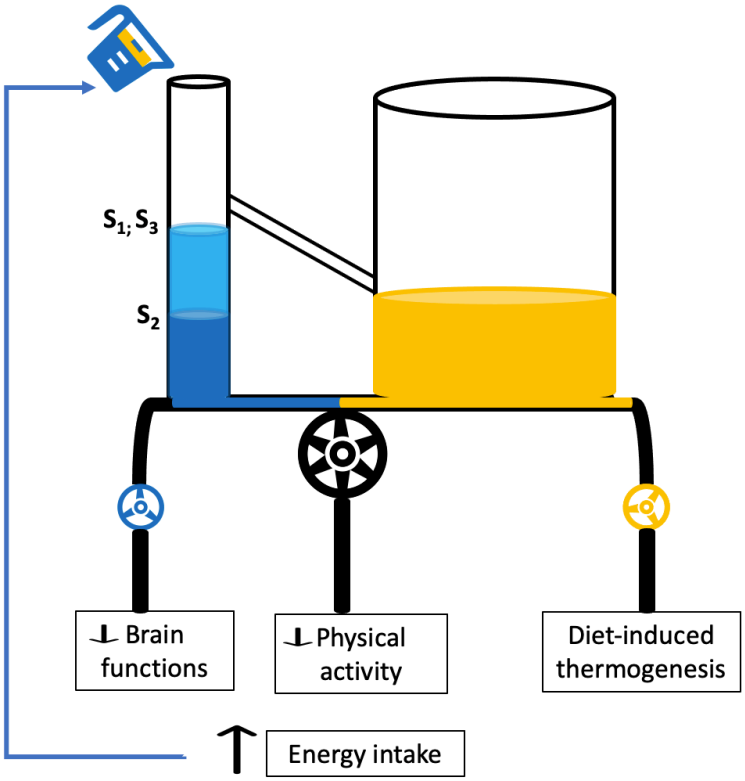
interventions (e.g., exercise and nutrition) and the initial stages of weight loss, carbohydrate stores are more likely to fluctuate than fat stores.

The glucostatic theory was the first to propose a role for glucose in energy intake regulation (Mayer, 1953). It has been shown in animals (Louis-Sylvestre & Le Magnen, 1980) and humans (Campfield et al., 1992a, 1996; Campfield & Smith, 2003; Kovacs et al., 2002; Melanson, Westerterp-Plantenga, Campfield, et al., 1999a; Melanson, Westerterp-Plantenga, Saris, et al., 1999; Wyatt et al., 2021), that variations in blood glucose levels are correlated with appetite and the initiation of food intake. In this regard, a study led by Campfield (Campfield et al., 1996) was the first to utilise continuous glucose monitoring in humans. Here 18 healthy participants, isolated from food and time cues, were assessed after spending the night in a laboratory. In a fasting state, appetite and blood glucose were tracked until the participants requested a meal. 83% of changes in participants' appetite ratings and meal initiation were preceded by brief glycemia declines (about 10% of baseline levels). However, the glucostatic theory does not consider the interdependence between substrate utilisation under different conditions, such as energy deficits or low glycogen levels (Iwayama et al., 2021).

Afterwards, another carbohydrate-related theory tried to expand this concept, with glycogen as a cornerstone. Jean Flatt (Flatt J. P., 1987) hypothesised that to preserve glucose availability, an inverse relationship between glucose/glycogen storage and food intake would exist. Flatt explained the glycogenostatic theory using a hydraulic model that involved two water reservoirs, one small for "sugar/glucose" and a large one for "fat," which moved three turbines –physical activity, brain function, and diet-induced thermogenesis. When glucose goes down, the hydrostatic pressure decreases, resulting in a low power output to feed the

turbines. This stimulated food intake to refill glucose levels and restore the correct function of the system (as shown in Figure 1.3). Nevertheless, even with the integration of lipid turnover, this theory ignores the mechanism that regulates the "fat" reservoir, assuming a passive regulation by calories and fat intake. However, as is well known, leptin, discovered a few years later (Zhang Y et al., 1994), plays a crucial role in signalling the energy stored in adipose tissue.

Figure 1.3. Glycogenstatic theory. *This hydraulic model represents how the energy intake is determined by*



changes in the content of two reservoirs that (the small in blue = sugar; and the big in yellow = fats) feed three turbines (blue turbine = brain activity; black turbine = physical activity; yellow turbine = diet-induced thermogenesis). When sugar levels fall from S1 to S2, the hydraulic pressure is reduced, downregulating the brain´s functions and physical activity. Consequently, the body increases food intake to recover the sugar levels (from S2 to S3). It is noteworthy that meals derived sugars and fats are stored in their respective reservoir, achieving the substrate balance again. Adapted from (Flatt J. P., 1987)

To test the glycogenstatic theory in humans, a group in the United States developed a carefully designed randomised crossover study (Snitker et al., 1997). Eight men stayed in a laboratory for 8-10 days to manipulate glycogen levels by applying different diets, exercise, and intravenous infusion protocols. In the first three days, they received a maintenance diet (50% carbohydrates, 30% fats, 20% proteins), after which they completed a glycogen-depleting exercise bout on a bike. Followed by a 3-day diet plan to increase muscle glycogen stores or keep them low. The high glycogen protocol consisted of 75% carbohydrate, 10% fat, 15% protein, plus 1.5 L intravenous glucose. The low glycogen condition consisted of 10% carbohydrate, 75% fat, and 15% protein plus 0.464 L of intravenous lipids. At this point, the vastus lateralis muscle glycogen was measured (high glycogen arm = 426 ± 87 mmol/kg dry muscle; low glycogen arm = 296 ±56 mmol/kg dry muscle). Then, both conditions were allowed to eat *ad libitum* for the next 48 hours. The results showed that *ad libitum* food intake was not affected by either of the treatments between day 1 and day 2. Lipid intake was reduced in the high glycogen arm from day 1 to day 2, and carbohydrate intake was unaffected. However, it is worth noting that a higher carbohydrate balance on day 1 was associated with a lower energy intake on day 2 (accounting for 9% of the variance).

Even if this study was adequately designed, some limitations are important to mention. First, the sample size was small and homogeneous (eight healthy white men); second, the liver's glycogen content was not assessed, so changes in this organ require further research. The conclusion obtained in this study suggests that daily carbohydrate balance is mainly achieved by reducing oxidation rates more than by increasing food intake, aligning with others (W. G. H. Abbott et al., 1988; Hopkins et al., 2011a; Shetty et al., 1994; Stubbs et al., 1993; Stubbs, Harbron, et al., 1995). Nevertheless, all the available data is based on muscle glycogen,

ignoring the possible influence of liver glycogen on energy balance (Edinburgh et al., 2019; Hopkins, 2019; Hopkins et al., 2014a; López-Soldado et al., 2015, 2017). Additionally, it is unknown if, in the presence of high physical activity, the reduction in carbohydrate oxidation is still enough to restore carbohydrate balance.

In 1963, Russek proposed a possible role of liver glycogen in regulating food intake (i.e., hepatostatic theory)(Russek, 1963). The hepatostatic theory states that the liver provides information to the central nervous system on intracellular glucose concentration, which was later supported by others (Niijima, 1969, 1984). Moreover, Russek suggested that glucose could be critical in food intake regulation, as hypoglycaemia increases hunger while hyperglycaemia and insulin increase satiety. A few years after, Russek modified his theory (Russek, 1981), describing that glucose *per se* could not be the direct stimulus for hepatic glucose receptors, moving the focus to "certain metabolic parameters" related to glucose or glycogen metabolism (i.e., pyruvate). In this way, the decreased absorption of glucose and amino acids, or depletion of liver glycogen, can lead to an increase in hunger levels because of alterations in intracellular pyruvate. Currently, new evidence has shown a strong correlation between fasting hunger ratings and the liver's metabolic activity (Casanova et al., 2022), further underscoring the liver's role in regulating appetite.

Russek's theory's foremost critics were based on the absence of a total hunger abolition with liver denervation. However, contrary to what is believed, the pathways that deliver the liver's information to the central nervous system are not only mediated by the vagal nerve. Indeed, only the neurotomy of the splanchnic nerves and the celiac superior mesenteric ganglion blunted the satiating effect of glucose and the counterregulatory response to hypoglycaemia,

and not the vagal nerve denervation (Barja & Mathison, 1984; Delaere et al., 2013; Fujita & Donovan, 2005; Mithieux et al., 2005; Soty et al., 2017; S. M. Ward et al., 2003). Furthermore, the liver, under certain circumstances, is more sensitive than the brain in detecting slow-onset hypoglycaemia (Soty et al., 2017). When experiencing systemic hypoglycaemia, maintaining portal vein euglycemia can suppress the counter-regulatory hormonal response, which increases epinephrine, norepinephrine, and glucagon (Donovan, Cane, et al., 1991; Donovan et al., 1994; Donovan, Halter, et al., 1991). On the other hand, portal hypoglycaemia is enough to trigger the counterregulatory hormones, even with systemic euglycemia (Lamarche et al., 1996). It is noteworthy that these counterregulatory responses are partially dependent on the liver´s glycogen levels. In a depleted state, the liver's endogenous glucose production is reduced, possibly leading to disturbances in glycaemic control (Winnick et al., 2016), which could modify energy intake.

Also, it is possible to find indirect evidence linking the liver with changes in food intake. In a study led by Hopkins (Hopkins et al., 2014a), women living with overweight and obesity completed either an overnight-fasted exercise bout (400 Kcal at 70% of the maximal estimated heart rate) or the equivalent time resting. After 60 minutes of recovery, food intake was assessed by an *ad libitum* meal test. Although the difference between treatments did not represent statistical significance, the between-individual variability was high (-234.3 to 278.5 Kcal). The only factor partially explaining the variance was carbohydrate oxidation during exercise, accounting for 37%. It is well known that maintaining normal blood glucose levels during exercise mainly depends on liver glycogenolysis. However, overnight fasting can reduce liver glycogen by 30-50% (Hultman & Nilsson, 1971). Reduced liver glycogen levels

may have affected the outcomes, but it cannot be confirmed as liver glycogen content was not measured. Further investigation is needed to clarify this aspect.

Something similar was seen in Edinburgh's research (Edinburgh et al., 2019), where 12 healthy, physically active men were recruited to determine the effects of skipping breakfast on 24-hour energy balance. The first arm of the protocol consisted of having breakfast followed by a resting period; the second was having breakfast and performing 60 minutes of cycling at 50% of peak power; the third was skipping breakfast and cycling using the same protocol. To assess energy intake, they completed an *ad libitum* meal test post-exercise, and then they were asked to eat the remaining meals of the day from a package provided by the investigators. Also, the energy expenditure and substrate utilisation were measured by indirect calorimetry during the exercise trial, infused [6,6-2H2] glucose tracers and a combined sensors (accelerometry plus heart rate sensor) device during free-living conditions.

The results showed that the energy intake during the *ad libitum* lunch test was increased in the exercise group that skipped breakfast, with an average of 166 kcal compared with the fed arm. However, this increase was insufficient to compensate for the energy deficit from skipping breakfast (430 kcal), leading to a - 400 kcal energy balance. To address the differences observed in the *ad libitum* meal test and within other variables, the researchers tested the correlation between plasma glucose utilisation and food intake, finding that a higher plasma glucose utilisation was associated with increased food intake.

Further evidence of the influence of carbohydrates on energy balance comes from mid-term studies (Martin et al., 2019; Polidori et al., 2016). The (Martin et al., 2019) study showed an increasing relationship between exercise doses (8 or 20 Kcal *per* kg body weight *per* week, between 65-85% VO_2max) and energy compensation after 24 weeks of training. An interesting feature of this study is that the energy compensators tended to have metabolic syndrome at baseline, showed blunted increments of VO_2max, had pronounced reductions in fasting glucose, and reported increasing cravings for sweets compared with their counterpart. Additionally, the study of sodium-glucose cotransporters-2 inhibitors and energy compensation has shed light on the relationship between increased energy output (glycosuria), weight loss, and food intake (Polidori et al., 2016). Here, glycosuria was increased passively (90 grams/day), simulating an enhanced energy output while participants remained blinded—avoiding behavioural compensations (i.e., one good habit replaces a bad one). The results showed that even blinded, the participants increased their food intake spontaneously (100 kcal per kg of body weight lost). However, it is impossible to distinguish if the increases in energy intake were mediated by the negative energy- or carbohydrate balance. Additionally, no physical activity energy expenditure measures were obtained during this trial, but new data has shown reductions in physical activity levels when glucose-lowering drugs are incorporated as a treatment (Yates et al., 2022)

Altogether, the links between carbohydrate availability/metabolism and energy balance can lead to design-specific studies testing the effects of manipulating carbohydrate availability (e.g., reduced intake or increased utilisation) on short-to-mid-term energy compensations, trying to distinguish the impact of the different carbohydrate sources.

1.5.2 Fat-free mass and energy balance regulation

Research has shown a strong correlation between FFM, RMR, and EI when individuals are in energy balance (Hopkins et al., 2016). Commonly, individuals with higher FFM display increased energy intake, primarily due to the mediation of a higher RMR (Stubbs et al., 2018). However, the correlation between energy expenditure and intake weakens considerably in the presence of high-fat mass (Casanova et al., 2021). Alternatively, other hypotheses have proposed different mechanisms to explain the influence of FFM on EI (Grannell et al., 2019), however they are out of the scope of this review.

During negative energy balance, evidence suggests a direct relationship between FFM loss, hunger, and desire to eat in men (Turicchi, Driscoll, et al., 2020). Likewise, the re-evaluation of the Minnesota semi-starvation study (Dulloo et al., 1997; Keys et al., 1950) identified that the after-dieting hyperphagia expression only finished when the pre-weight loss FFM levels were reinstated. Furthermore, the FFM loss correlates with a disproportionate decrease in RMR (adaptive thermogenesis) after adjusting to body mass changes (Müller et al., 2015). However, the reduction in RMR cannot be entirely attributed to the loss of highly active tissue (e.g., liver, kidneys, heart, etc.), as most of the tissue lost is composed of glycogen, proteins, and water excretion (Heymsfield et al., 2011). Alternatively, it has been suggested that adaptive thermogenesis could be linked to a glycogen-based “set-point” (Müller et al., 2016). If the latter is true, interventions utilising physical activity/exercise in the presence of reduced carbohydrate availability, have the potential to produce sharp reductions in glycogen stores, prompting substantial energy compensation responses.

The short-term nature of adaptive thermogenesis highlights that mild changes in FFM or its composition could modulate energy balance (Heymsfield et al., 2022). However, to test this hypothesis, more studies using high-resolution body composition analyses under strictly controlled conditions are required.

1.5.3. Energy-constrained model and energy balance regulation

Some recent studies have proposed another perspective related to where the body centres energy balance control. Because food intake is always mediated by behaviour, the latest theories centre the control of energy balance to the modulation of energy expenditure above energy intake (M. J. Müller & Geisler, 2017; Manfred J. Müller et al., 2016). Thus, with any energy balance alterations, the body will defend itself by the only direct mechanism available, modifying the energy expenditure to achieve a "new" energy balance.

Herman Pontzer's energy-constrained model (Pontzer, 2015) and the ActivityStat hypothesis (Gomersall et al., 2013) suggest a limit to the total amount of energy a person can expend. This means that, for example, engaging in exercise will not result in a linear, additive increase in energy expenditure (Pontzer et al., 2016). However, this is still debated (Gonzalez et al., 2023; E. Robinson & Stensel, 2023). The mechanisms are supposed to be driven by an energy balance's central control, which will redistribute the energy by downregulating other physiological functions (e.g., immune- or reproductive functions) to try to reduce the total energy expenditure (Pontzer, 2018).

Likewise, some studies involving high physical activity levels have supported a ceiling on energy expenditure. After an initial increase in energy expenditure due to physical activity, the total energy expenditure will plateau without further rising (Thurber et al., 2019; Westerterp et al., 1992), however it is still possible that reductions in other physical activities could account for these missing calories (Mansfeldt & Magkos, 2023). Furthermore, if these efforts to increase physical activity remain over time, the energy expenditure will be modulated by increasing mechanic/metabolic efficiency to compensate and preserve energy for other functions (e.g., immune, reproduction and stress responses).

1.6. Summary

Obesity and overweight are common health issues worldwide, and many approaches are used to prevent and manage them. However, most of these strategies lack long-term effectiveness due to energy compensation. This means that weight management therapies are often compromised by a decrease in energy expenditure and an increase in appetite or food intake, resulting in difficulties with weight loss and maintenance.

While there are theories about the mechanisms that regulate energy balance (homeostatic and non-homeostatic), none can explain all the issues. Therefore, further exploring mechanisms that modulate energy balance using different time scales could lead to developing more accurate and personalised strategies to counteract the effects of energy compensations. In this context, due to the propensity of carbohydrate metabolism and stores to fluctuate acutely after interventions with exercise and nutrition, this macronutrient could be involved in modulating energy balance components in the short term. Consequently, additional research on this topic could help to shed light on short-term energy balance fluctuations.

1.7 Aims and objectives of this thesis.

The overall aim of the thesis is to understand better how, from a metabolic perspective, dietary carbohydrates and physical activity/exercise interact in the short-term to modulate energy balance components (such as energy expenditure, energy intake, and appetite). The characterisation of these responses will help inform the development of integrated strategies (exercise plus nutrition) to manage energy balance.

The specific objectives of the experimental chapters of this thesis were to:

1. To investigate the effects of an overnight-fasted aerobic exercise session followed by a high carbohydrate/low fat or a low carbohydrate /high fat energy replacement on short-term energy balance components in physically active normal-weight individuals (Chapter 3).
2. To characterise the responses of energy balance components after overnight-fasted or carbohydrate-fed exercise bouts in physically active normal-weight individuals (Chapter 4).
3. To explore the associations between habitual diet, in terms of energy and nutrient intake, and physical activity energy expenditure (AEE) levels within the UK Biobank population (Chapter 5).

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Chapter 2. General Methods

This chapter will describe the rationale, principles and limitations of the methods used in this thesis. The detailed protocols are described later in the corresponding experimental chapters unless necessary to explain the principles behind each method.

2.1 Body composition—Bioelectrical Impedance Analysis (BIA)

BIA was used in chapters 3 and 4 to characterise participants' body composition. This method has been used since the 1980s, to assess body composition. BIA has shown a high correlation between the impedance quotient and measures of FFM, total body water, and body cell mass (Lukaski et al., 1985). The device measures the electrical impedance (i.e., the total opposition to an alternating current flow) and based on this, it can derivate a resistance number (the opposition to a current due to the resistivity of body fluids).

Theoretically, the volume of a conductor relates to its length and impedance. The latter varies according to the conductor resistivity and volume. The conductor in the body is represented by total body water (TBW), and usually, FFM is used as a surrogate for it. So, it can be assumed that the volume of the conductor (V) (i.e., FFM volume) can be calculated by utilising equation (1). Where the resistivity of the conductor (measured in ohm cm) is represented by p, and the conductor's length (measured in cm) is denoted by L. In the case of human body measurements, stature is used as an approximation for the true conductive length. The electrical resistance of the conductor (measured in ohm) is represented by R (Lukaski et al., 1985; L. C. Ward, 2019).

(1) $V = p\,(L^2/R)$

BIA can be performed utilising single or multiple frequencies. Commonly, single-frequency devices utilise a 50kHz frequency, which crosses the intra and extracellular fluid. On the other side, multifrequency approaches use a wide range of frequencies allowing the differentiation of ECW and ICW, consequently favouring a better estimation of body composition (U. Kyle, 2004). During this thesis, a tetrapolar hand-to-foot device was used (Bodystat Quadscan, 4000). The tetrapolar BIA has been shown to be a valid and reliable method to assess body composition in healthy people under controlled conditions (Lukaski et al., 1986).

The use of BIA devices has gained popularity because they are affordable, easy to transport, non-invasive, and require minimal technical training and time commitment. Also, they do not release any radiation compared with other methods (e.g., DEXA scan). On the other hand, they have a few limitations. First, as it is an indirect body composition method, it is based on some assumptions. Indeed, it measures resistance and converts it to total body water, then calculates a value for FFM based on the assumption that FFM has a hydration fraction of 0.73. Consequently, changes in hydration levels can impact the results (U. G. Kyle et al., 2004). Second, the algorithms utilised the average resistivity of different tissues/segments of the body, assuming that the body is a cylinder of uniform circumference. Third, the results are based on population-based resistivity measures, increasing the estimation error.

In preparation for chapters 3 and 4, participants were instructed to restrain alcohol intake and strenuous physical activity 48 hours before to minimise the risk of dehydration. All the tests were performed first thing in the morning after overnight fasting. The impact of any potential errors associated with the device's estimates was minimised as all the comparisons were made within-individual.

2.2 Indirect calorimetry:

2.2.1 Principles

Chapters 3 and 4 of this thesis used Indirect Calorimetry (IC) to estimate energy and substrate utilisation during both rest and exercise. Measurement of energy expenditure (EE) has been a fundamental part of nutrition and metabolism research. The gold standard method to assess EE is direct calorimetry. However, this process requires staying in a confined space and an expensive setup. To address these limitations, indirect calorimetry (IC) has been used as a valid alternative, as it has shown high correlations with values obtained through direct calorimetry (Jéquier & Felber, 1987). IC measures O_2 consumption and CO_2 production and based on different equations EE and substrate utilisation can be calculated. This tool is highly adaptable and can be used in a range of scenarios, including during exercise, rest, and critical care situations.

IC assumes that the gas exchange at the mouth reflects closely the gas exchange in the periphery at cellular levels. The amount of O_2 consumed and CO_2 production varies according to the substrate utilised. The utilisation of carbohydrates and fats differs mainly as the metabolism of a mol of glucose compared to a mol of fat utilises less oxygen and

produces relatively more CO_2. To quantify the utilisation of protein for energy production, urinary nitrogen excretion is commonly utilised. However, it has been established that not measuring urine nitrogen does not lead to significant errors in estimating substrate and EE, since the contribution of protein oxidation to energy production is negligible (Frayn, 1983). The ratio of O_2 and CO_2 in the expired breath is known as RER (i.e., respiratory exchange ratio). The RER is assumed as a proxy of the respiratory quotient (RQ). The RQ usually is measured in a specific tissue by the A-V method. Here, the O_2 and CO_2 concentrations from the arterial bed are compared with the O_2 and CO_2 on the venous bed, to account for the difference.

2.2.2 Assumptions:

To calculate EE and substrate metabolism, it is assumed that certain conditions are accomplished. The first one is that the O_2 and CO_2 measured on the expired gas come mainly from the oxidation of fat and carbohydrate and, in less proportion, protein oxidation. However, other processes like gluconeogenesis, lipogenesis and ketogenesis are known to consume oxygen as well.

Second, the bicarbonate pools in the body are stable. During exercise (above maximum lactate steady state (MLSS)) or certain levels of physiological stress (big burns or critical illness), the increased glycolytic flux produces higher levels of carbonic acid by the tamponing of hydron ions by bicarbonate. Then, carbonic acid dissociates into CO_2 and water, which can, in turn, alter the substrate and energy calculations.

During chapters 3 and 4, to standardise and minimise the influence of diet and other oxygen-consuming processes, participants were tested after 10-12 hours of overnight fasting. The exercise sessions were also conducted in accordance with IC assumptions, with a prescribed intensity (i.e., 70%VO_2peak) below MLSS and constant monitoring using a metabolic cart.

2.2.5 IC during rest and exercise:

2.2.5.1. Douglas bags: In chapters 3 and 4 of this thesis, energy and substrate utilisation during resting conditions were obtained by the combination of Douglas bags and gas analysers. Douglas bags (DB) are known to be one of the most reliable methods to collect expired gas. Here, using a mouthpiece and a nose clip, the exhaled air is stored and mixed in bags varying in capacity, favouring more stable and accurate estimations of energy expenditure and substrate utilisation while analysing gas concentrations. Although it is a reliable method in resting and exercise conditions, some limitations must be considered before implementation. First, this method does not allow for obtaining instantaneous results. Second, it is more time-consuming than other methodologies (e.g., metabolic carts). Third, using a mouthpiece and a nose clip can lead to an overestimated RMR of up to 8% (Forse, 1993).

During chapters 3 and 4, the resting $\dot{V}O_2$ and $\dot{V}CO_2$ concentration levels were measured with gas analysers (MOXAR Respirometry System, AEI technologies, USA), previously calibrated with two known concentration gases. Gas volume and temperature were measured by a dry gas meter (Harvard Bioscience, inc., Germany) and a gas thermometer (electronic temperature instruments, ltd., UK). Then, corrections for standard temperature and

barometric pressure were performed. Based on these values, substrate utilisation and energy expenditure were estimated utilising established equations (Elia & Livesey, 1992), which have been shown to have lower error percentages for calculations of energy expenditure when considering different protein oxidation rates as compared to Weir equation (Weir, 1949).

2.2.5.2 Metabolic cart:

In chapters 3 and 4, substrate and energy utilisation during exercise ($\dot{V}O_2$peak test and steady state exercise) were obtained by using metabolic carts. Metabolic carts have been widely used in the field of metabolism research. They usually have the advantage of giving instantaneous data, and be less time-consuming, with the option to have high resolution and granular data by using breath-by-breath analyses or to average values according to user's needs. One disadvantage of these systems is that most of them have their own assumptions and algorithms to calculate data (depending on the brand and software). This is potentially an important source of error for estimating VE, as it is calculated based on the flow rates. However, if they are well-calibrated, validated and maintained, the chances to have inaccuracies are reduced.

In this thesis, Vyntus metabolic carts were used (Vyntus, Vyaire Medical, US) for experimental chapters 3 and 4. This system has a $\dot{V}O_2$ electrochemical analyser and an infrared $\dot{V}CO_2$ analyser, with overall accuracy showing average absolute percentage error levels < 5% for gas exchange, substrate, and energy estimation outcomes(Van Hooren et al., 2023). To estimate substrate utilisation and energy expenditure during exercise, the equation of (Jeukendrup & Wallis, 2005) was utilised. This equation incorporated muscle

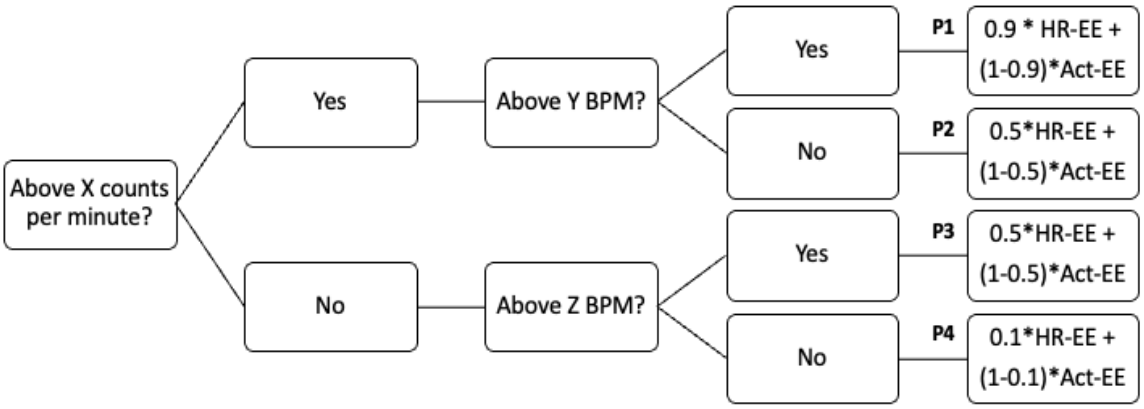
glycogen utilisation within the energy yield processes, contrasting with others that only considered glucose contribution (Ferrannini, 1988; Péronnet & Massicotte, 1991), leading to more accurate substrate oxidation rate calculations.

2.3 Activity energy expenditure during free-living conditions —ActiHeart (AHR)

The calculation of energy expenditure during free living conditions has been a challenge in the context of nutritional and exercise sciences. Although doubly-labelled water has been recognised as the gold standard method to estimate energy expenditure outside of the laboratory (Schoeller & van Santen, 1982), a few wearable devices has been shown to be reliable and cost-effective alternatives (O'Driscoll et al., 2020). In chapters 3 and 4 of this thesis, total- and activity-energy expenditure during free-living conditions were obtained from the data collected by Actiheart. This wearable device is the one with the highest correlation levels for activity- and total- energy expenditure, compared with the values measured by doubly-labelled water (Brage et al., 2015). The Actiheart utilised a previously validated algorithm based on a branched model (Brage et al., 2007) that combines the synchronised data from heart rate and accelerometry to estimate the total- and activity-energy expenditure. For improved accuracy, individualised calibration curves were built based on the values of heart rate and $\dot{V}O_2$ obtained in the submaximal and maximal exercise tests. Also, the measured RMR was included to improve further energy expenditure estimation. The participant's anthropometric information (i.e., body mass and height), date of birth, sex, sleeping- and maximum- heart rates are introduced into the software for data processing.

The Actiheart, according to its branched algorithm model, relies differentially on accelerometry or heart rate data depending on the intensity of physical activity (See fig. 2.1). During low-intensity physical activities, the AHR relies more on accelerometry, and during moderate to vigorous-intensity physical activities, the weighting of the heart rate for the algorithm increases, which favours a better estimation of physical activity energy expenditure. This is due to heart rate-based estimations tend to overestimate AEE, and accelerometry-based estimations tend to underestimate AEE (Crouter et al., 2008). For the accelerometry data, the main limitation is biomechanical as these devices are not able to differentiate between different activities (e.g., inclined vs flat walk/run, cycling, and weight-bearing activities). For heart rate data, the limitation is the biological variance, where differences by sex, age, BMI, training state, environmental temperature, hydration, and the quantity of working muscle mass during an activity can modify the relation between heart rate and EE.

The weighting of heart rate and accelerometry for the branched model depends on the activity (count per minute) and heart rate flex points (figure 2.1). Both show the cut-off where energy expenditure prediction switches between a linear projection from the origin to the activity and the HR EE equations. The activity flex point is a fabric-estimated cut-off point during specific tasks (e.g., walking and running). The flex HR point is the average of the highest HR above sleep during resting and the lowest HR during the incremental exercise treadmill test. The Transition HR is the average between the highest walking and slowest jogging HR during the same test (Brage et al., 2004).



X = X value has been chosen to ensure that "no movement" (including noise) will be quantified by the two lower boxes
Y = Flex HR: average above sleep of the highest HR during rest and the lowest HR during incremental exercise treadmill test.
Z = Transition HR: the average between the highest walking and slowest jogging HR.
HR-EE = Relationship between heart rate and energy expenditure.
Act-EE = Relationship between accelerometry and energy expenditure.

Figure 2.1. P1-4 are weighting factors that will determine the weighting given in the energy expenditure calculation to the values derived from HR-EE and Act-EE. X is used to distinguish between activity and no activity. In the presence of activity, Y is a heart rate threshold used to distinguish mainly between walking and running. When no activity is detected according to X, Z is used to distinguish between true HR increase due to activity or other factors (e.g., stress). Adapted from (Brage et al., 2004)

2.4 Energy intake assessment

2.4.1 Food diary

To estimate energy intake during free-living conditions, different methodologies can be used. However, none has been recognised as "gold standard". In this thesis, food diary records plus the provision of a food package were used in chapter 3. Participants were only allowed to eat from the food package provided and asked to bring back all food leftovers. Although it is known that this methodology usually tends to underreport food intake and can influence eating behaviour (observation/reactivity effect), it has shown moderate to high reproducibility (Tremblay et al., 1983). Indeed, food diaries combined with the

provision of a food package increase the reliability of food dietary records (Chaput et al., 2016; McNeil et al., 2012). Also, compared with 24-hour food dietary recall, food diaries tend to produce more accurate values for energy intake estimations, as it does not rely heavily on memory and should be completed at the time that the food has been eaten (prospective method). Furthermore, even if dietary records accuracy is not high, the concurrent measurement of energy expenditure can provide extra information to test the feasibility of the dietary records (Goldberg et al., 1991) during weight stability.

In this thesis, participants were instructed on how to complete food diary records during the free-living period (i.e., around 3 days). The mean reported food intake was calculated directly from the information collected from the food diaries (in chapter 3). Furthermore, the investigators subtracted the food leftovers from the total caloric content of the food package, allowing the cross-checking of the information obtained. In chapter 4, the calculation of the food intake by accounting for food leftovers was improved by providing three separated daily packages and asking participants to return the leftovers in the corresponding bag. Allowing the calculation of a daily energy intake instead of the cumulative energy intake.

2.4.2 Preload and *Ad libitum* meal tests

The preload ad libitum meal test has been extensively used in nutritional sciences to quantify energy and nutrient intake within laboratories. The highly controlled nature of this approach allows to obtain accurate and reproducible results for food intake (Gregersen et al., 2008; Horner et al., 2014; Laan et al., 2010). This test gives the participants a load (i.e., solid or liquid food), which is matched in almost every but one characteristic (e.g., different

energy content or nutritional composition) and then measures the satiety effects on total food intake during an ad libitum meal test (i.e., buffet or single course style). The standardisation of both preload and meal test is essential, as it has been shown that differences in the type or palatability of the meal tests can affect the results(Raynor & Epstein, 2000; T. Robinson et al., 2005). Furthermore, if the aim is to test the physiological effects of a specific preload, it is commonly applied a covert/placebo approach, where participants and the investigator are blinded, avoiding behavioural compensations.

This methodology has shown consistent results when an intra-individual repeated measure approach has been used under strictly controlled baseline conditions (energy balance status, feeding status, avoiding physical activity the day before, etc.) (Livingstone et al., 2000). Also, it is very important to set right the time between the preload and the meal test. This is since a short time between the preload and meal test can be utilised when the purpose is to test the sensorial properties of the preload. Conversely, a long time between them (~4 hours) would be better applied to test the post-absorptive/metabolic properties of the preload (J. Blundell et al., 2010a)

In this thesis, in chapter 4, the preload plus an ad libitum meal methodology was used to test the effects of a carbohydrate-based drink against a non-caloric placebo on energy balance behaviours. Here, after overnight fasting, one of the two drinks was consumed, and 4.75 hours later, a single course ad libitum meal test was performed. Participants were offered a warm portion of food and instructed to eat until they “felt comfortably full”. They were frequently provided with more food until they refer having finished. The test was completed in isolation, forbidding the use of cell phones and water consumption. All the

food was weighed before and after, allowing the calculation of the energy and macronutrient intake.

2.5 Dietary restraint levels:

Within the eating behaviour traits, the relation between disinhibition and restraint levels seems to modulate energy intake. Restrained eaters, as measured by the DEBQ, are defined as those individuals who can consistently restrict their food intake (Laessle et al., 1989). Indeed, they tend to eat less than unrestrained eaters under similar circumstances (Lluch et al., 2000; Polivy et al., 2020). Furthermore, it has been shown that restrained eaters tend to underreport energy intake more compared to unrestrained eaters (Olea López & Johnson, 2016; Rennie et al., 2006a). For these reasons, to ensure that participants in the present studies (chapters 3 and 4) did not over-monitor their diet, their restraint levels were assessed by utilising the Dutch Eating Behaviour Questionnaire's restraint subscale (DEBQ-R) (Van Strien et al., 1986). This questionnaire has good test-retest reliability and high internal consistency (Allison et al., 1992). The restrain subscale has 10 Likert questions, scored from 1 to 5. The minimum score possible is 8, and the maximum is 50. Only participants with average values below 3.0 were allowed to participate in the study (Rennie, Siervo and Jebb, 2006). This was done to eliminate the confounding effects of restraint levels on the study outcomes and to isolate the impact of the intervention on energy intake. As restrain levels seem to mediate the effect of fat mass and fat-free mass on energy intake (Hopkins, Finlayson, et al., 2019).

2.6 Appetite VAS

Visual analogue scales (VASs) have been extensively used as a valid and reliable psychometric tool to assess appetite levels (De Graaf, 1993; Rogers & Blundell, 1979; Stubbs et al., 2000a). These scales collect information on an individual's sensations and have been found to be reproducible when used after a meal or exercise intervention (Flint et al., 2000; Goltz et al., 2018; Laan et al., 2010). VASs are 100 mm horizontal lines with two anchors at either end. This line represents a continuum where participants are requested to mark a line according to the intensity of the subjective sensation at that specific time. Then, the sensation can be quantified.

In chapters 3 and 4 of the present thesis, the questions included were: How hungry do you feel now? How strong is your desire to eat? How full do you feel now? and how much do you think you can eat now? To allow the participants to answer the scales on their computers/mobiles/tablets, the investigator configurated an online questionnaire. The data were collected and managed using REDcap electronic data capture tools hosted at the University of Birmingham, UK (Harris et al., 2009). REDcap was programmed to email the questionnaires daily to the participants on a pre-specified schedule.

2.7 Blood Sampling & biochemical Assays.

In chapters 3 and 4 of this thesis, venous blood samples were obtained and collected into pre-chilled 6 ml EDTA tubes to analyse blood metabolites and hormones. Blood samples were taken by venepuncture and cannulation from the antecubital vein. A protease inhibitor (Pefabloc® SC, Merck, Germany) was utilised to preserve the integrity of the samples for the subsequent measurement of appetite-related hormone concentrations. Tubes were kept on ice until they were centrifugated at 4 ºC, 1500 g, for 10 minutes. Plasma aliquots were stored at – 70ºC until they were analysed.

Plasma samples were analysed using an automated clinical analyser (RX Daytona+ Randox, London, UK) to determine glucose (Glucose hexokinase, Randox, UK) and lactate (Lactate Dehydrogenase, Randox, UK) concentrations. The automated clinical analyser operates on the principles of measuring the absorbance of light coupled to NAD/NADH concentrations.

The manufacturer's instructions were followed, and calibration and quality checks were performed before each metabolite was analysed. An example of how an automated clinical analyser works is provided using a Lactate Kit (Randox in London, UK). The plasma sample was incubated with lactate oxidase, which broke down lactate into pyruvate and hydrogen peroxide. The reaction of hydrogen peroxide with a hydrogen donor was catalysed by the peroxidase enzyme, resulting in a purple product. The intensity of the purple product was finally measured by the automated analyser, and the metabolite concentration in the plasma solution was provided based on the calibration curve.

In chapter 3, insulin, acylated ghrelin, and leptin, sandwich direct-type ELISA assays were used (EMD Millipore Corporation, Missouri, USA), and in chapter 4, an ultrasensitive insulin ELISA kit (Mercodia, Uppsala, Sweden). All biochemical analysis was performed in duplicate, for intra-assay CVs, please see table 2.1 For a better understanding of ELISA assays, serum insulin is provided as an example. Serum insulin concentration was determined in duplicate using a commercially available kit (EZHI-14 k Human Insulin, Millipore, Missouri, USA). The ELISA method employs a direct sandwich technique with two monoclonal antibodies directed against the insulin molecule, located at different sites. The first antibody is bound at the bottom of a 96-well pre-titered microplate, where the sample is added. Additionally, biotinylated anti-insulin antibodies conjugated with horseradish peroxidase are added, binding to the insulin molecule's second site.

Throughout the incubation phase of this test, insulin within the sample joins with both antibodies. Any peroxidase-conjugated antibody that is not bound is washed away. The attached insulin is then identified through a reaction with 3,3',5,5'-tetramethylbenzidine (TMB) and the reaction is halted by the addition of hydrochloric acid. The absorbency at 450 nm is measured using spectrophotometry to determine enzyme activity by a Biotek 800 Absorbance Reader (Biotek Instruments, USA). After the acidification of the formed products, an increase in absorbency indicates the amount of captured human insulin in the unknown sample. This quantity can be obtained by interpolation from a reference curve created using reference standards that have known concentrations of human insulin.

Table 2.1 Measurement of variation.

	Intra-assay coefficient of variation (CVs) (%)
Plasma insulin	10[a] and 2.6[b]
Plasma leptin	2
Plasma Glucose	1
Plasma Lactate	1.3

Intra-assay CVs were obtained from technical repeats of the same analysis/plate. [a] Value obtained for (EMD Millipore Corporation, Missouri, USA) ELISA assay in chapter 3, and [b] ultrasensitive insulin ELISA assay (Mercodia, Uppsala, Sweden) in chapter 4.

2.8 Continuous Glucose Monitoring (CGM)

Continuous glucose monitoring has been an important part of the management of diabetes and the study of metabolism in the last years. These devices can measure the interstitial glucose concentration every five minutes. They work by inserting a transcutaneous amperometric sensor, which has a filament (catalytic biosensor) that is placed in the interstitial fluid. Here, the glucose oxidase enzyme reacts in contact with glucose, and then it translates into a current and values for glucose concentration (Monnier et al., 2020). Commonly, these sensors can be placed on the arms or abdomen. However, more accurate readings are obtained from the arms (Garg et al., 2022). Previously, most of the brands required their devices to be calibrated against capillary measures of glucose. However, new devices come factory calibrated (e.g., DEXCOM G7 and Freestyle libre 2), reducing the burden on the user. Most of the sensors have a life of between 7 to 14 days, and then the sensor must be replaced.

In Chapter 4, the CGM Dexcom G7 was utilised to monitor blood glucose levels mainly during free-living conditions. The mean absolute relative differences (MARD) between the Dexcom G7 CGM and repeated venous blood sampling measured with reference systems

have been reported to be 8.2% and 9.1% for the sensors placed on the arm and abdomen, respectively (Garg et al., 2022). This metric reflects a high level of accuracy, although accuracy tends to be slightly reduced right after inserting the sensor, during hypoglycaemia, and after rapid changes in glucose concentrations (Garg et al., 2022). During exercise, higher MARDs (around 13.63%) have been reported for different devices in the diabetic population (Moser et al., 2020). The same metrics for a healthy population remain to be tested.

Some artefacts can be produced after the insertion of the sensor, as the site suffers a small trauma affecting the readings. Although now, some brands have modified their algorithms to account for these inaccuracies. Indeed, the Dexcom CGM G7 has been approved by the FDA to make clinical management decisions in type 1 and 2 diabetes. Another artefact can be produced by increasing the pressure on the sensor, as the local blood flow can be reduced. Similar problems can happen with localised changes in temperature and their impact on blood flow. Furthermore, the interaction of the sensor with different compounds can produce erratic readings. For example, acetaminophen and ascorbic acid tend to show higher glucose readings (Battelino et al., 2023). However, Dexcom G7 CGM has managed to overcome these problems (Garg et al., 2022).

In chapter 4, the investigators arranged a visit to fit the Dexcom G7 CGM (Dexcom, UK) on one of the participants' arms at least 48 hours before the next visit. This remained in place until the end of each experimental trial. The sensor performed a glucose reading every five minutes, and the participants received a blinded receiver to avoid any changes in behaviour.

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Chapter 3: Post-exercise dietary macronutrient composition modulates components of energy balance in young, physically active adults.

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3.1 Abstract:

The effectiveness of exercise to reduce body mass is typically modest, partially due to energy compensation responses which may be linked to energy substrate availability around exercise. The present study aimed to investigate the effect of manipulating post-exercise energy substrate availability (high carbohydrate/low fat [HCLF] or low carbohydrate/high fat [LCHF] energy replacement) on energy balance components in the short-term (i.e., appetite, energy intake (EI) and energy expenditure (EE)).

Methods: Appetite, EI, activity- and total- EE were measured in twelve healthy, young (21.0 ± 2.3 years) physically active participants (10 men, 2 women) on two occasions across 4 days after a 75-minute run and an isocaloric energy replacement drink (HCLF and LCHF). Appetite was measured daily by visual analogue scales, EI was calculated by subtracting the energy content of food leftovers from a provided food package, activity- and total- EE determined by heart-rate accelerometery.

Results: Composite appetite ratings between days were lower in HCLF (62.4 ± 12) compared to LCHF (68.3 ± 8.9 mm; p = 0.048). No differences between conditions were detected for EI. Cumulative activity-EE (HCLF = 20.9 ± 3.7, LCHF = 16.9 ± 3.1 MJ; p = 0.037), but not total-EE (HCLF = 44.6 ± 7.7, LCHF = 39.9 ± 4.7 MJ; p = 0.060), was higher for the HCLF condition than the LCHF across the measurement period.

Conclusion: Compared with low carbohydrate/high fat, **i**mmediate post-exercise energy replacement with a high carbohydrate/low fat drink resulted in higher short-term activity energy expenditure and lower appetite ratings.

Abbreviations:

ANOVA Analysis of variance

AEE Activity energy expenditure

BF% Body fat percentage

BM Body mass

BMI Body mass index

DEBQ Dutch eating behaviour questionnaire

ECRs Energy compensation responses

EDTA Ethylenediamine tetra-acetic acid

EI_{diary} Energy intake from food diaries

$EI_{leftovers}$ Energy intake from food leftovers

HCLF High carbohydrate low fat

HR Heart rate

LCHF Low carbohydrate high fat

RMR Resting metabolic rate

RER Respiratory exchange ratio

SD Standard deviation

TEE Total energy expenditure

VAS Visual analogue scale

VO_2peak Peak oxygen uptake

3.2 Introduction

The prevalence of overweight and obesity has risen in the last few decades, impacting the health of around 2 billion people worldwide (Afshin et al., 2017; World Health Organization, 2016). Overweight and obesity are commonly treated with lifestyle interventions, including exercise and diet. However, the efficacy of exercise and/or diet interventions on body-mass regulation varies among individuals (N. A. King et al., 2008) and such interventions often do not lead to the predicted body mass changes (J. L. Dorling et al., 2020). While this could be partially explained by a lack of compliance, physiological factors triggering energy compensation responses (ECRs) (i.e., increases in energy intake and/or reductions in energy expenditure components) could also play an important role (Aronne et al., 2021b). A better understanding of ECRs could therefore lead to more effective strategies for controlling body mass.

ECRs are commonly described in long-term energy restriction, where reductions in fat- and fat-free mass lead to more than predicted decreases in energy expenditure, concomitant with increases in hunger and food intake, changes in the hormonal milieu and substrate utilisation (Aronne et al., 2021b; Müller et al., 2016). On the other hand, ECRs could also be partially driven by short-term changes in substrate balances (e.g., carbohydrates and proteins) (Flatt J. P., 1987; Mayer, 1953; Mellinkoff et al., 1956). Indeed, changes in energy intake and/or energy expenditure following hours to days of exercise- and nutrition-interventions have been described even without body mass changes (Galgani & Ravussin, 2008; Hopkins et al., 2011b; Müller, Geisler, Heymsfield, & Bosy-westphal, 2018). However, these phenomena remain to be fully understood, and more research is warranted.

Regarding carbohydrate metabolism, the glyco- and glycogeno-static theories (Flatt, 1987; Flatt J. P., 1987; Mayer, 1953) posit that blood glucose and liver and/or muscle glycogen levels regulate food intake. These carbohydrate stores are relatively limited, essential for controlling glycaemia, and can be substantially affected by exercise and nutrition in the short term. As a result, reducing carbohydrate stores could increase food intake and lower physical activity levels. While this makes intuitive sense, evidence from studies manipulating nutrition only have not fully supported this notion (Hengist et al., 2022; Shetty et al., 1994; Stubbs et al., 1993; Stubbs, Harbron, et al., 1995; Stubbs, Ritz, et al., 1995). For instance, a metabolic ward study by Stubbs and colleagues (Stubbs, Ritz, et al., 1995) showed a mild negative relationship between energy balance and carbohydrate balance during seven days of eating diets (*ad libitum)* with different levels of carbohydrates. Carbohydrate balance explained 5.5% of the variance in the subsequent day´s energy balance. However, against a background of physical exercise, which has the potential to reduce carbohydrate availability markedly, the data suggest carbohydrates may exert a stronger regulatory role on ECRs (Alméras et al., 1995; Snitker et al., 1997; Tremblay et al., 1994).

In the context of exercise interventions, multiple lines of evidence have linked carbohydrates and ECRs (Alméras et al., 1995; Snitker et al., 1997; Tremblay et al., 1994). Tremblay and colleagues investigated combining a 60-minute run with three *ad libitum* diets differing in the fat-to-carbohydrate distribution and found that only the diets with high carbohydrate content led to a negative energy balance within the next 48 hours (Tremblay et al., 1994). However, the design does not allow for distinguishing between the effects of the exercise-induced energy deficit and substrate utilisation on energy balance. In this sense, stronger associations between carbohydrate metabolism and energy intake have

been observed in situations where carbohydrate availability is even more limited (e.g., overnight-fasted exercise protocols). For instance, Hopkins and colleagues showed that in women with obesity, after 60 minutes of exercise cessation, 37% of the variance in food intake during an *ad libitum* meal was explained by carbohydrate oxidation during exercise (Hopkins et al., 2014b). Furthermore, Edinburgh and colleagues., showed that in lean participants, blood glucose used during exercise was correlated with energy intake during a meal test performed two hours after finishing exercising (Edinburgh et al., 2019). On the other hand, carbohydrate availability has been linked to some extent with changes in energy expenditure, particularly in the physical activity domain. For example, during prolonged fasting periods, where carbohydrate availability is limited, physical activity levels tend to decrease spontaneously (Betts et al., 2014; Chowdhury et al., 2016a; Farooq et al., 2021a; Lessan et al., 2018; Templeman et al., 2021) However, whether physical activity can be affected by higher carbohydrate demands and lower carbohydrate availability (e.g., with exercise and diet interventions) remains to be directly tested.

At present, no studies have expanded on the impact of overnight-fasted exercise and carbohydrate metabolism on short-term energy intake and physical activity, even though energy balance changes can have a lag time of days (Bray et al., 2008; Champagne et al., 2013b). Therefore, the present study aimed to investigate the effects of an overnight-fasted aerobic exercise session followed by high carbohydrate/low fat or low carbohydrate/high fat energy replacement on short-term (i.e., across 4 days) energy balance components (i.e., appetite, energy intake and energy expenditure) in physically active normal-weight participants. It was hypothesised that compared with low carbohydrate/high fat (LCHF), high carbohydrate/low fat (HCLF) energy replacement after exercise would: (a) reduce appetite

and energy intake and (b) increase physical activity and total energy expenditure during free-living conditions.

3.3. Materials and methods:

Participants:

Twelve healthy, physically active participants (10 men and two women) provided written informed consent and completed the study, which was approved by the local ethics committee of the University of Birmingham, UK (code: ERN_20-1826). Inclusion criteria were men or women, self-reported healthy, low food restraint levels (<3.0 in the DEBQ) (Rennie et al., 2006a) $\dot{V}O_2$peak > 50 and 45 ml·kg-$^{-1}$·min^{-1} for men and women respectively, 18-45 years old, BMI < 27.5 kg ·m^{-2}. Participants who were dieting, smoking, taking medications, pregnant, irregularly menstruating, or lactating were excluded. The final participants' characteristics were age - 21.0 ± 2.3 years (mean ± standard deviation); body mass (BM) - 72.8 ± 7.9 kg; body mass index (BMI) 22.9 ± 1.5 kg · m^{-2}; body fat percentage (BF%) - 14.3 ± 5.6 %; peak oxygen uptake ($\dot{V}O_2$peak) - 56.0 ± 6.4 ml · kg^{-1} · min^{-1}; resting metabolic rate (RMR) – 7.94 ± 1.48 MJ · day^{-1}; Dutch eating behaviour questionnaire (DEBQ-R) score (Van Strien et al., 1986): 1.9 ± 0.4.

Experimental design:

Following the preliminary screening, every volunteer participated in a double-blinded randomised crossover design. Participants were randomised and counterbalanced to each condition by a third-party utilising www.randomizer.com. A familiarisation session and two trials were completed separated by one week of washout for men and four weeks for women (for the two women included, the trials were performed to match the same phase

of their menstrual cycle). The experimental sessions were performed on the same weekdays to decrease the effect of daily life routine changes. To standardise nutrition before the experimental days, participants recorded their food intake and were asked to replicate it for the second experimental condition. Caffeine and alcohol intake was avoided 24 hours before the trials, and strenuous physical activity for 48 hours.

For every trial, the participants arrived at the laboratory ~ 8 am in an overnight-fasted state (10-12 hours since last meal). Following the baseline measurements (i.e., appetite visual analogue scale (VAS), anthropometry, RMR, and a blood sample), a 75-minute run was completed, followed by a LCHF or HCLF drink and a 4-hour recovery within the laboratory. Subsequently, the participants left the facilities with a food package and returned on the morning of the fourth day, where the baseline measurements were repeated.

During the free-living time between the baseline- and follow-up visit, participants were requested to only eat from the food provided, to precisely complete a food diary record, and return all food leftovers. Participants wore an Actiheart device (Camntech, Cambridge, UK) to assess total- and activity-energy expenditure, only taking it off during water-related activities. To determine changes in daily appetite levels, participants received an email with a link to answer an appetite VAS before having breakfast.

After the washout period, the participants underwent the identical experimental condition using a different recovery drink. The drinks were designed to replace the energy expended during the run, differing only in the macronutrient distribution, but were carefully matched in flavour and texture.

Preliminary testing and familiarisation visit

Participants attended the laboratory, and after providing written informed consent and answering a general health screening questionnaire and the DEBQ, the RMR, anthropometry, and $\dot{V}O_2$peak were assessed**.** Next, a 20-minute familiarisation run was performed on a treadmill at the selected exercise intensity. At the end of this visit, the participants had to wear an Actiheart for at least 24 hours to estimate energy needs based on energy expenditure and develop an individualised food menu.

Resting metabolic rate:

The RMR was estimated by indirect calorimetry by a 30-minute protocol (Cranlea Douglas bags system, Birmingham, UK). Participants laid down for a 15-minute stabilisation period, followed by a 15-minute breath sample collection period. Then, the $\dot{V}O_2$ and $\dot{V}CO_2$ concentration levels were measured with gas analysers (MOXAR Respirometry System, AEI technologies, USA), previously calibrated with two known concentration gases. Gas volume and temperature were measured by a dry gas meter (Harvard Bioscience, inc., Germany) and a gas thermometer (electronic temperature instruments, ltd., UK). Then, corrections for standard temperature and barometric pressure were performed. Based on these values, substrate utilisation and energy expenditure were estimated utilising established equations (Elia & Livesey, 1992).

Anthropometry:

Body mass (Ohoaus Champ II scale, Cole-Parmer, UK) and height (SECA 213 stadiometer, UK) were measured to the closest 0.05 kg and 0.01 m, respectively.

Submaximal and maximal exercise testing:

A submaximal running test was performed to establish the relationship between exercise intensity and oxygen uptake, determining the speed necessary to elicit 70% of the $\dot{V}O_2$peak. After 3-5 minutes of warm-up, participants completed four 4-minute stages commencing typically between 7-8 km/hr and with stepped increases of 2 km/hr. $\dot{V}O_2$ and $\dot{V}CO_2$ production were measured by a metabolic cart (Vyntus, Vyaire Medical, IL, US) previously calibrated for volume and gas concentrations following manufacturer recommendations. Heart rate was constantly monitored during the test (Polar H10 heart rate monitor, Finland).

After resting for 20 minutes, a maximal test was performed to determine participants' $\dot{V}O_2$peak. The initial speed was 2 km/hr less than the last stage on the submaximal test, with an initial slope of 1%. Every minute the slope increased by one degree until reaching volitional fatigue. Participants were verbally encouraged during the test. The test finished once participants could not continue or if the maximal criteria variables were achieved for $\dot{V}O_2$, heart rate (HR), and respiratory exchange ratio (RER) (i.e., no further increase in $\dot{V}O_2$ (< 2 ml $\cdot$ kg^{-1} $\cdot$ min^{-1}), HR ± 10 beats of the age estimated HR max, and RER >1.1).

Experimental trials (Fig.3.1)

Baseline visit:

Participants arrived at the laboratory ~ at 8:00 am and were asked to void their bladder. Then they completed the first appetite VAS and body mass was measured while wearing light clothes. RMR was assessed by a 30-minute protocol utilising Douglas bags and indirect calorimetry. Body composition was then estimated by bioelectrical impedance (Bodystat Quadscan 4000, Isle of Man) and a blood sample was obtained by venepuncture.

For the treadmill run, participants started with a 5-minute warm-up followed by 75minutes at a running speed designed to elicit 70% $\dot{V}O_2$peak. Every 15 minutes, a three-minute breath sample was collected and analysed to ensure that the targeted intensity was achieved (LCHF: 72.5 ± 3.8 & HCLF: 72.3 ± 3.2% $\dot{V}O_2$peak; p = 0.829) (Vyntus, Vyaire Medical, IL, US). The heart rate and 6-20 Borg perceived exertion were measured throughout the protocol.

Once participants finished running, body mass and appetite were assessed again, a blood sample was taken by inserting a cannula into the antecubital vein, and the Actiheart was placed on the chest. Afterwards, participants had 10 minutes to change before staying in a research kitchen for the next four hours. Within the kitchen, they drank two bottles of the designated drink, separated by an hour. After 15 minutes of finishing the first bottle, a third appetite VAS was completed. During the recovery, a blood sample and appetite VAS were obtained hourly.

At the end of the four hours, participants left the building with a food package to cover the rest of that day and the next two days. Participants were requested to complete an appetite VAS before breakfast and a food record daily, wear the Actiheart for the whole -living period and bring back food leftovers on the morning of the fourth day.

Follow-up visit:

After finishing the free-living period, participants returned to the lab on the morning of the fourth day (~ 8 am) in an overnight-fasted state. Here, the last appetite VAS was completed, followed by measuring body mass, RMR and body composition. Finally, a blood sample was taken, the food leftovers were collected, and Actiheart data was downloaded.

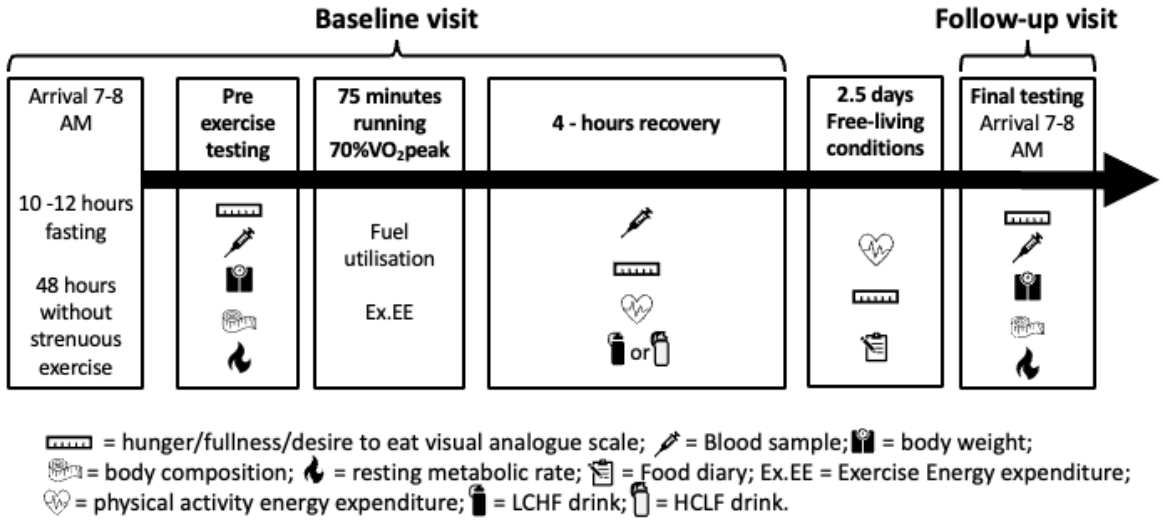

Figure 3.1 Schematic overview of the experimental days

Nutritional manipulation

Participants were given one of the two isocaloric drinks (4.45 ± 0.6 MJ) after exercising. One was LCHF (5.3%, 87.7%, 7.0% of protein, fats, and carbohydrate, respectively), and the other was HCLF (7.3%, 0.5%, 92.2% of protein, fats, and carbohydrates, respectively). The drinks matched the energy expended during the running bout and were prepared by a trained third person who was not otherwise involved in the research. The LCHF drink was composed of semi-skimmed milk, double cream, and sucralose. The HCLF drink was made from semi-skimmed milk, sucrose and maltodextrin. To avoid visual recognition, the drinks were provided in opaque bottles.

For the free-living period, participants were instructed to eat "as much or as little as they want" but only from the food provided, which covered 125% of their total estimated energy needs (i.e., 1.25 x total energy needs —obtained by combining the RMR and the physical activity energy expenditure obtained by the Actiheart). Participants had the option to request additional food from the investigators if necessary. The average macronutrient distribution was 45%, 18%, and 37% of energy from carbohydrates, proteins, and fats. Two breakfasts, three lunches, three dinners and three snack bags were provided. The food provided and the ingredients utilised for the preparation of the drinks were obtained from commercially available products (Tesco, UK). The main meals (i.e., lunch and dinner) were "ready-to-eat food", and the labelled nutritional information was utilised to calculate energy intake.

Energy Intake: *from diaries and leftovers.*

Participants were instructed to complete food diary records during the free-living period. For this purpose, the investigator taught participants how to complete the paper-based form accurately. This approach was combined with the provision of food by the investigators to increase the reliability of dietary food records (McNeil et al., 2012). The mean reported energy intake was obtained directly from the food diaries (EI_{diary}). Furthermore, to calculate the energy intake more objectively, the investigators subtracted the food leftovers from the total caloric content of the food provided ($EI_{leftovers}$). For comparison purposes, EI_{diary} and $EI_{leftovers}$ outcomes are taken as cumulative values on each trial (i.e., the sum of food´s caloric content across the days). For the analysis, the drinks' energy content is not considered within the cumulative EI.

Appetite VAS assessment

In the present study, the questions included were: How hungry do you feel now? How strong is your desire to eat? And how full do you feel now? These questions were answered by marking a 100 mm line using anchor statements as previously validated (Stubbs et al., 2000a) in the laboratory and during free-living conditions. The investigator configurated an online questionnaire to allow the participants to answer the scales on their computers/mobiles/tablets. The data were collected and managed using REDcap electronic data capture tools hosted at the University of Birmingham, UK (Harris et al., 2009). For free-living conditions, REDcap was programmed to email the questionnaires daily to the participants on a pre-specified schedule. The three questions were analysed individually and as a composite.

$$appetite\ composite = \frac{\mathrm{H.H} + \mathrm{H.SD} + (100 - \mathrm{H.F})}{3}$$

H.H = how hungry do you feel?; H.SD = How strong is your desire to eat?; H.F = how full do you feel? All the values are expressed in mm

Exercise *energy expenditure within the lab.*

For estimating energy expenditure and substrate utilisation during the 75-minute run at 70% $\dot{V}O_2$peak (energy expenditure: 4.58± 0.64 MJ; fat oxidation: 48 ± 15 g; carbohydrate oxidation: 153 ± 31 g), a 3-minute breath sample was obtained every 15 minutes by indirect calorimetry utilising a metabolic cart (Vyntus, Vyaire Medical, IL, US). The values were calculated utilising the equations of Jeukendrup & Wallis (Jeukendrup & Wallis, 2005). For the analysis, exercise energy expenditure is not considered within the cumulative energy expenditure data.

Total- and activity-energy expenditure in free-living conditions (TEE & AEE)

The total- and activity-energy expenditure during free-living conditions was obtained from the data collected by the Actiheart (Brage et al., 2015). Participants were instructed to wear the device all the time, from finishing the run until the morning they returned to the laboratory. They were only allowed to take it off during aquatic activities. The Actiheart utilised a previously validated algorithm based on a branched model (Brage et al., 2007) that combines heart rate and accelerometry to estimate the total- and activity-energy expenditure. The device was configurated to collect data every 15 seconds.

The participant's anthropometric information (i.e., body mass and height), date of birth, sex, sleeping- and maximum-heart rates were programmed into the software for data processing. For improved accuracy, individualised calibration curves were built based on the

values of heart rate and $\dot{V}O_2$ obtained in the submaximal and maximal exercise tests. Also, the measured RMR was included to improve further energy expenditure estimation on every trial. For interpretation, as done with energy intake, TEE and AEE outcomes are reported as cumulative values on each trial (i.e., the sum of TEE and AEE values across the days). Only participants without episodes of “non-wearing time", as detected by the Actiheart 5 software (i.e., no periods of more than 2 hours of non-wearing), were included in the analysis.

Blood sampling & biochemical analyses

Venous blood samples were obtained and collected into pre-chilled 6 ml EDTA tubes. Blood samples were taken by venepuncture from the antecubital vein at baseline; and by cannulation after finishing the exercise, at 1, 2, 3, and 4 hours. A protease inhibitor (Pefabloc® SC, Merck, Germany) was utilised to preserve the integrity of the samples for the subsequent measurement of appetite-related hormone concentrations. Tubes were kept on ice until they were centrifugated at 4 ºC, 1500 g, for 10 minutes. Plasma aliquots were stored at – 70ºC until they were analysed.

Plasma samples were analysed in using an automated clinical analyser (RX Daytona+ Randox, London, UK) to determine glucose (Glucose hexokinase, Randox, UK) and lactate (Lactate Dehydrogenase, Randox, UK) concentrations. For insulin, acylated ghrelin, and leptin, ELISA assays were used (EMD Millipore Corporation, Missouri, USA). All biochemical analysis was performed in duplicate.

3.4 Statistics

The sample size was selected (n = 12) to be comparable with previous research investigating the impact of short-term exercise and nutrition manipulation on energy balance (Edinburgh et al., 2019; Hopkins et al., 2014b). Unless otherwise stated, the statistical analyses included 12 participants. Data are described as mean ± standard deviation (SD). For cumulative values of energy intake, activity- and total- energy expenditure, a paired t-test was performed to compare mean values. The energy expenditure analysis account for all the data collected after the exercise bout, whereas the energy intake was calculated from the free-living period only. A two-way repeated-measures ANOVA was utilised for daily-appetite, activity- and total-energy expenditure, blood hormones and metabolites. For time x condition interactions, a Holm-Bonferroni *post hoc* was performed. Data were tested for sphericity with Mauchly´s test. For the variables where the sphericity assumption was violated, a Greenhouse-Geisser correction was performed. Change in insulin was calculated for each trial and compared by paired t-test between conditions due to significant baseline differences. Statistical significance was set at $p < 0.05$. Statistics were performed using JASP 0.16.3 software and graphics with Graphpad PRISM 9.4.1.

3.5 Results

Energy and macronutrient intake

The mean cumulative EI_{diary} for the LCHF and HCLF conditions were 37.5 ± 7.6 and 38.7 ± 7.3 MJ (p=0.425). The mean $EI_{leftovers}$ for the LCHF and HCLF conditions were 40.2 ± 9.7 and 40.3 ± 8.7 MJ (p= 0.925), respectively (see Fig. 3.2). The mean cumulative data for carbohydrates, proteins, fats, and fibre intake did not show significant differences between conditions (table 3.1)

Table 3.1. Cumulative macronutrient intake for each condition

	LCHF	**HCLF**
Carbohydrates (g)	1124 ± 275 (47 ± 12 % EI)	1129 ± 251 (47 ± 11 % EI)
Proteins (g)	395 ± 63 (16 ± 3 % EI)	398 ± 51 (14 ± 2 % EI)
Fats (g)	352 ± 103 (33 ± 10 % EI)	352 ± 94 (33 ± 9 % EI)
Fibre (g)	126 ± 34 (4 ± 1 % EI)	126 ± 28 (4 ± 1 % EI)

Values are presented as mean ± SD in grams and as percentage of EI (% EI). No statistically significant differences between conditions were detected ($p > 0.05$). The data were calculated based on the information obtained after accounting for food leftovers.

Cumulative total-and activity-energy expenditure

Data on n = 8 is presented as four participants were excluded from the analysis, as they had not worn the Actiheart for sufficient periods during the trials. The mean cumulative TEE for the LCHF and HCLF conditions were 39.9 ± 4.7 & 44.6 ± 7.7 MJ (p = .060). The corresponding values for AEE were 16.9 ± 3.1 & 20.9 ± 3.7 MJ (p = .037) for the LCHF and HCLF conditions (See fig. 3.2). The breakdown of these data is shown in table 3.2.

Table 3.2. Total daily energy expenditure & daily activity energy expenditure corresponding to each condition.

	AEE (MJ) [a]		*TDEE (MJ)* [a]	
	LCHF	HCLF [b]	LCHF	HCLF
Day 1	4.6 ± 1.4	5.1 ± 1.6	9.7 ± 2.0	10.3 ± 2.5
Day 2	5.8 ± 2.4	6.6 ± 2.4	14.7 ± 2.7	15.6 ± 3.2
Day 3	6.5 ± 2.1	9.3 ± 2.5	15.5 ± 2.7	18.7 ± 4.0

Data are presented as mean ± SD; N = 8. A repeated measured ANOVA was performed for AEE = Activity energy expenditure and TDEE = Total daily energy expenditure. [a] Time effect; [b] Condition effect. Day 1 is considered from after the run until midnight. Days 2 and 3 run from midnight until midnight.

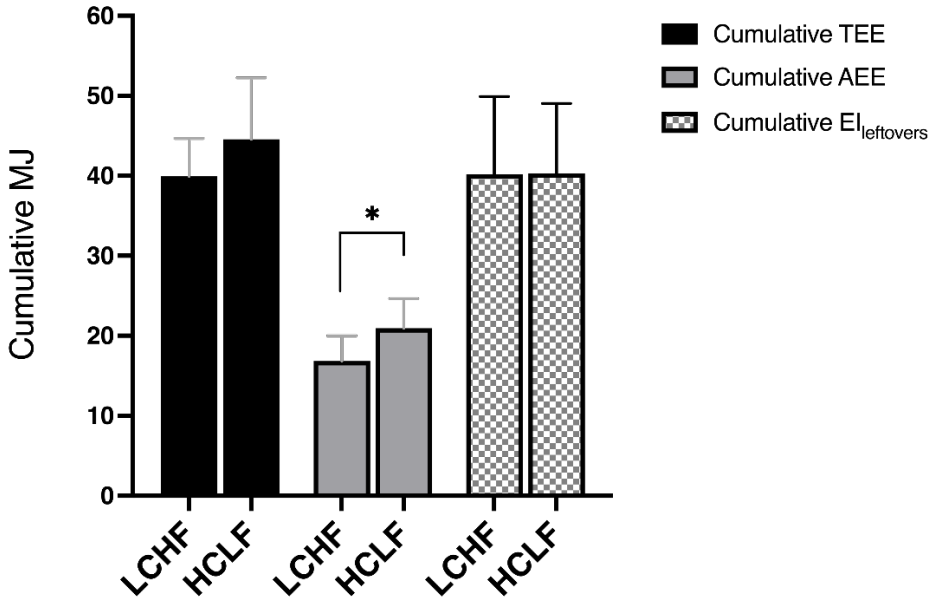

Figure 3.2. Cumulative TEE, AEE, and $EI_{leftovers}$. Values are presented as mean ± SD. TEE = total energy expenditure (n = 8); AEE = activity energy expenditure (n = 8); $EI_{leftovers}$ = Calculated energy intake from leftovers (n = 12). * p = 0.037

Appetite Composite

Up to five participants were excluded from these analyses because they did not fully complete the appetite VAS. During the recovery within the lab, there was a time effect (p = 0.038) (Fig. 3.3) but no condition (p =0.181) or time x condition effects (p = 0.775). For the comparison between days, the LCHF condition presented higher levels of fasted appetite composite than the HCLF (LCHF = 68.3 ± 8.9, HCLF = 62.4 ± 12 mm, p = 0.048; fig. 3.4. A). When the questions were analysed separately, statistically significant differences were only found for "How strong is your desire to eat" with lower values for the HCLF condition (LCHF = 67.6 ± 8.8, HCLF = 63.4 ± 12.4 mm; p = 0.023; fig. 4.D).

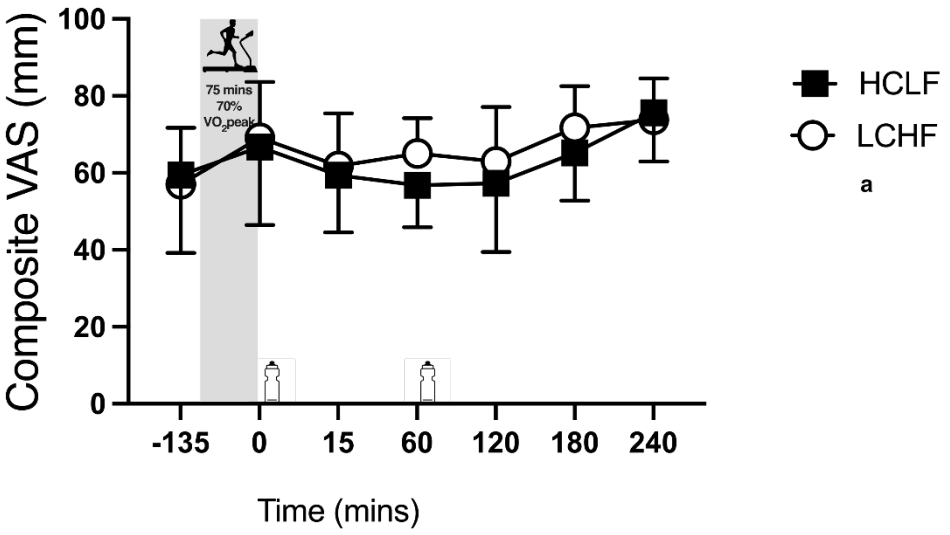

Figure 3.3. Appetite VAS composite within the laboratory. n = 9 Data are presented in mean ± SD. A two-way repeated measure ANOVA was performed. [a] time effect.

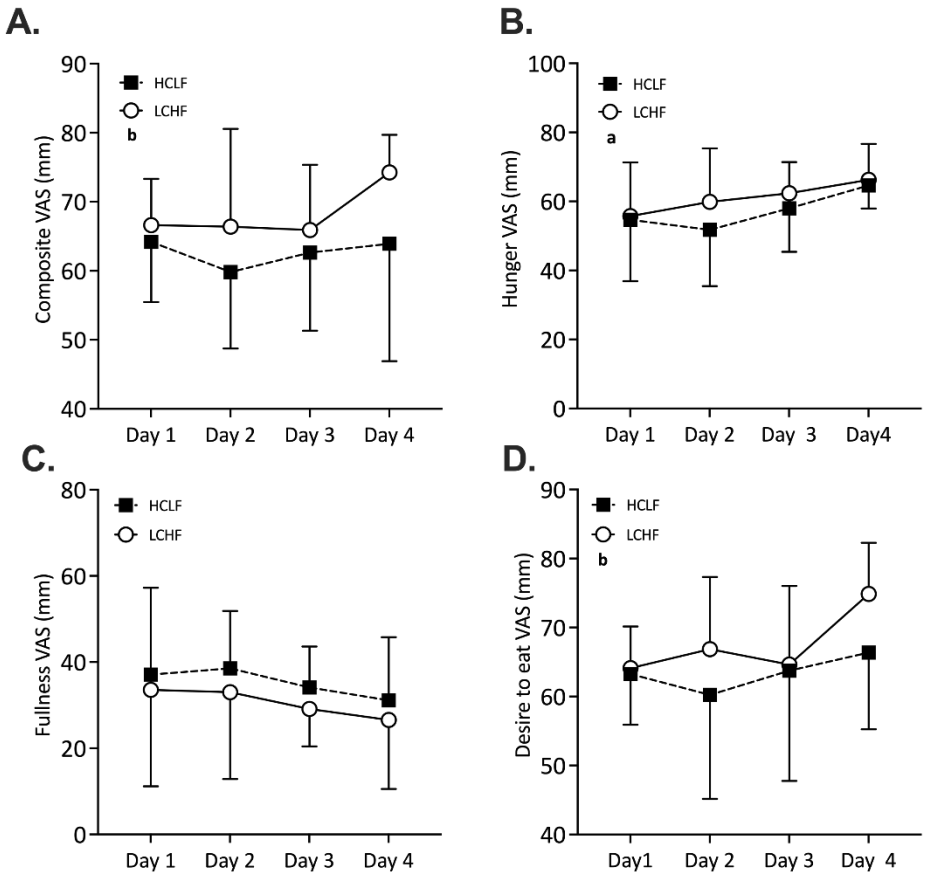

Figure 3.4. Appetite VAS. A) appetite composite n = 7; B) How hungry do you feel? n = 8; C) How full do you feel? n = 9; D) How strong is your desire to eat? n = 8. Data are presented in mean ± SD. A two-way repeated measure ANOVA was performed. [a] time effect; [b] condition effect.

Blood metabolites and hormones:

Glucose:

The effects of the intervention on blood glucose concentrations during the lab recovery were affected by time (P = <0.001) but not by condition (P = 0.335), and there was a time x condition interaction (P = <0.001) (Fig 3.5.A). *Post hoc* analyses showed that within the lab, the HCLF condition presented lower glucose levels compared with LCHF 4 hours after the exercise (5.07 ± 0.28 LCHF vs 3.72 ± 0.96 HCLF mmol · L^{-1} P < 0.001). For the comparison between day 1 and day 4, no differences were detected (Fig 3.5.B).

Insulin:

For the values obtained during the lab recovery period, there were differences by time (p = <0.001), condition (p = <0.001), and time x condition (p = <0.001). *Post hoc* analyses showed significant differences between conditions at hour 1 (10.8 ± 8.8 LCHF vs 56.1 ± 29.4 HCLF µU· ml^{-1}; p = <0.001), hour 2 (9.5 ± 6.5 LCHF vs 58.9 ± 33.5 HCLF µU· ml^{-1}; p = <0.001), and hour 3 (6.3 ± 3.6 LCHF ± 40.7 ± 26.4 HCLF µU· ml^{-1}; p = 0.02) (Fig 3.5.C). For the comparison between day 1 and day 4, there were no differences between conditions (p = 0.489) (Fig 3.5.D).

Lactate:

For blood lactate concentration during the lab recovery, there were differences by condition (P = <0.001), and time x condition (P = < 0.001), but not by time (P = 0.063). *Post hoc* analyses showed significant differences between conditions at hour 1 (0.89 ± 0.13 LCHF vs 2.09 ± 0.68 HCLF mmol · L^{-1}; p = <0.001), hour 2 (0.78 ± 0.09 LCHF vs 1.91 ± 0.53 HCLF mmol · L^{-1}; p = <0.001), and hour 3 (0.75 ± 0.06 LCHF vs 1.52 ± 0.37 HCLF mmol · L^{-1}; p = 0.04) (Fig 3.5.E). There were no differences in the comparisons between day 1 and day 4 (Fig 3.5.F).

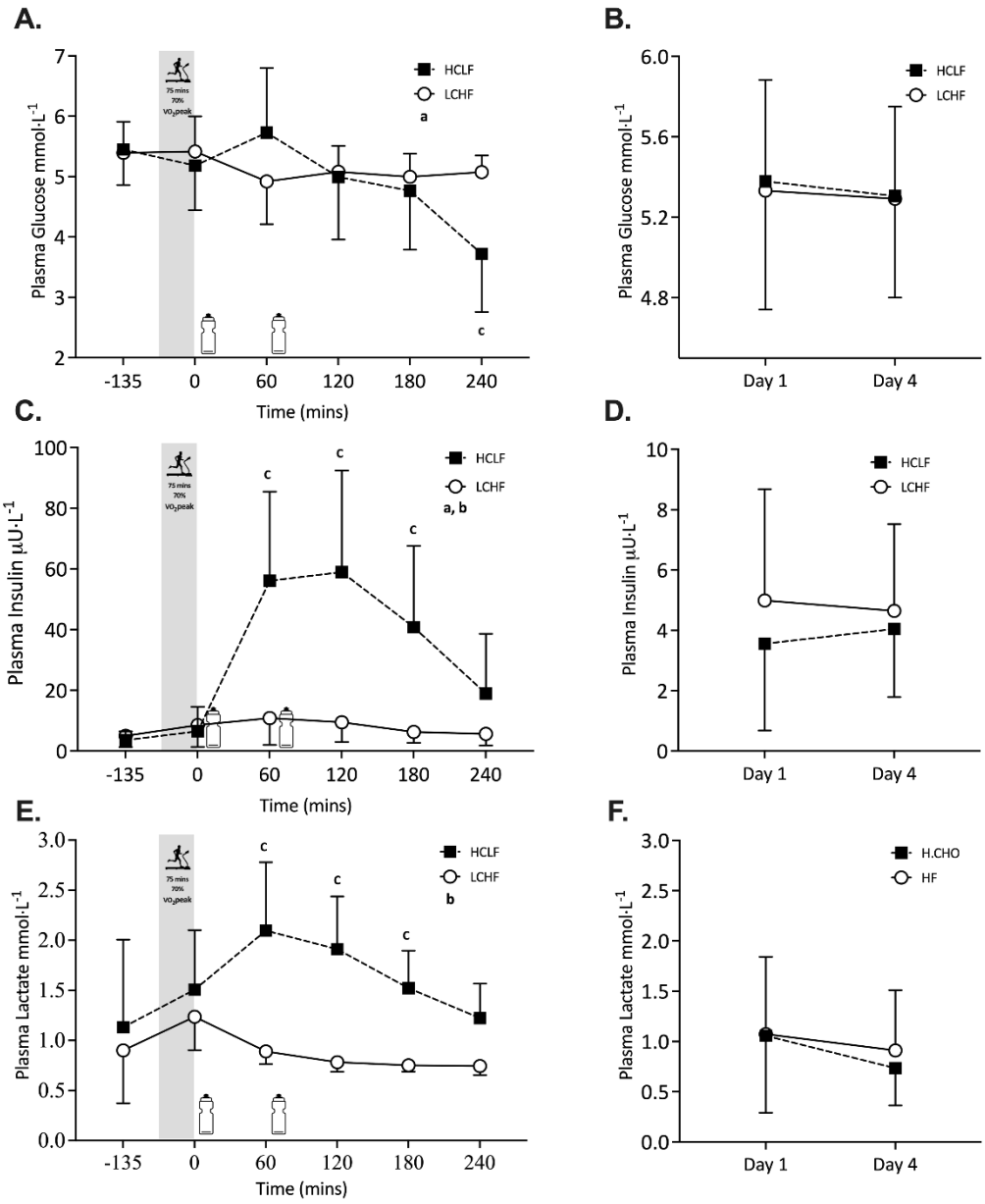

Figure 3.5 Glucose A-B, insulin C-D, and lactate E-F concentrations values for the comparisons within the laboratory and between days. Values are presented as mean ± SD. A two-way repeated measure ANOVA was performed. [a] time effect; [b] condition effect; [c] Time x condition interaction.

Acylated- ghrelin:

The acylated ghrelin concentrations were measured on days 1 and 4. Here, significant differences were observed by condition (p = 0.013) and time x condition interaction (p = 0.039), where the HCLF condition showed higher concentrations than the LCHF condition. *Post hoc* analyses showed differences by condition on day 4 (329± 164 LCHF vs 518 ± 267 HCLF pg · mL^{-1}; p = 0.008) (Fig. 3.6).

Leptin:

For leptin concentration, there were no differences by time (p = 0.223), condition (p= 0.583) and time x condition interaction (p = 0.335) (Fig 3.6).

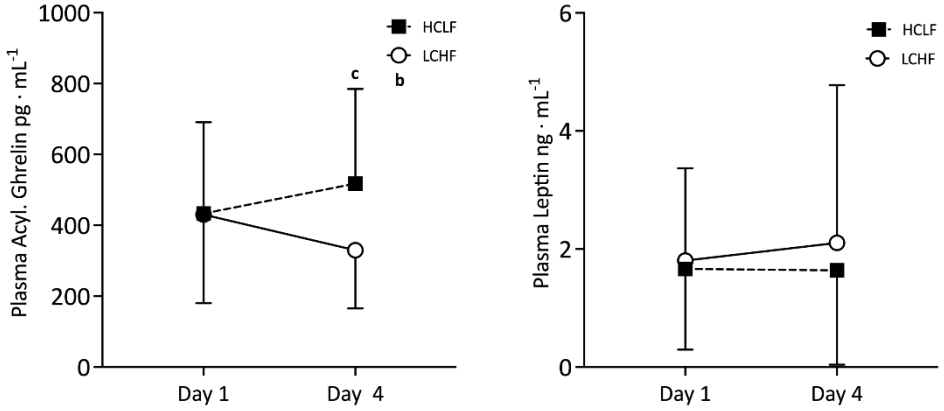

Figure 3.6. Acylated ghrelin and leptin plasma concentration values for the comparisons between days. Values are presented as mean ± SD. [b] condition effect; [c] Time x condition interaction.

3.6 Discussion:

The present study aimed to investigate the effects of an aerobic exercise session followed by HCLF or LCHF energy replacement on short-term (i.e., across 4 days) energy balance components (i.e., appetite, energy intake and energy expenditure) in physically active normal-weight participants. Here, the HCLF condition led to higher AEE and fasted acylated ghrelin levels and reduced fasted-appetite composite rating compared with the LCHF condition, although no differences in energy intake were observed between conditions.

As above, a key finding in the present study was higher levels of AEE for the HCLF condition. TEE was not significantly increased in HCLF, which could suggest compensatory adaptations as implied by the constrained energy hypothesis (Pontzer, 2015). However, this seems unlikely given TEE alterations were directionally consistent with AEE (i.e., increased in HCLF). Regardless, the present results agree with one of the study hypotheses and provide evidence that post-exercise dietary macronutrient composition can modulate AEE. Based on the glycogenostatic theory, the lower AEE in the LCHF condition could be explained by the reduced carbohydrate availability, as has been observed with other interventions combining overnight-fasting plus moderate to vigorous exercise (Hultman & Nilsson, 1971). Although studies testing these effects on AEE directly are lacking, indirect evidence pointing to low carbohydrate availability and reduced AEE can be found in the context of fasting studies (Betts et al., 2014; Chowdhury et al., 2016a; Farooq et al., 2021a; Lessan et al., 2018; Templeman et al., 2021). In this sense, it has been reported that overnight-fasted exercise can lead to higher glycaemic variability (Nygaard et al., 2017) and lower counterregulatory response to hypoglycaemia (Galassetti, Mann, Tate, Neill, Costa, et al., 2001). Consequently, if carbohydrate stores are not replenished after exercise, endogenous glucose production

can be affected (Kishore et al., 2006), triggering strategies to regulate glycaemia, such as reduced muscle glucose uptake (Gregory et al., 2017; Meyer et al., 2005) and increased hunger. Altogether, it could lead to decreased physical activity until glycaemic control is achieved again. On the other hand, it cannot be discarded that the observed LCHF results are driven by acute fat intake, as it could also modify glycaemic control and peripheral glucose metabolism (Hernández et al., 2017; Sarabhai et al., 2020, 2022). Indeed, the type of fat intake by itself (saturated v. monounsaturated) can have different effects on physical activity and resting energy expenditure levels in healthy people, where a higher proportion of monounsaturated fat has led to higher energy expenditure (Kien et al., 2013). If others corroborate that post-exercise macronutrient intake modulates AEE without modifying EI, it could open opportunities to develop nutritional-based approaches to minimise ECR in the context of exercise training and weight management programmes.

The appetite data collected in the present study showed that participants in the HCLF condition presented lower fasted-appetite levels in the days after the intervention. As no appetite-VAS differences between conditions were seen during the early recovery period within the laboratory, the direct satiating effect of the nutrients should not have influenced the results. Whether this points to a further influence of carbohydrate availability on short-medium-term appetite regulation is unclear. However, indirect evidence linking higher carbohydrate availability after exercising and reduced energy intake between days suggest a modulatory role (Alméras et al., 1995; Snitker et al., 1997; Tremblay et al., 1994). Notwithstanding, even if there was a reduction in appetite ratings, the mean ~ 6 mm differences were modest and did not modify food intake. This agrees with the literature, which reports that a minimum 15 mm change in appetite VAS would be required to increase

food intake (Sadoul et al., 2014). Within the appetite-related hormones, the levels of acylated ghrelin were higher for the HCLF compared to the LCHF condition. This finding contradicts what was found for appetite, as it is well-known that acylated ghrelin is an orexigenic hormone. Nonetheless, it should be recognised that ghrelin is only one hormone from an array that influence appetite, and it is not uncommon to find uncoupled responses between endocrine signalling, appetite, and energy intake (J. A. King et al., 2015). Even though the energy intake was not different between groups, the present data could help inform nutritional strategies to modulate appetite during high physical activity periods while eating *ad libitum*.

Despite the investigators' efforts, the present study is not free of limitations. It is important to mention that valuable information regarding physical activity and appetite during the free-living period could not be obtained. Four participants did not wear the device as requested and did not reply to the appetite-VAS, despite frequent reminders. Another limitation is the relatively homogenous population group, which limits the generalisation of the results. On the other hand, it is also possible to point out strengths. The design of the present study, combining lab and free-living conditions, gives control for the main manipulation (corroborated by the blood markers) and adds ecological validity, allowing capture of spontaneous behavioural changes after a controlled intervention. A 2.5 days free-living period allowed us to capture changes in physical activity that were not captured by shorter studies, suggesting the need to monitor longer after lifestyle interventions. Moreover, participants completed the trials matching the same days of the week between conditions, reducing the influence of routine changes on our results. Furthermore, limiting food variety helped avoid nutrient-different influences on appetite and food

overconsumption. Indeed, there were no differences in macronutrients (table 3.1) or caloric intake between conditions, isolating the energy replacement protocols' effects to explain the results.

In summary, this study shows that when the energy expended during exercise is replaced with different macronutrients, an HCLF composition favours higher levels of AEE and lower appetite ratings in the short term. However, as the present study did not include direct measurements of substrate availability (e.g., liver/muscle glycogen, continuous glucose monitoring, etc.), it is not feasible to offer stronger mechanistic insights. Future research should focus on studying the relationship between carbohydrate pools (blood glucose, liver and muscle glycogen) and their direct/indirect effects on energy balance components, with the potential to inform better nutritional strategies for physically active people where energy balance is a concern (e.g., weight loss programs, weight-categorised sports, etc.)

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Chapter 4: Effects of overnight-fasted versus fed-state exercise on the components of energy balance and interstitial glucose in healthy adults.

4.1 Abstract

Exercise is an essential component of body mass management interventions. Overnight-fasted exercise (FASTex) acutely enhances fat oxidation compared with fed exercise (FEDex). However, consistent FASTex training does not typically further enhance body mass or fat mass loss, suggesting the induction of energy compensation responses. The present study aimed to study the effects of FASTex or FEDex on the components of energy balance (i.e., energy intake (EI), energy expenditure (EE), and appetite) and interstitial glucose metrics across four days.

Methods: Twelve (10 men, 2 women) healthy, physically active participants were studied on two occasions across four days after a 75-minute run either FASTex or FEDex. EI was obtained after subtracting food leftovers from the food provided. Appetite was measured by visual analogue scales. Activity- and total- EE were estimated by the combination of heart rate and accelerometry. Lastly, continuous glucose monitoring was used to capture daily interstitial glucose metrics and Likert scales were utilised to quantify fatigue-, stress, sleep quality-, and muscle soreness levels.

Results: No differences between conditions were observed for EI ($p = 0.865$), activity- and total energy expenditure ($p = 0.223$ & $p = 0.136$, respectively), appetite ($p > 0.05$), and interstitial glucose ($p = 0.074$).

Conclusion: FASTex did not differ from FEDex in the response of components of energy balance or interstitial glucose across four days, suggesting that both approaches could be used interchangeably as exercise strategies for body mass management.

Abbreviations:

ANOVA Analysis of variance

AEE Activity energy expenditure

BF% Body fat percentage

BM Body mass

BMI Body mass index

CGM continuous glucose monitoring

CV glucose coefficient of variation

DEBQ Dutch eating behaviour questionnaire

EDTA Ethylenediamine tetra-acetic acid

EI Energy intake

FASTex overnight-fasted exercise

FEDex Fed exercise

FS Feeling scale

HR Heart rate

RMR Resting metabolic rate

RER Respiratory exchange ratio

SD Standard deviation

TEE Total energy expenditure

VAS Visual analogue scale

VO_2peak Peak oxygen uptake

4.2 Introduction

Regular exercise and a balanced diet are considered integral to body mass management (i.e., mass loss or maintenance). Accordingly, there are significant efforts to develop strategies to optimise the effects of exercise on health outcomes including the interaction between nutrition and exercise on energy balance. One such strategy is to perform aerobic exercise in the overnight-fasted (FASTex) versus fed state (FEDex), which has been shown to improve selected metabolic adaptations to aerobic exercise training, such as insulin sensitivity (Edinburgh et al., 2020; Van Proeyen et al., 2010; Wallis & Gonzalez, 2018). Acute FASTex studies (i.e., a single exercise bout) lead to negative energy balance in the immediate hours (i.e., ≤ 24-hours) after exercise (Frampton et al., 2022) but short-term interventions (i.e., ≤ six weeks) do not show superior effects of FASTex in body mass management (Edinburgh et al., 2020; Gillen et al., 2013; Schoenfeld et al., 2014). The discrepancy between what might be predicted from negative energy balance observed in acute FASTex studies (i.e., greater body mass loss) and general lack of efficacy on body mass outcomes may be related to laboratory studies that do not reflect free-living conditions. Energy compensation responses counteracting any energy deficit created that manifest across a time-frame later than those studied to date in acute interventions (i.e., single exercise bout with ≤ 24-h follow-up) could also be important (i.e., changes in appetite, energy intake, activity- and resting energy expenditure)(Aronne et al., 2021b).

Only a limited number of studies have analysed the effects of FASTex on the components of daily energy balance. A systematic review (Frampton, Edinburgh, et al., 2021) described that as compared to FEDex, a single bout of FASTex has been shown to generate an acute reduction in energy intake (≤ 24 hours). However, it also reduces post-exercise energy

expenditure, which suggests a compensatory response. Unfortunately, no studies have assessed changes in activity- and total- daily energy expenditure in free-living conditions beyond the first few hours after exercise (~6 hours). It is conceivable that changes in appetite, energy intake or expenditure may occur in the day or up to three days after exercise (Bray et al., 2008; Champagne et al., 2013a; Podestá D et al., 2023). Collectively, while FASTex may favourably impact energy intake acutely, subsequent energy compensations affecting appetite, energy intake and/or energy expenditure could potentially limit the overall changes in energy balance over a more extended period.

Acutely, the reduction in energy intake with FASTex has been linked to the lack of opportunity to replace breakfast and the transient exercise-induced appetite suppression (J. Dorling et al., 2018; Schubert et al., 2013, 2014; Thackray & Stensel, 2023). Metabolically, one potential modulator of appetite and food intake could be postprandial and postabsorptive decreases in blood glucose concentrations (Campfield et al., 1992b; Wyatt et al., 2021), implying that the body's carbohydrate availability may be important. It has been suggested that reduced carbohydrate availability can also increase appetite by the associated reduction in carbohydrate oxidation rates (i.e., glucopenic effects) (Melanson, Westerterp-Plantenga, Campfield, et al., 1999b). Notably, FASTex typically significantly reduces the body's limited carbohydrate reserves (i.e., liver and muscle glycogen) and utilisation (Iwayama et al., 2021, 2023), increasing the reliance on fat oxidation (Iwayama et al., 2015, 2017). The reduced carbohydrate stores could translate into challenges to maintaining blood glucose concentration and stability in the days after FASTex (DuBose et al., 2021; Frampton et al., 2021), leading to compensatory increases in appetite or energy intake. Furthermore, decreased glucose availability has been suggested to reduce

spontaneous physical activity, compromising daily energy expenditure (Betts et al., 2014; Chowdhury et al., 2016a; Farooq et al., 2021b; Lessan et al., 2018; Podestá D et al., 2023; Smith et al., 2017; Templeman et al., 2021). Nevertheless, whether circulating glucose availability differs after FASTex or FEDex during an extended free-living period remains to be determined.

The present study aimed to investigate the effects of a single bout of FASTex versus FEDex on the components of energy balance and blood glucose metrics across four days to provide further insights into the apparent discrepancy between acute and short-term studies of energy balance responses. Compared to FEDex, it was hypothesised that FASTex would show greater energy intake and/or appetite and decrease energy expenditure across four days.

4.3 Material and methods

Participants

The study was approved by the ethics committee of the University of Birmingham, UK (ERN_22-0367). Twelve healthy, physically active participants were recruited (10 men and two women), providing written informed consent. Inclusion criteria were men or women, self-reported healthy, low food restraint levels (< 3.0 in the Dutch eating behaviour questionnaire (Rennie et al., 2006b)), VO_2peak > 50 and 45 $ml \cdot kg^{-1} \cdot min^{-1}$ for men and women respectively, 18–45 years old, Body mass index (BMI) <27.5 $kg \cdot m^{-2}$. Participants who were dieting, smoking, taking medications, pregnant, irregularly menstruating, or lactating were excluded.

The characteristics of the participants included were: age 22.6 + 1.2 years (mean ± standard deviation (SD)); VO_2peak 58.7 ± 7.2 ml·kg^{-1}·min $^{-1}$; body mass (BM) 70.8 ± 10.8 kg; Body Mass Index (BMI) 22.5 ± 2.8 kg · m^{-2}; Body fat percentage (BF%) 14.5 ± 4.9 %; resting metabolic rate (RMR) 1856 ± 255 kcal.day^{-1}; DEBQ score 1.8 ± 0.5 (Van Strien et al., 1986).

Experimental design

Participants performed exercise having consumed either a carbohydrate-based or placebo drink in a randomised counterbalanced double-blind crossover study. After completing the preparticipation screening, the main study involved two different experimental periods, separated by one week. Each 4-day period included metabolic (i.e., blood samples and RMR), body composition, and appetite assessments, the corresponding drink, an exercise bout, an *ad libitum* meal test, and a follow-up free-living conditions period. A schematic overview of the study is provided below (Fig. 4.1). The exercise intervention consisted of 75 minutes of running at 70% of peak oxygen uptake (VO_2peak). The exercise was followed by a recovery period where participants stayed in the laboratory for three hours, and at hour 2, they were asked to complete an *ad libitum* meal test.

After leaving the laboratory, a 2.5-day free-living follow-up period started, including assessments of appetite (visual analogue scales (VAS)), fatigue-, stress-, sleep quality- and muscle soreness- (1 to 5 Likert scales), food intake (food was provided), interstitial glucose concentration (by CGM) and energy expenditure (estimated from heart rate and accelerometry by the Actiheart 5 (Camntech, Cambridge, UK)). On the morning of day 4, participants returned the accelerometer, CGM, and the food leftovers and underwent the final metabolic, body composition, the four Likert scales, and appetite assessments.

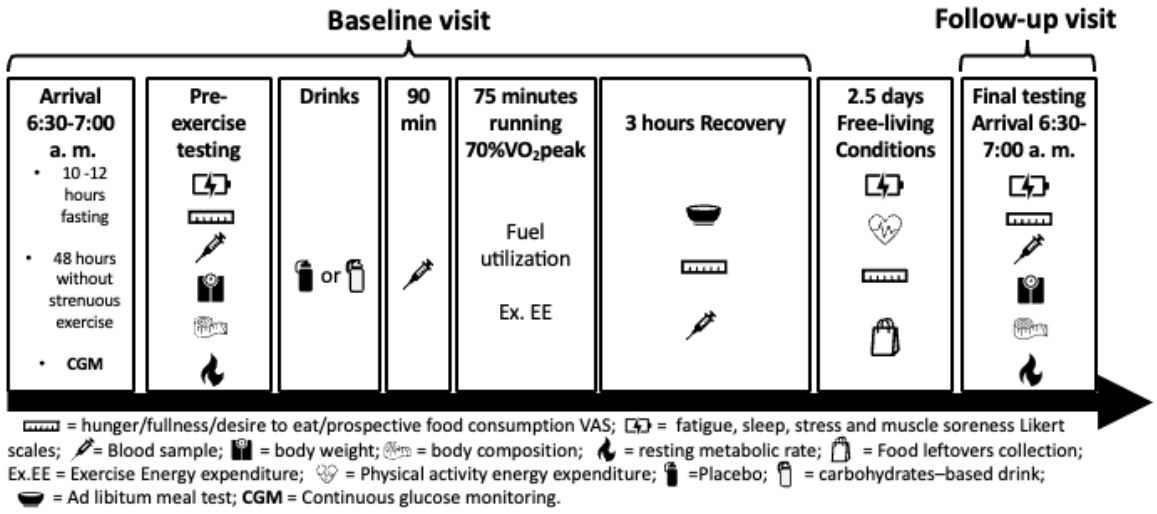

Figure 4.1. Schematic overview of the experimental trials.

Screening and baseline measurements:

Participants arrived at the laboratory at a convenient time after reading a participant information sheet. After providing written informed consent, they completed general health screening and food restraint questionnaires (DEBQ). Then, body mass, RMR and VO_2peak were determined followed by an exercise familiarisation and placement of the Actiheart. Before the next visit, investigators coordinated with participants to return the food diary and the Actiheart monitor to estimate each person's energy needs and provide a tailored diet prescription.

Resting metabolic rate

The RMR was determined by indirect calorimetry using a 30-minute protocol (Cranlea Douglas bags system, Birmingham, UK). Participants remained supine for a 15-minute stabilisation period, followed by a 15-minute breath sample collection period. The O_2 and

CO_2 concentration levels were measured with gas analysers (MOXAR Respirometry System, AEI technologies, USA), previously calibrated with two gas mixtures of known concentrations. Gas volume and temperature were measured by a dry gas meter (Harvard Bioscience, inc., Germany) and a gas thermometer (Electronic Temperature Instruments, ltd., UK), with corrections for standard temperature and barometric pressure performed. Based on these values, VO_2 and VCO_2 were subsequently determined, and substrate utilisation and energy expenditure were estimated utilising established equations (Elia & Livesey, 1992).

Anthropometry

Body mass (Ohoaus Champ II scale, Cole-Parmer, UK) and height (SECA 213 stadiometer, UK) were measured to the closest 0.05 kg and 0.01 m, respectively.

Submaximal and maximal exercise testing

A submaximal running test was performed to establish the relationship between exercise intensity and oxygen uptake, determining the speed necessary to elicit 70% VO_2peak. After 3–5 min of warm-up, participants completed four 4-min stages commencing typically between 7 and 8 $km \cdot hr^{-1}$ and with stepped increases of 2 km/hr. VO_2 and VCO_2 were measured by a metabolic cart (Vyntus, Vyaire Medical, IL, US) previously calibrated for volume and gas concentrations following manufacturer recommendations. Heart rate was continuously monitored during the test (Polar H10 heart rate monitor, Finland). After resting for 20 minutes, a maximal test was performed to determine participants' VO_2peak. The initial speed was 2 km/hr less than the last stage on the submaximal test, with an initial slope of 1%. Every minute, the slope increased by one degree until reaching volitional

fatigue. Participants were verbally encouraged during the test. The test finished once participants could not continue or if the maximal criteria variables were achieved for VO_2, heart rate (HR), and respiratory exchange ratio (RER) (i.e., no further increase in VO_2 [< 2 ml $\cdot$ kg^{-1} $\cdot$ min^{-1}], HR ± 10 beats of the age estimated HR max, and RER > 1.1).

Experimental trials

Baseline visit:

Participants were randomly assigned to one of two conditions (FASTex or FEDex) by a third person (www.randomizer.com). They arrived at ~7 am at the laboratory after 10-12 hours of overnight fasting and avoiding any strenuous exercise in the previous 48 hours, and caffeine and alcohol intake during the previous 24 hours (a 24-hour food dietary record was requested to standardise the diet prior to the trials). To allow for stabilisation of the device and its outputs, participants were asked to wear the CGM at least 24 hours before this visit.

After arriving in the laboratory, participants were assessed for body mass, body composition (Bioelectrical impedance analysis, (Bodystat Quadscan, 4000)), the Likert scales, overnight-fasted appetite VAS, followed by the RMR. Then, a 10 ml blood sample was obtained by cannulation of the antecubital vein.

Immediately after the resting measurements, the participants were provided with the corresponding drink (carbohydrate-based or placebo). For the FEDex trial, they were provided with 500 ml of carbohydrate-based drink (a blend of 100 grams of sucrose & 50 grams of maltodextrin). Meanwhile, the FASTex trial utilised a non-caloric 500 ml drink (sucralose and water) matched by volume and flavour to be indistinguishable from each

other. Then, participants rested for 90 minutes before exercising. Further blood samples were taken at minute 30 and 90 after drink provision.

Next (i.e. 90 min after drink provision), 75 min running at 70% VO_2peak was performed, whilst measuring respiratory gas exchange to determine the energy expenditure and carbohydrate/fat utilisation. The HR, RPE, and Feeling scale (FS) were monitored every 15 minutes (Hardy & Rejeski, 1989). Immediately after exercise the Actiheart device was installed. During exercise and recovery, water intake was allowed. After finishing the exercise trial, the participant stayed inside the laboratory for the next 3 hours. At the beginning of this period, the next blood sample was taken, and appetite was assessed every thirty minutes. After passing two hours post-exercise, participants completed an "*ad libitum* meal test", followed by the last appetite VAS within the laboratory thirty minutes after finishing the meal test.

Before leaving the laboratory, all the participants were provided with a daily food package to eat during the experimental trials (they were only allowed to eat from the food provided) and requested to collect and bring back any food leftover in the corresponding bag.

Follow-up visit

After completing the 2.5-day follow-up, the participants returned to reassess most of the variables (RMR, blood samples, Likert scales, fasted appetite VAS, body mass and composition) and to return the Actiheart, CGM, and the remaining food. Also, an exit interview was conducted to assess if participants could recognise the different drinks. After a washout period (one week), the next trial for the same participant was performed. For

females, laboratory visit and follow-up period was conducted during the same phase of the menstrual cycle. All the experimental sessions for the same participant were performed on the same weekdays to decrease the effect of daily life routine changes.

Assessment of eating behaviour:

Ad libitum meal test:

Participants were sitting in the research kitchen where investigators constantly provided them with warm food (Pasta Bolognese, Sainsbury, UK (47% carbohydrate, 33 % fat, 18 % protein, 2% fibre)), with instructions to eat until they felt "comfortably full", and then the test finished. The investigators accounted for the food intake by weighing the food provided and the leftovers. During the meal test, participants were alone, without using mobile phones, and no water consumption was allowed.

Free-living conditions feeding:

Each participant was provided with a complete feeding plan for the next ~2.5 days. The energy needs were calculated according to the baseline RMR and AEE in free-living conditions, as estimated by the Actiheart. Every food package contained three main meals (reaching 75-80% of total daily energy needs) and snacks to achieve 135% of the daily energy requirements. Participants were instructed to request more food if necessary. They were instructed to eat "as much or as little they want". After finishing the free-living period, participants returned the daily food leftovers. This approach has worked successfully in a recent study (Podestá D et al., 2023). All the food provided was obtained from commercial distributors and stored following the manufacturer's recommendation. The diet's

macronutrient distribution of the food provided were 50% carbohydrates, 15% proteins and 35% fats.

Energy intake

To calculate the energy intake, the investigators subtracted the daily food leftovers from the daily total caloric content of the food provided. For the analysis, the *ad libitum* meal test is considered within the EI. The pre-exercise drinks' energy and nutrient content are not considered within the EI calculations.

Appetite VAS & Fatigue-, sleep quality-, muscle soreness-, stress- levels Likert scales

In the present study, for appetite assessment, the questions included were: How hungry do you feel now? How strong is your desire to eat? How much do you think you could eat right now? And how full do you feel now? These questions were answered by marking a 100 mm line using anchor statements as previously validated (Stubbs et al., 2000b), in the laboratory and during free-living conditions.

For fatigue, sleep quality, stress, and muscle soreness, a 1 to 5 Likert scale was utilised (Hooper & Mackinnon, 1995). The questions were: How are your fatigue levels now? How are your stress levels now? How was your sleep quality last night? How are your muscle soreness levels now?

The investigator configured an online questionnaire to allow the participants to answer the scales on their computers/mobiles/tablets. The data were collected and managed using REDcap electronic data capture tools hosted at the University of Birmingham, UK (Harris et

al., 2009). For free-living conditions, REDcap was programmed to email the questionnaires daily to the participants on a pre-specified schedule. The questions were analysed individually (Likert scales) and as a composite (VAS appetite).

$$appetite\ composite = \frac{\mathrm{H.H} + \mathrm{H.SD} + \mathrm{HM} + (100 - \mathrm{H.F})}{4}$$

HH = How hungry do you feel?; H.SD = How strong is your desire to eat?; HF = How full do you feel? HM = How much do you think you could eat right now?; All the values are expressed in mm.

Exercise energy expenditure and substrate utilisation within the lab

For estimating energy expenditure and substrate utilisation during the 75-minute run at 70% VO_2peak, a 3-minute breath sample was obtained every 15 min by indirect calorimetry utilising a metabolic cart (Vyntus, Vyaire Medical, IL, US). The values were calculated utilising the equations of Jeukendrup & Wallis (Jeukendrup & Wallis, 2005). For the analysis, exercise energy expenditure is not considered within the energy expenditure data.

Total and activity energy expenditure in free-living conditions (TEE & AEE)

The total- and activity-energy expenditure during free-living conditions was obtained from the data collected by the Actiheart (Brage et al., 2015). Participants were instructed to wear the device all the time, from finishing the run until the morning they returned to the laboratory. The Actiheart utilised a previously validated algorithm based on a branched model (Brage et al., 2007) that combines heart rate and accelerometry to estimate the total- and activity-energy expenditure. The device was configured to collect data every 15 s.

The participant's anthropometric information (i.e., body mass and height), date of birth, sex, sleeping- and maximum-heart rates were programmed into the software for data

processing. For improved accuracy, individualised calibration curves were built based on the values of heart rate and VO_2 obtained in the submaximal and maximal exercise tests. Also, the average of the two RMR measures in each trial was included to improve further energy expenditure estimation. Only participants without episodes of "non-wearing time", as detected by the Actiheart 5 software (i.e., no periods of more than two hours of non-wearing), were included in the analysis.

Blood sampling & biochemical analyses.

Venous blood samples were obtained and collected into pre-chilled 6 ml EDTA tubes. Blood samples were taken by cannulation from the antecubital vein at baseline, 30 and 90 minutes after the drink, after exercising, and two hours after exercising. Tubes were kept on ice until they were centrifugated at 4 ◦C, 1500 g, for 10 min. Plasma aliquots were stored at – 70◦C until they were analysed.

Plasma samples were analysed using an automated clinical analyser (RX Daytona + Randox, London, UK) to determine glucose (Glucose hexokinase, Randox, UK) and lactate (Lactate Dehydrogenase, Randox, UK) concentrations. For insulin, ultrasensitive ELISA assays were used (Mercodia, Uppsala, Sweden). All biochemical analysis was performed in duplicate.

Continuous glucose monitoring (CGM)

To continuously monitor interstitial glucose levels during the trials, a continuous glucose monitor (Dexcom G7, Dexcom, inc. USA) was placed on one of the participant's arms at least 24 hours before the start of each experimental trial, measuring glucose concentrations every 5 minutes. The participants were provided with a blinded receiver. The Dexcom Clarity

platform was utilised to process the data. The variables analysed included daily- mean glucose, mean glucose standard deviation (Glucose SD), and glucose coefficient of variation (calculated as glucose SD/mean glucose (%CV)). The data collected included three completed days, starting from midnight before the baseline visits until midnight before the follow-up visits, on each trial.

4.4 Statistics

The sample size was selected (n = 12) to be comparable with previous research investigating the impact of short-term exercise and nutrition manipulation on energy balance (Edinburgh et al., 2019; Podestá D et al., 2023). Unless otherwise stated, the statistical analyses included 12 participants. Data are described as mean ± standard deviation. The energy expenditure analyses account for all the data collected after the exercise bout and the energy intake was calculated from the *ad libitum* meal test plus the free-living period. Paired t-tests were used for exercise energy expenditure, % VO_2peak, RER, carbohydrate- and fat-oxidation rates, RPE, heart rate, and FS during exercise. A two-way repeated-measures ANOVA was utilised for daily- appetite, energy intake, Likert scales, activity- and total-energy expenditure, CGM data, and blood samples. For time x condition interactions, a Holm-Bonferroni *post hoc* was performed. Data were tested for sphericity with Mauchlý s test. For the variables where the sphericity assumption was violated, a Greenhouse–Geisser correction was performed. For the fatigue questionnaire, due to significant baseline differences, changes in fatigue levels from baseline were calculated for each trial and compared by paired *t-tests* between conditions. Statistical significance was set at $p < 0.05$. Statistics were performed using JASP 0.17.1 software and graphics with Graphpad PRISM 10.0.3.

4.5 Results

Exercise intervention

For exercise energy expenditure and percentage VO_2peak, no differences were observed between conditions (p = 0.138 & p = 0.078, respectively). For the RER and carbohydrate oxidation, higher values were observed during the FEDex condition (p < 0.001), while fat oxidation was higher for the FASTex (p < 0.001). Heart rate and rate of perceived exertion (RPE) were different between conditions, with lower values for the FEDex condition (p = 0.032 & p = 0.022, respectively). No differences between conditions were observed for the FS (p = 0.066) (for specific information, see Table 4.1).

Table 4.1. Exercise VO_2peak, energy expenditure, carbohydrate and fat oxidation, RER, heart rate, RPE and FS.

	FEDex	*FASTex*
% VO_2 peak	*70.7 ± 5.5*	*73.0 ± 5.0*
Energy expenditure (Kcal)	*1098 ± 207*	*1128 ± 218*
Carbohydrate oxidation (g min^{-1})	*2.72 ± 0.52**	*2.12 ± 0.45*
Fat oxidation (g min^{-1})	*0.36 ± 0.18**	*0.66 ± 0.21*
RER	*0.93 ± 0.02**	*0.87 ± 0.02*
HR (bpm)	*152 ± 9**	*155 ± 8*
RPE	*11.8 ± 1.7**	*12.5 ± 1.7*
FS	*2.12 ± 1.26*	*1.45 ± 1.45*

*Data are presented as mean ±SD. N = 12. * p <0.05*

Energy- and macronutrient- intake

No main effects for time, condition or time x condition interaction were observed for daily energy- (see Fig. 4.2. A, $p = 0.865$), carbohydrate-, protein-, and fat- intake (see Table 2, $p > 0.05$). For fibre intake, a main effect of time was observed ($p = 0.004$) (see Table 4.2).

Table 4.2. Daily macronutrient intake for both conditions.

	Carbohydrate (g)		*Protein (g)*		*Fat(g)*		*Fibre(g)*[a]	
	FEDex	FASTex	FEDex	FASTex	FEDex	FASTex	FEDex	FASTex
Day 1	372 ± 118	385 ± 110	147 ± 52	150 ± 42	144 ± 58	150 ± 42	35 ± 13	38 ± 11
Day 2	391± 113	383 ± 97	159 ± 38	159 ± 30	147 ± 48	147 ± 35	44 ± 13	43 ± 13
Day 3	394 ± 92	388 ± 103	157 ± 36	157 ± 33	148 ± 35	148 ± 37	44 ± 11	41± 16

Data are presented as mean ± SD. N = 12. [a] Time effect. Day 1 includes the energy and macronutrients from the *ad libitum* meal test.

Activity- and Total- Energy Expenditure

Data on 11 participants are presented as one participants' data was excluded due to insufficient Actiheart sensor wear-time. For TDEE (Fig. 4.2. B), time ($p < 0.001$) and time x condition interaction ($p = 0.017$) effects were observed, without a condition effect ($p = 0.136$). Subsequent post-hoc analysis did not show a difference between conditions at any time point ($p > 0.05$). For daily AEE (Fig. 4.2. C), time ($p < 0.001$) and time x condition interaction ($p = 0.018$) effects were observed. However, no main effect of condition was detected ($p= 0.223$). Post hoc analysis did not show any differences between conditions at any time point ($p > 0.05$).

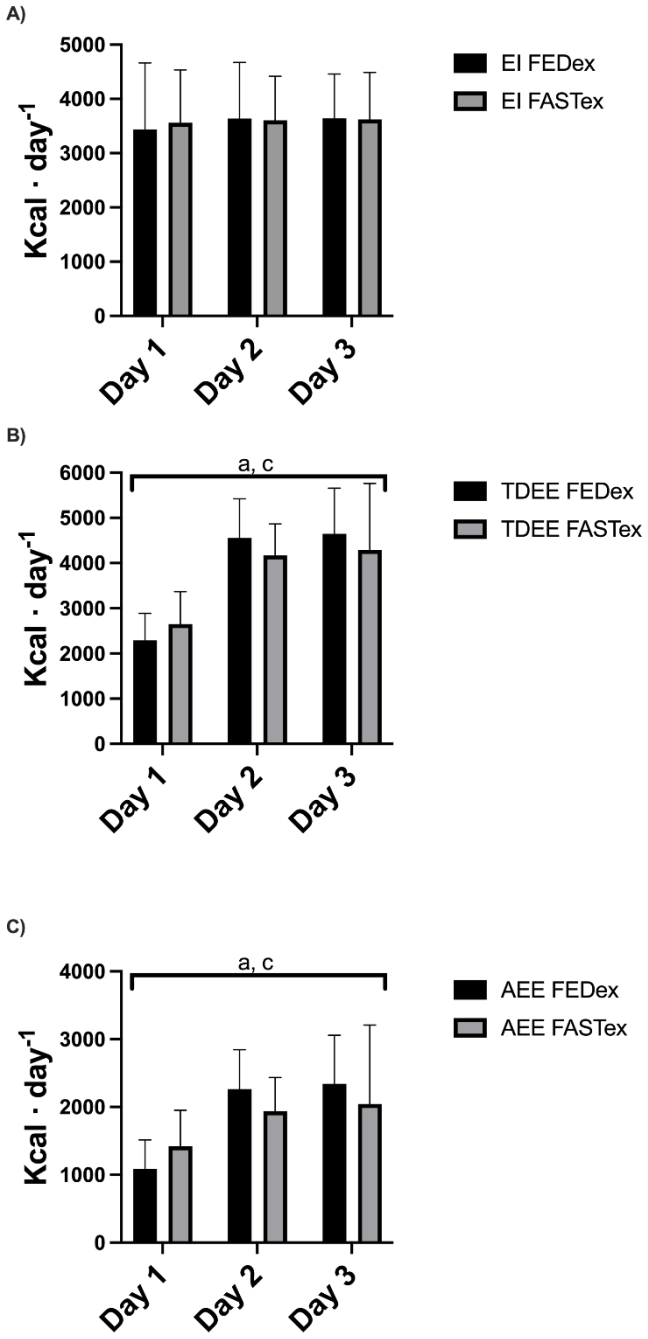

Figure 4.2. Comparison of the components of energy balance for both conditions. Data are presented as mean ± SD. A) EI = Energy intake (N =12). B) TDEE = total daily energy expenditure (N =11). C) AEE = Activity energy expenditure (N = 11). [a] Time effect. [c] Time x condition interaction effect. For TDEE and AEE day 1 is considered from after the run until midnight. Days 2 and 3 run from midnight until midnight.

Appetite

For the appetite VAS composite within the laboratory (Fig. 4.3. A), although a time effect was observed ($p < 0.01$), no condition or time x condition interaction effects were detected ($p > 0.05$). Similarly, no time, condition, or time x condition interaction effects were shown for appetite VAS between days (Fig. 4.3. B) ($p > 0.05$).

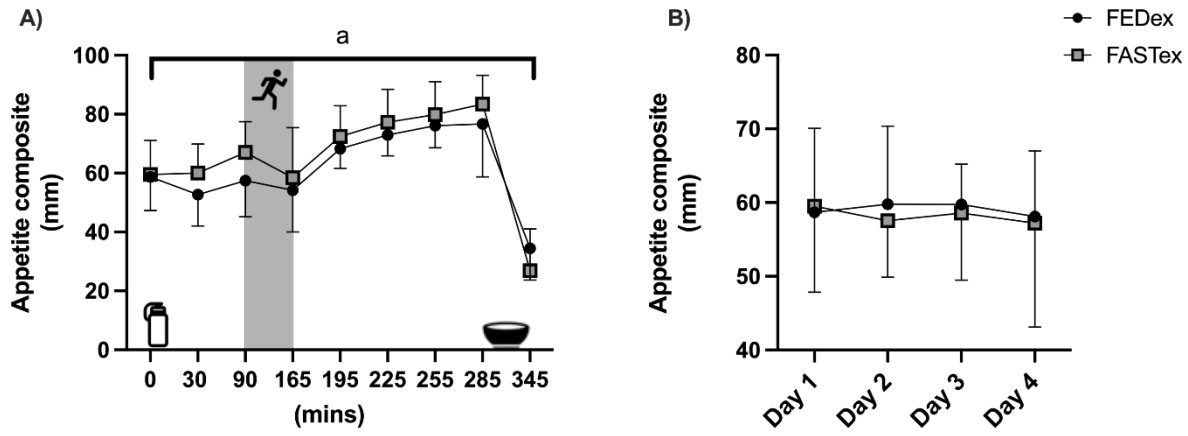

Figure. 4.3. **Composite appetite VAS within the lab (A) and between days (B).** N= 11. Data are presented in mean ± SD. The bottle represents the drink, the grey shaded area represents the 75-minute exercise bout, and the bowl represents the *ad libitum* meal test. [a] time effect.

Fatigue, sleep quality, stress, and muscle soreness levels

For fatigue (Fig. 4.4. A) and stress levels (Fig. 4.4. B), no differences were observed between conditions ($p > 0.05$). Only sleep quality (Fig. 4.4. C) differed between conditions ($p = 0.014$), with better sleep quality for the FEDex condition. Furthermore, a time effect was observed for muscle soreness (Fig. 4.4. D).

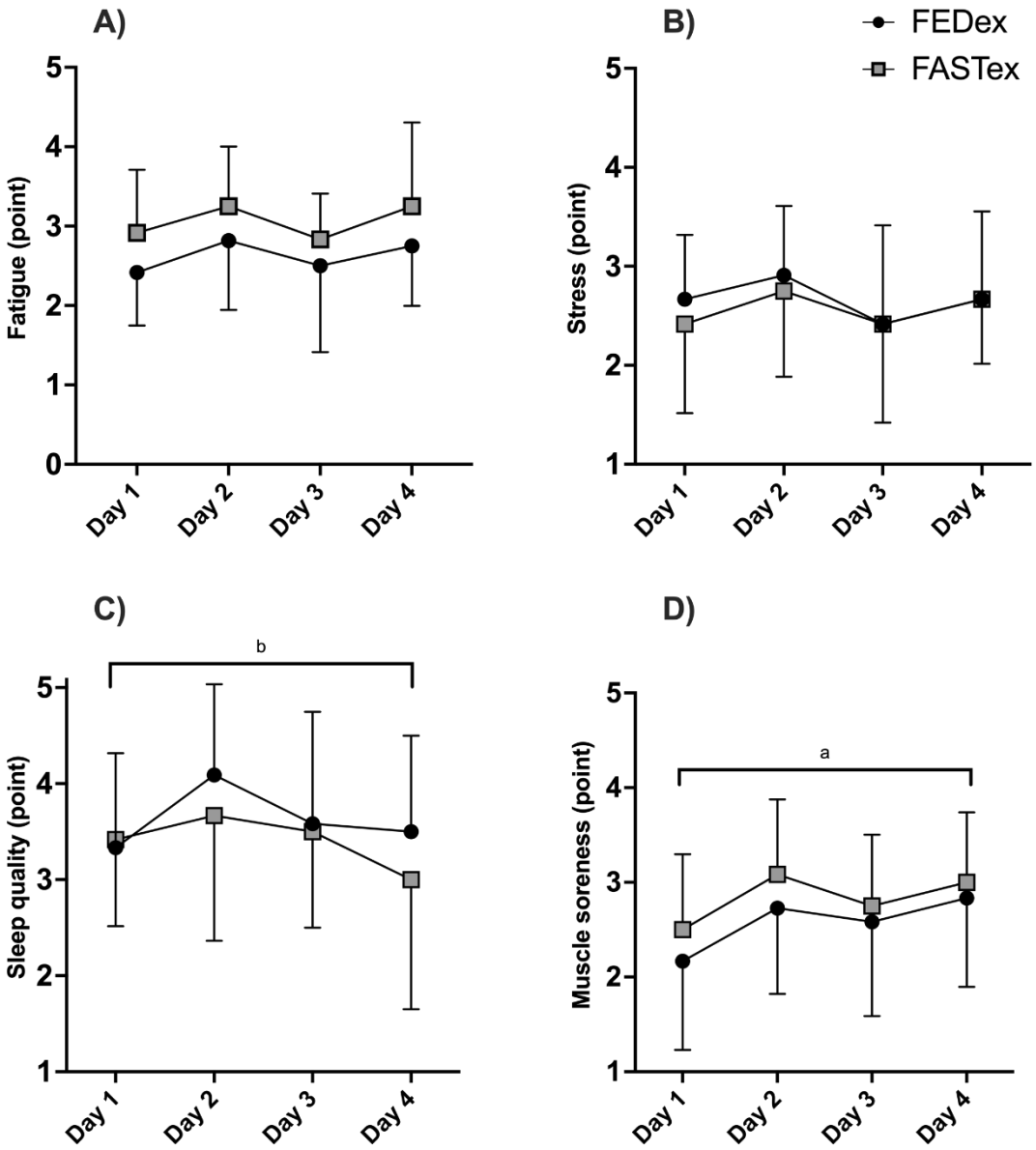

Figure 4.4. **Self-reported fatigue (A), stress (B), sleep quality (C), and muscle soreness (D) levels between days (N= 11).** Data are presented in mean ±SD. [a] time effect; [b] condition effect.

Blood metabolites and hormones

Glucose

For blood glucose concentrations measured within the laboratory period (Fig. 4.5. A), time ($p = 0.002$) and time x condition interaction ($p < 0.001$) effects were observed. *Post hoc* analysis showed during the FEDex condition the blood glucose was higher 30 minutes ($p < 0.001$), and lower 90 mins ($p = 0.014$) after the drink compared with the FASTex condition. For glucose samples obtained between days (Fig. 4.5. B), no condition or time x condition interaction effects were observed ($p > 0.05$). However, a time effect was observed ($p = 0.038$)

Insulin

For blood insulin concentrations measured within the laboratory period (Fig. 4.5. C), time ($p = 0.003$), condition ($p < 0.001$), and time x condition interaction effects were observed ($p = 0.003$). *Post hoc* analyses showed higher insulin values 30 mins after the drink for the FEDex condition ($p < 0.001$). For insulin between days (Fig. 4.5. D), while a time effect was shown ($p = 0.013$), no condition or time x condition interaction effects were observed ($p > 0.05$).

Lactate

For Lactate within the lab (Fig. 4.5. E), time ($p =0.001$), condition ($p < 0.001$), and time x condition interaction ($p < 0.001$) effects were shown. *Post hoc* analyses showed higher lactate values for the FEDex condition at 30 ($p < 0.001$) and 90 ($p < 0.001$) minutes after the drink. For lactate between days (Fig. 4.5. F), no time, condition, or time x condition effects were observed ($p > 0.05$).

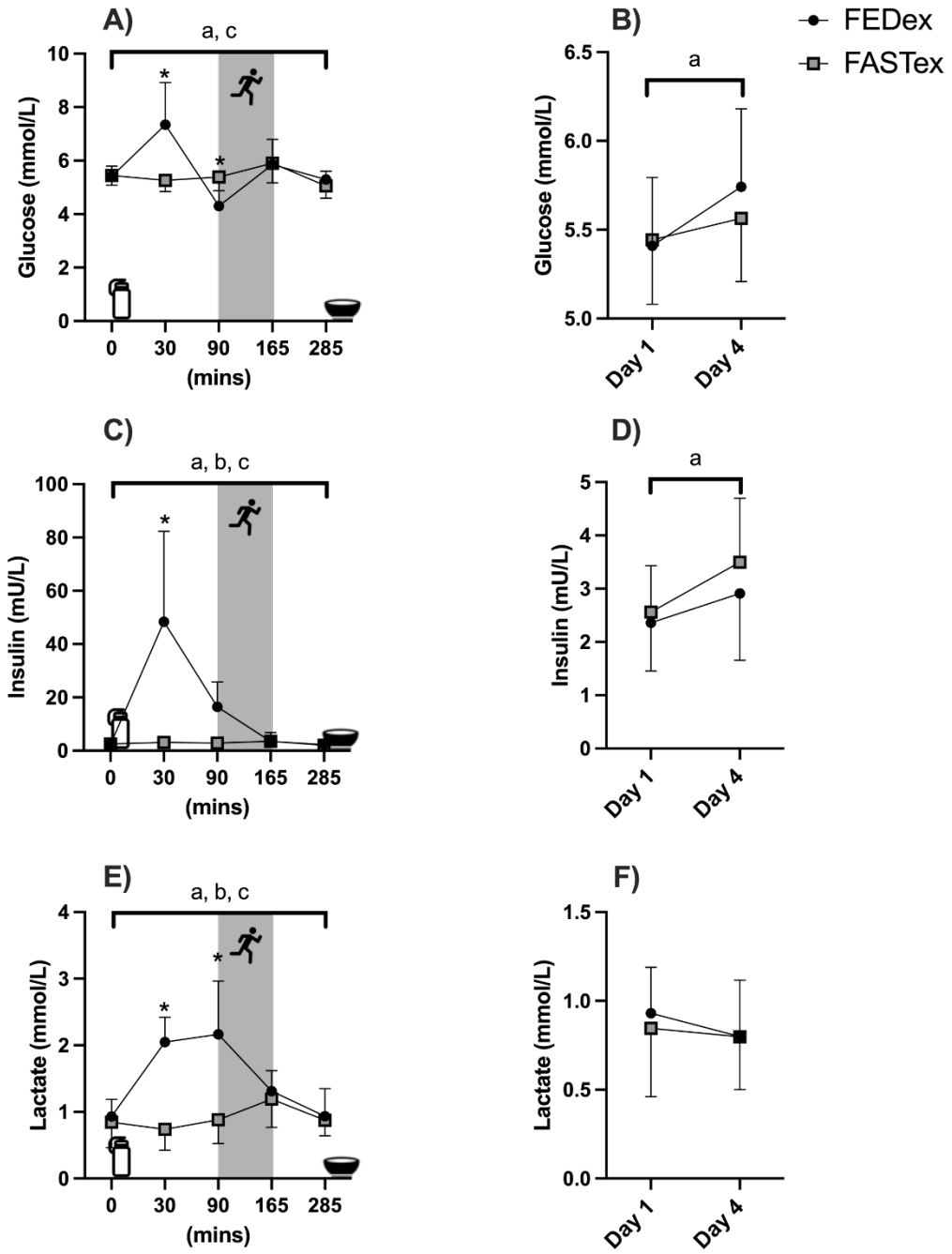

Figure 4.5. **Blood glucose (A, B), insulin (C, D), and lactate (E, F) concentrations within the lab (N= 10) and between days (N =11).** Data are presented in mean ±SD. The bottle represents the corresponding beverage, the grey shaded area represents the 75-minute exercise bout, and the bowl represents the ad libitum meal test. [a] time effect. [b] condition effect. [c] condition interaction. * Represents differences at specific time points according to Holm & Bonferroni *post hoc* tests.

CGM

The continuous glucose monitoring data are presented for 11 participants, due to one participant's device stopped working during one trial (Fig. 4.6). For daily- mean glucose levels (Fig. 4.6. A), mean glucose SD (Fig. 4.6. B), and glucose CV (Fig. 4.6. C), no time (p = 0.923; p = 0.834; p = 0.929, respectively), condition (p = 0.074; p = 0.088; 0.609, respectively) or time x condition interaction effects (p = 0.455; p = 0.088; p = 0.078, respectively) were observed.

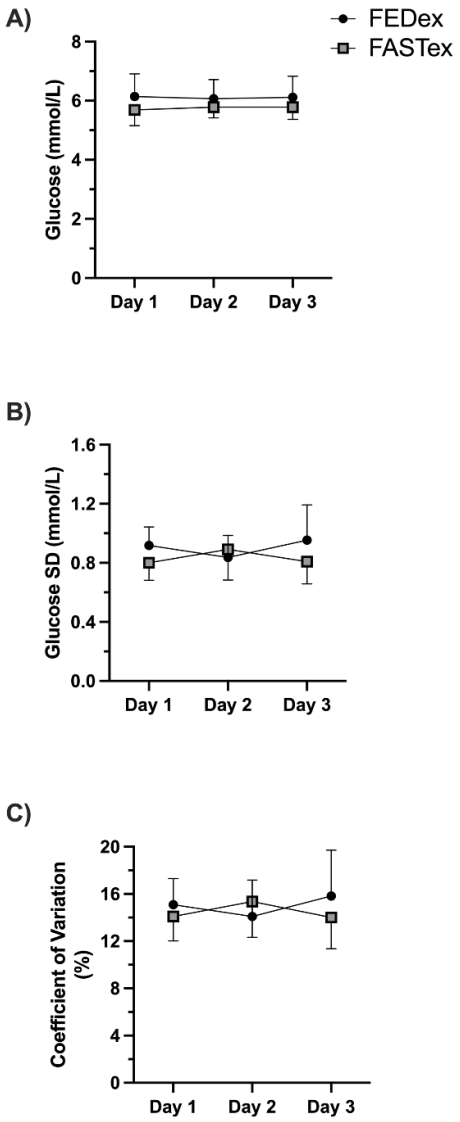

Figure 4.6. **Daily CGM mean glucose (A), mean glucose SD (B), and CV (C).** Data are presented in mean ±SD. (N= 11). SD = standard deviation. CV = % coefficient of variation.

4.6 Discussion

The current study investigated, in physically active individuals, the effects of performing an aerobic exercise session in an overnight-fasted (FASTex) or fed state (FEDex) on energy balance components across four days (i.e., appetite, energy intake and expenditure). Additionally, the effects of the two conditions on interstitial glucose concentrations were tested. No differences between conditions were observed in appetite, energy intake, energy expenditure, or interstitial glucose concentrations despite clear differences in the metabolic response to exercise between conditions. The results are discussed within the context of previous research exploring the impact of FASTex vs. FEDex in acute exercise and short-term exercise training interventions.

Contrary to the hypothesis, no differences between conditions were observed for energy intake or appetite within and outside the laboratory. These results agree with the literature for energy intake, showing no compensation within the first 24 hours following FASTex vs FEDex—even with exercise performed during the evening (Slater et al., 2023). Acutely, this could be a matter of timing, where the lack of time within daily routines avoids introducing another mealtime. Regarding appetite, no differences were observed between conditions, consistent with the energy intake data. Commonly, acute appetite suppression has been described after moderate to vigorous exercise sessions (Hu et al., 2023; Thackray & Stensel, 2023), followed by increased hunger levels in the next hours if the session was performed overnight-fasted exercise (Frampton et al., 2022) compared to a carbohydrate fed exercise (Frampton et al., 2023). In the present study, within the laboratory, despite the differences in the feeding state, the interventions did not significantly affect appetite. This could be attributed to the fact that to favour the blinding process, and opposite to the studies

showing differences in appetite, the present intervention used liquid- instead of solid food (Bachman et al., 2016; Deighton et al., 2012; Frampton et al., 2022; Griffiths et al., 2020; Hunschede et al., 2015; Veasey et al., 2015).

The present work extends the time frame of observations in previous studies to demonstrate FASTex vs. FEDex also did not differentially affect appetite and energy intake for up to 2.5 days of free-living conditions after the exercise intervention. Considering previous studies (Campfield et al., 1996; Wyatt et al., 2021) and integrating the present CGM metrics, the absence of differences between conditions on glucose mean, SD or CV may provide some explanation. Previous research has reported increased glucose variability after performing FASTex (Nygaard et al., 2017) along with alterations in post-exercise counterregulatory responses after performing prolonged exercise during the following hours (Galassetti, Mann, Tate, Neill, Wasserman, et al., 2001; Meyer et al., 2005) and day (Galassetti, Mann, Tate, Neill, Costa, et al., 2001). However, as stated, circulating glucose availability was similar between FASTex and FEDex, so whether differences in glucose availability is relevant for appetite or energy intake modulation cannot be determined from the present study.

The present investigation also provided an extended time frame (i.e., beyond 24 h after the exercise bout) from previous studies for the measurement of AEE and/or TDEE (Frampton, Edinburgh, et al., 2021). Despite not finding clear differences in energy expenditure after FASTex vs. FEDex the conditions showed opposing patterns for AEE and TDEE. That is, the FASTex condition tended to induce higher levels of AEE and TDEE during day one with lower values on days two and three compared with FEDex —possibly counteracting any treatment

effect on short-term energy balance. Notwithstanding, it is essential to highlight that the energy expenditure data for day one only accounted for the time after exercising onwards. Before this (i.e., between the drink and the exercise bout), participants were asked to remain within the lab, which could have limited free movement and possibly masked any influence of the differences in blood glucose levels on AEE, as suggested by others (Betts et al., 2014; Chowdhury et al., 2016b; Farooq et al., 2021b; Lessan et al., 2018; Podestá D et al., 2023; Smith et al., 2017; Templeman et al., 2021). Collectively, regardless of not having differences between conditions in the components of energy balance, the FASTex did not produce any increase in appetite or energy intake nor a reduction in energy expenditure during the trials. Although this scenario would favour an acute negative energy balance, no extra benefits in body mass regulation have been reported with FASTex (Edinburgh et al., 2020; Gillen et al., 2013; Schoenfeld et al., 2014), suggesting that energy compensation responses would be evident in more extended periods.

During the free-living condition period, fatigue, stress, sleep quality and muscle soreness levels were assessed by self-reported ratings. As with the main outcomes, these subjective variables did not show significant differences between conditions apart from sleep quality, where the FEDex trial expressed higher scores (i.e., better sleep quality). Although explaining the mechanisms behind these results is beyond the scope of this article, differences in sleep quality have been shown with diets varying in carbohydrate intake (Benton et al., 2022). In the current study, differences in carbohydrate intake were a side effect of the carbohydrate-based beverage on the FEDex condition. Whether these differences in sleep quality can favour more physically active patterns in the long term is still a matter of debate (Kline, 2014).

While the present study employed a careful design, it is essential to note that the results were obtained from a homogenous sample (mainly white, physically active males), limiting the generalisation of the present results, and warranting testing these outcomes in other populations of interest (e.g., overweight/obesity or weight-based sports categories). On the other hand, it is possible to underscore that the combined nature of the experimental design (i.e., controlled laboratory and free-living conditions) helped to expand the current literature available in terms of time frame as only acute energy balance data (up to 24 hours) were published previously. Moreover, as no energy intake, nutrient intake, or energy expenditure data differences were observed, any result found here can be linked to manipulating the pre-exercise feeding state. To control for the influence of food variety on appetite and energy intake, participants were provided precisely with the same foods for both trials (except for the drink). Also, the participants completed the experimental trials matching the same days of the week (avoiding inherent changes in behaviour due to the weekly routine), and females completed the trials during the same self-reported phase of the menstrual cycle.

In summary, despite the present study showing no differences in energy balance components after performing FASTex or FEDex, it expanded the time frame from the previously available data. From a practical perspective, although the FASTex condition did not produce either increases in appetite and energy intake or decreases in energy expenditure, the literature shows no additional benefit of FASTex vs FEDex to favour body mass regulation. The latter highlights the need for well-controlled studies using extended periods to determine how and when compensatory responses appear.

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Chapter 5: Associations between habitual diet and physical activity energy expenditure in the UK Biobank cohort.

For the present study, Dr Daniel Perez Zapata from the University of Birmingham, UK, provided biostatistical support. The research question, hypothesis, and the models tested were designed by Israel Podesta D, who also interpreted the data.

5.1 Abstract

Introduction: There is data that shows that physical activity energy expenditure (AEE) can independently predict a small percentage of energy intake (EI). Additionally, indirect evidence has suggested that dietary nutrient intake/availability can influence both the EI and AEE. However, the association between habitual diet and AEE levels has not been tested. Therefore, the present study aimed to explore the association between habitual dietary intake (nutrient and energy) and AEE levels in the UK Biobank cohort population.

Methods: 52,643 participants (mean 56 years old; 57% females), including at least two 24-hour dietary recall and derived accelerometry measures, were included from the UK Biobank cohort. AEE was derived by using a previously validated equation from wrist accelerometry. Mixed effects models were utilised to assess the association between carbohydrates-, protein-, fat- and energy intake with AEE levels.

Results: After addressing collinearity, the mixed effects model showed that Model 2 (R2m = 0.017; R2c = 0.758; AIC = 148,542), including only habitual carbohydrate intake ($\beta = 0.129$; $p < 0.01$), was able to predict a 1.7% AEE variance comparable with Model 1, which included habitual carbohydrate- ($\beta = 0.127$; $p < 0.01$) and energy intake ($\beta = 0.002$; $p > 0.05$) (R2m = 0.017; R2c = 0.859; AIC = 148,552).

Conclusion: The present study suggests that habitual carbohydrate intake can predict around 1.7% of the variance in AEE levels in the UK Biobank cohort population. The addition of energy intake as an independent variable did not enhance the predictability, indicating that habitual carbohydrate intake could predict AEE beyond their energy content.

Abbreviations

AEE activity energy expenditure

AIC Akaike Index Criterion

BMI Body Mass Index

EI energy intake

NHS National Health Service

RMR Resting Metabolic Rate

RQ Respiratory Quotient

R2c Condition r-squared

R2m Marginal r-squared

SD Standard Deviation

VIF Variance Inflation Factor

5.2 Introduction.

Obesity and overweight have been rising worldwide in the last few decades. Although obesity is a well-known multicausal disease (e.g., social, environmental, lifestyle, health, etc.) (Schwartz et al., 2017), fundamentally, it results from a chronic imbalance between energy- intake (EI) and expenditure (EE) that leads to fat mass accumulation. According to some models, a daily 30-50 kcal mismatch between EI and EE is associated with 0.5 kg of body weight gain within a year (Speakman et al., 2011b). Such small differences that accumulate over time point to the need to consider the regulation of energy balance (and its components) in acute and long-term contexts.

The relationship between energy intake and expenditure is reciprocal (i.e., they can influence each other). In this regard, it has been shown that up to 47% of the variance in EI across two weeks can be explained by resting energy expenditure (RMR) in males and females eating *ad libitum* (Hopkins et al., 2016). However, this relationship seems weaker with higher body fat mass (Casanova et al., 2021). Additionally, independent effects of physical activity energy expenditure (AEE) on EI have been shown by (Hopkins, Duarte, et al., 2019). Here, the authors showed a mild but significant independent role for AEE as a predictor of EI, explaining around 3% of the variance for this variable. Furthermore, other studies (Beaulieu et al., 2016b; Edholm, 1977; Edholm et al., 1955, 1970; Hägele et al., 2019b; Mayer et al., 1956; Shook et al., 2015) have shown that the association between EE and EI seems to closely match each other when moderate to high physical activity levels are present (i.e., at high energy flux (Bosy-Westphal et al., 2021)). In this sense, it has been observed that physically active people tend to have a higher drive to eat but also increased

satiety, keeping them close to energy balance (J. E. Blundell & Beaulieu, 2023; N. A. King et al., 2009). However, the inference that observed EI increases as a consequence of AEE (i.e., for energy or nutrient replenishment) fails to consider the alternative (i.e., changes in energy or nutrient availability influence AEE), which warrants further studies.

Whether EI and/or the component nutrients could modify AEE levels have rarely been tested. In the study led by Hopkins et al. 2019, the authors tested the effects of EI on AEE mediated by fat mass, fat-free mass and RMR as an alternative model. They hypothesised that increased EI would result in increased fat mass and decreased AEE. Still, this model failed to support the hypothesis due to unsuitable model fit metrics (i.e., the variables included in the model do not fit the observed data well, and further refinements for the model are needed). This may suggest that the effects of EI on AEE are not mediated by body composition changes but rather by habitual energy and/or nutrient availability for physical activity. The latter has been further supported by the indirect evidence linking higher respiratory quotients (RQ) and carbohydrate oxidation with higher EI (Flatt, 1987; Hopkins et al., 2011a; Pannacciulli et al., 2007; Piaggi et al., 2015) and lower carbohydrate/energy availability with reduced AEE (Betts et al., 2014; Chowdhury et al., 2016b; Farooq et al., 2021b; Kien et al., 2013; Lessan et al., 2018; Podestá D et al., 2023; Templeman et al., 2021; Yates et al., 2022). However, testing this alternative hypothesis (i.e., that EI and/or nutrient intake predict AEE levels) would require modelling this association, including nutrients and energy as predictor variables for AEE. Since conducting long-term dietary interventions is challenging, one way to overcome these difficulties is to use cohort data.

Therefore, the present investigation aimed to explore the associations between habitual diet, in terms of energy and nutrient intake, and AEE levels within the UK Biobank cohort population. It was hypothesised that the habitual amount of dietary nutrients (i.e., carbohydrates, fat, and protein) would predict AEE variance levels beyond their energy content.

5.3 Methods

The present study was a retrospective, population-based cohort study of the UK Biobank participants. The UK biobank enrolled 500,000 individuals aged 40-69 years by postal invitations from March 2006 to July 2010, described in detail elsewhere (Sudlow et al., 2015). Briefly, the participants underwent physical examinations and anthropometric assessments by trained personnel and filled out touchscreen questionnaires (including dietary information). Between June 2013 and December 2015, a subsample (n = 103,670) of the UK Biobank agreed to wear an Axivity AX3 accelerometer (Axivity, Newcastle, UK) (Doherty et al., 2017). All participants provided written consent for data collection, analysis, and linkage, and the study received ethical approval from the UK National Health Service (NHS) National Research Ethics Service (11/NW/0382). Only participants with at least two 24-hour dietary recalls and derived accelerometry data available were included in the analyses.

Accelerometry measurement and processing.

Participants in the accelerometry subsample were requested to wear the AX3 Axivity device on their dominant wrist continuously for seven days (Doherty et al., 2017). The devices were calibrated to local gravity (van Hees et al., 2014), recording raw data at a 100Hz resolution.

A low pass filter of 20 Hz was applied to eliminate machinery noise. The proportion of time spent at different movement intensities throughout the day for each participant was determined by summarising the movement-related acceleration calculated as the vector magnitude minus gravitational acceleration in 5-second epochs. Extended periods of non-wearing the device were identified (≥ 60 minutes) (van Hees et al., 2013). Then, the missing data was imputed using the average of a similar time of day vector magnitude and intensity distribution points with one minute granularity. From the 103,612 participants with derived accelerometry available, those with deficient calibration and insufficient wearing time (i.e., a lack of data in at least a one-hour period within a 24-hour cycle or less than 72 hours of wearing time) were excluded, leaving 96,664 participants in the analysis. A previously validated population-specific equation (White et al., 2016, 2019) was applied to obtain AEE, converting the time spent in the different movement intensity categories into energy expenditure.

24-hour dietary recall

This questionnaire was first introduced for the last 70,000 participants during the assessment visits (UK Biobank, 2012). It was also emailed to be completed remotely for ~320,00 participants who provided valid email addresses (online version previously validated (Liu et al., 2011)). Additionally, participants were invited on four separate occasions over one year (between Feb 2011 and April 2012) to complete the questionnaire to account for seasonal variation in dietary intake and to provide an average measure for everyone (i.e., as a marker of habitual intake). This questionnaire included questions about the intake of foods and beverages consumed in the previous 24 hours. The quantity of each food, drink, and nutrient consumed was calculated by multiplying the portion size by the

number of portions consumed based on the food composition table by McCance and Widdowson's "The Composition of Foods 6th edition (2002)" (Food Standards Agency, 2002).

Characterisation of the sample:

All the descriptive data (age, sex, ethnicity, mobility limitation/long-standing illness, and medications) were obtained from the baseline visits to the health centres between 2006 and 2010.

From those participants with available derived mean daily accelerometry (96,664), only 52,643 registered at least two 24-hour dietary recalls at different time points. Then, the associations between daily- nutrients (carbohydrates, protein, fat (grams/day)) and dietary energy intake (kJ/day) with physical activity energy expenditure (kJ/kg/day) were tested.

5.4 Statistical analysis

Data are reported as mean ± SD. Pearson correlations were used to assess the association between carbohydrates, protein, fat, energy content, and AEE. Based on previous data (Hopkins et al., 2019), a random intercept model was fitted with mean daily AEE as a dependent variable, carbohydrates, fat, protein, and energy content as independent variables (i.e., fixed effects) and an "intercept term" was introduced to address the dependency of the aggregated data obtained from the same individual (i.e., random effects). These models were selected for their capacity to handle missing values and the violation of the independence of data principle (Brown, 2021). Multicollinearity (i.e., when two or more independent (predictor) variables are correlated with each other) was pre-screened by inspecting Pearson's r correlation levels and calculating the variance inflation

factor (VIF) for the models developed, to avoid the inflation of the estimators' variances and to evaluate the relative contribution of each independent variable in the model (Khalili et al., 2021). VIF is a diagnostic tool commonly used within regression and mixed effects model to detect multicollinearity. VIF helps to quantify how much of this correlation inflates the variance of a specific coefficient's estimate. It essentially compares the variance of a coefficient's estimate when estimated in the full model (with all variables) to the variance if it were estimated alone (without the influence of other variables) (Hair Jr. JF et al., 2019). VIF above 5 indicate the presence of multicollinearity in a model, however, lower VIF values do not fully discard the presence of multicollinearity. Consequently, it is recommended to test the bivariate correlation between the same variables to corroborate the results. Within the present study, as the initial model, including all the nutrients, showed values of VIF > 5 for all three nutrients and Person's correlation coefficients > 0.85, the two nutrients with the highest VIF were removed (i.e., protein and fat) (Hair Jr. JF et al., 2019). Then, the two possible models were tested. Model 1 included carbohydrates, energy intake, and the intercept, and Model 2 included carbohydrates plus the intercept as variables. The calculation of the marginal r-squared (R2m), conditional r-squared (R2c) (Nakagawa & Schielzeth, 2013), and the Akaike information criterion (AIC) (Cavanaugh & Neath, 2019) were performed to compare the models.

The marginal r-squared represents the predictability based only on the fixed effects, while the conditional r-squared states the predictability explained by the random and fixed effects together. The AIC is a tool used to compare the relative quality of different statistical models for a given set of data (i.e., the model that offers a better balance between fitting the data and complexity). More complex models with many parameters tend to fit the training data

better but might struggle to generalise to unseen data (overfitting). AIC penalises models with a higher number of parameters. Lower AIC scores indicate a better model according to the AIC criterion (Cavanaugh & Neath, 2019). In the present study, the model with the lowest AIC was selected, as a lower AIC reflects a better trade-off between goodness of fit and lower complexity for the models (i.e., more parsimonious). All analyses were performed using R studio (version 2023.09.0+463) and the lme4 package (version 1.1-35.1).

5.5 Results

Sample characteristics (See table 5.1):

Out of the 52,643 individuals who were included in the study, 57% were females. Fifty-eight point three per cent of the sample had a BMI ≥ 25 kg/m^2. From this sample, 97.5% of the participants identified themselves as having a white ethnic background. Furthermore, 27.6% of the sample reported living with long-standing- illness, disability, or infirmity. Among the female and male participants, 26.1% and 29.6%, respectively, were taking at least one medication for cholesterol and/or blood pressure and/or diabetes and/or hormones.

***Table 5.1**. Descriptive statistics of the population. Data presented as mean ± SD, n, and %.*

Characteristic	***Overall*** **(n = 52,643)**
Females (n)	*29,963*
Age (y)	*56.2 ± 7.8*
Height (cm)	*169.3 ± 9.1*
Body mass (kg)	*76.2 ± 15.3*
BMI (kg/m^2)	*26.5 ± 4.8*
Overweight, n (%) *(25-29.9 kg/m^2)*	*21,121 (40.2)*
Obesity, n (%) *(≥ 30 kg/m^2)*	*9,534 (18.1)*

Associations between habitual dietary nutrients, energy intake, and AEE.

Mean values for daily carbohydrates, protein, fat, energy intake, and AEE are described in Table 5.2. As can be observed in Fig.5.1, statistically significant positive associations were observed between carbohydrates ($r = 0.129$, $p < 0.001$), protein ($r = 0.109$, $p < 0.001$), fat ($r = 0.118$, $p < 0.001$), energy intake ($r = 0.094$, $p < 0.001$) and AEE. Also, high Pearson's r-coefficient values were observed between the independent variables (see Table 5.3). To test the overall associations between carbohydrates-, protein-, fat-, energy intake and AEE, a random intercept model was developed, including all four dietary variables as independent predictors and AEE as the dependent variable. After testing for multicollinearity and based on a priori expectations, protein (VIF = 8.96) and fat (VIF = 9.42) were excluded from the model, as they showed the highest VIF.

Table 5.2. Descriptive statistics for carbohydrates, protein, fat, energy intake and AEE.

	Carbohydrates (grams/day)	*Protein (grams/day)*	*Fat (grams/day)*	*Energy intake (kJ/day)*	*AEE (kJ/kg/day)*
Mean ± SD	*254 (69)*	*81 (20)*	*74 (25)*	*8676 (2128)*	*46.1 (13.9)*
Median	*249*	*79*	*69*	*8463*	*44.6*
min	*3.8*	*0*	*0*	*198*	*1.04*
max	*990*	*271*	*361*	*33008*	*322*

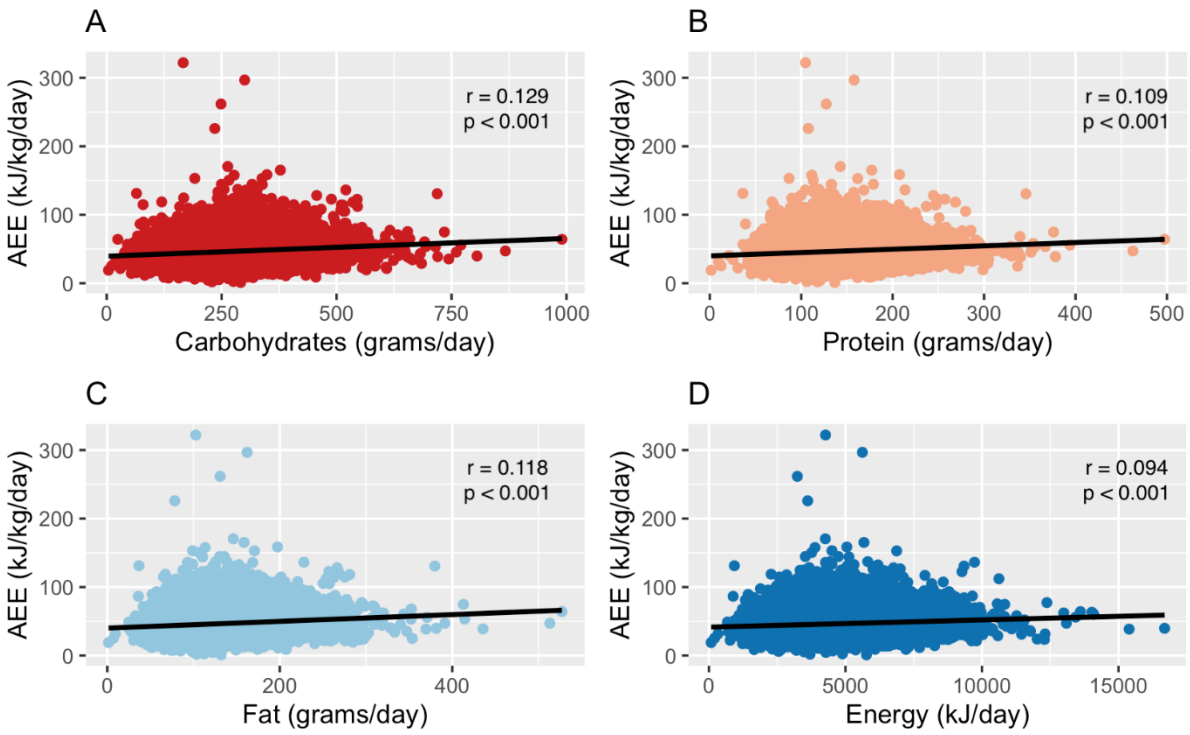

Figure 5.1. Pearson's r correlations between AEE and habitual dietary carbohydrates (A), protein (B), fat (C), and energy intake (D). All the associations were statistically significant, p <0.001.

Table 5.3. Pearson's r coefficients for the associations between carbohydrates, protein, fat, and energy intake.

	Carbohydrates	*Protein*	*Fat*	*Energy intake*
Carbohydrates	*1*	*0.845****	*0.856****	*0.719****
Protein	*0.845 *****	*1*	*0.936****	*0.556****
Fat	*0.856 *****	*0.936****	*1*	*0.609****
Energy intake	*0.719 *****	*0.556****	*0.609****	*1*

*** p <0.001*

Habitual carbohydrate- and energy intake as predictors of AEE.

From the two models, Model 1 included the intercept, carbohydrate- and energy intake as predictor variables, and Model 2 only included the intercept and carbohydrates to predict AEE. For Model 1 and Model 2, the Random effects (i.e., intercept) explained 84.3 % (SD 91.2) and 74.1% (SD 86.1) of the variances for AEE. The fixed effects (i.e., carbohydrate intake and/or energy intake) for each model are described in Table 5.4. The R2m and R2c for Model 1 were 0.017 and 0.859, and for Model 2, 0.017 and 0.758, respectively. The Akaike information criterion (AIC) was 148,552.9 for Model 1 and 148,542.7 for Model 2.

Table 5.4. Summary of the variables included in Models 1 and 2.

Models	Dependant	Independent	Estimate (b)	SE	t-value	P
Model 1	AEE	Intercept	-8.823 e-14	4.322 e-3	0.000	1.000
		Carbohydrates	1.268 e-01	6.216 e-3	20.402	<2 e-16***
		Energy intake	2.372 e-03	6.216 e-3	0.382	0.703
Model 2	AEE	Intercept	-8.763 e-14	4.322 e-3	0.000	1.000
		Carbohydrates	1.285 e-01	4.322 e-3	29.73	<2 e-16***

*** $p < 0.001$.

5.6 Discussion

The present study tested whether the components of the habitual diet (nutrients and energy intake) differentially explained the mean daily AEE in the UK Biobank cohort. The results show that habitual carbohydrate intake (Model 2) can explain 1.7% of the variance for AEE (R2m = 0.017; R2c = 0.758; AIC = 148,542). However, when energy intake was included in modelling this association (Model 1), it did not reach significance or improve the percentage of the variance explained by the fixed effects (R2m = 0.017; R2c = 0.859; AIC = 148,552). When comparing the models, the AIC suggests better goodness of fit for the model only including carbohydrate intake (Model 2), as it captures a comparable level of the variability of the data with lower complexity (i.e., Model 2 is more parsimonious).

While the present models exhibit a significant but weak predictability (1.7%) of AEE, these results are comparable in magnitude to those that have tested these associations in the opposite direction (Hopkins, Duarte, et al., 2019; Piaggi et al., 2015). For instance, Hopkins et al. have shown that AEE can independently predict a small percentage of energy intake (~3%) during free-living conditions. Similarly, Piaggi et al. found an association between AEE and energy intake while studying individuals within a metabolic chamber ($r = 0.33$, $p = 0.001$). Nevertheless, when AEE was included in a multivariable model along with 24-hour EE and RQ, this association was not significant anymore ($p = 0.39$). Despite the reported associations, no studies have tested this relationship in the opposite direction or including the energy sources (i.e., nutrients) as independent variables. To the authors' knowledge, the present study is novel in exploring these associations, where the influence of the habitual diet composition (nutrients and energy content) on the AEE levels was tested.

Based on the glycogenostatic theory of energy intake control (Flatt, 1987), which postulates that the quantity of energy stored as carbohydrates within the body influences brain function, physical activity, and the thermic effect of food, these results may be explained. Higher habitual carbohydrate intake will favour a higher carbohydrate availability to be used during physical activity or brain functions. Also, indirect evidence has shown that dietary nutrient composition (Kien et al., 2013; Podestá D et al., 2023) or energy availability (Betts et al., 2014; Chowdhury et al., 2016a; Farooq et al., 2021a; Lessan et al., 2018; Templeman et al., 2021) could modulate AEE. In the present study, carbohydrate intake in isolation explained the same percentage of AEE variance (1.7%) as the model that also included energy intake. Based on the cited studies, the present results suggest that the association between habitual diet composition and AEE could be a joint response to the metabolic

effects of carbohydrate intake (e.g., increasing carbohydrate oxidation, changes in glycaemia, fuelling the brain) and the energy provided, where energy intake seems to be permissive with carbohydrates intake.

It is important to note that although our hypothesis and the timing of the data collection (diet (2009-2012) and accelerometry (2013-2015)) suggest a directionality (i.e., the influence of diet on AEE), it is still possible that this relationship could be at least bidirectional. In that case, the results could be interpreted as an attempt by the body to replace AEE preferentially with carbohydrates instead of a non-specific EI (Pomerleau et al., 2004; Verger et al., 1992) as the AEE increases. These suggestions would fit with other studies, as it can be argued that a tighter EI/AEE response is observed at higher physical activity levels (Beaulieu et al., 2016b, 2018), where carbohydrates are the primary fuel. While a nutrient-specific replacement intuitively makes sense, there is insufficient evidence to support any macronutrient preference based on physical activity levels consistently (Beaulieu et al., 2016b; Elder & Roberts, 2008; Hopkins et al., 2011a). For example, from the research exploring the eating patterns of active individuals compared to those of inactive, there are studies suggesting that active individuals show increased carbohydrate and reduced fat intake (Catenacci et al., 2014; Jago et al., 2005; Van Walleghen et al., 2007), while others have shown increased protein intake (Jokisch et al., 2012). This discrepancy underscores the need for more studies to test directionality.

Regarding the influence of the random effects (i.e., the between individual differences) on the predictability of the models, it was estimated that individuals' traits explain substantial amounts of the AEE variance but with high variability (Model 1: 84.3% (SD 91.8) and Model

2: 74.1% (SD 86.1)). Among the individual traits that can influence AEE, body size and composition, age, genetic predisposition, and mobility limitations or illness have been identified as the most influential (Westerterp, 2013). In the present sample, participants were between 40 and 69 years old; 57% were females, 58% were living with overweight or obesity, 28% were living with a long-standing illness or mobility limitation, and 26% of the females and 30% of males were taking medications for cholesterol and/or blood pressure and/or diabetes and/or hormones. Consequently, the present results should only be interpreted considering the characteristics of the current population, and further studies are needed to address the differences associated with each specific characteristic as a moderator factor.

In summary, these exploratory data suggest that habitual carbohydrate intake can predict around 1.7 % of the AEE levels variance in the UK Biobank cohort population. The addition of energy intake as an independent variable in modelling this association did not enhance the predictability, suggesting that the observed results could be more related to metabolic changes rather than an isolated increase in energy availability. Future work should continue exploring these associations to determine if different conditions (e.g., metabolic disease, drugs, the extremes of the physical activity spectrum) modulate these relationships.

5.7 References

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Chapter 6: General Discussion.

The overarching theme of the present thesis was to understand better how, from a metabolic perspective, dietary carbohydrates and physical activity/exercise interact in the short term to modulate energy balance components (such as energy expenditure, energy intake, and appetite). This spawned the generation of three aims:

1. To investigate the effects of an overnight-fasted aerobic exercise session followed by a high carbohydrate/low fat or a low carbohydrate /high-fat energy replacement on short-term energy balance components in physically active normal-weight individuals (Chapter 3).

2. To characterise the responses of energy balance components after overnight-fasted or carbohydrate-fed exercise bouts in physically active normal-weight individuals (Chapter 4).

3. To explore the associations between habitual diet, in terms of energy and nutrient intake, and physical activity energy expenditure (AEE) levels within the UK Biobank population (chapter 5).

This general discussion section will include a succinct summary of the most relevant findings of each chapter in respect of their specific aims. General topics that have been common across the chapters of this thesis will be addressed in independent sections. Finally, the limitations and suggestions for future work will be discussed.

6.1 Summary of findings

Chapter 3 explored the short-term effects of replacing exercise energy expenditure with a high carbohydrate/low fat or a high fat/low carbohydrate drink on energy balance components, addressing the corresponding aim. After the exercise energy expenditure was replaced with two different drinks, participants were studied across four days, where appetite, energy intake, activity- and total energy expenditure were evaluated. This chapter showed two novel findings. The increased cumulative activity energy expenditure and decreased appetite for the high carbohydrate low-fat treatment (compared with the low carbohydrate/high fat condition), without differences in energy intake between conditions. Therefore, it showed that a nutritionally different energy replacement post-exercise could lead to divergent responses on energy balance and appetite components, addressing the first thesis aim.

In Chapter 4 the effects of a carbohydrate-fed (FEDex) versus an overnight-fasted (FASTex) exercise bout on different components of energy balance were investigated. This included examining the responses of appetite, energy intake, activity- and total- energy expenditure, and interstitial glucose metrics across four days. Although no significant differences between the two conditions, the study helped to expand the previously published data time frame, providing a more comprehensive understanding of acute and long-term energy balance studies. The findings show that as compared to the FEDex, a single FASTex session did not modify energy balance components or interstitial glucose differently, suggesting that both approaches could be used interchangeably as exercise strategies for body mass management.

Chapter 5 investigated the relationship between the habitual diet of individuals, considering both nutrients and energy intake, and their physical activity energy expenditure (AEE). The study was conducted using data from the UK Biobank cohort, and after addressing collinearity, a Random intercept model was fitted from 52,643 participants. The study tested the predictability of two models - one including carbohydrates and energy intake, and the other including only carbohydrates - on the AEE levels. Although both models predicted around the same percentage of the variance (~2%) for AEE, the model that only included carbohydrates presented a better goodness of fit/complexity ratio according to the Akaike Index Criterion (AIC). These findings showed that habitual carbohydrate intake weakly but significantly predicts AEE levels in the UK Biobank cohort, suggesting that the effects of carbohydrates are not only due to their energy content but also their metabolic effects.

6.2 Common topics and emergent themes across chapters.

6.2.1 Carbohydrate availability and physical activity levels.

Aligning broadly with the overarching theme of this thesis, the availability of carbohydrates around exercise was indirectly linked to physical activity energy expenditure (AEE). Previously, some studies have suggested a possible role of carbohydrate availability in explaining differences in physical activity levels after dietary manipulations (Andriessen et al., 2023; Betts et al., 2014; Chowdhury et al., 2016a; Farooq et al., 2021b; Lessan et al., 2018). In this context, several dietary and physical activity interventions have shown the potential to reduce acutely the limited carbohydrates stores within the body (Hultman & Nilsson, 1971; Iwayama et al., 2021, 2023; Kojima et al., 2020; Nilsson et al., 1973; Nilsson & Hultman, 1973), reducing their utilisation (W. G. Abbott et al., 1988; Andriessen et al., 2023;

Shetty et al., 1994; Stubbs et al., 1993) and possibly impacting physical activity. This has been partially supported by a study in a metabolic chamber, where reduced carbohydrate oxidation and respiratory exchange ratio were associated with lower daytime physical activity counts while fasting (Andriessen et al., 2023). However, none of the previous studies manipulated carbohydrate availability directly to test if these effects were directly related with changes in AEE.

In Chapter 3 higher AEE levels were observed following a High Carbohydrate/Low-Fat (HCLF) energy replacement compared with a Low Carbohydrate/High-Fat(LCHF) trial, without differences in cumulative energy- or nutrient intake, suggesting an association between post-exercise carbohydrate intake and AEE. It is worth considering that the HCLF drink provided on average 245 grams of carbohydrates post-exercise, which is equivalent to 21% of the cumulative carbohydrate intake in Chapter 3 and 65% of the daily carbohydrate intake in Chapter 4. On the other hand, the LCHF condition provided on average 104 g of fat acutely, of which 64 g were saturated fats. This is equivalent to 30% of the cumulative fat intake in Chapter 3 and 71% of the daily fat intake in Chapter 4. This highlights the magnitude of the intervention produced by the drinks in Chapter 3 and their possible metabolic consequences.

It is speculated that the physiological mechanisms linking carbohydrate availability and physical activity could be mediated by blood glucose levels. Reduced carbohydrate availability around exercise can increase glycaemic variability (Nygaard et al., 2017) and lower counterregulatory responses to hypoglycaemia (Galassetti, Mann, Tate, Neill, Costa, et al., 2001; Galassetti, Mann, Tate, Neill, Wasserman, et al., 2001) by reducing endogenous

glucose production (Kishore et al., 2006). This can lead to reductions in peripheral blood glucose uptake and oxidation (Gregory et al., 2017; Meyer et al., 2005) and increases in hunger (Dewan et al., 2004) as strategies to regulate glycaemia. As a result, it could lead to decreased physical activity until glycaemic control is achieved again. Conversely, it cannot be excluded that the differences in AEE are the effects of the LCHF condition on glycaemic control and peripheral glucose metabolism (Hernández et al., 2017; Sarabhai et al., 2020, 2022), as it has been shown that high consumption of saturated fats can lead to lower resting- and activity EE (Kien et al., 2013), compared with monounsaturated fats. Unfortunately, in Chapter 3 no direct relationships between glycaemic control and AEE were possible to be tested, as in this study no monitoring of glycaemia was performed during free-living conditions. However, these results were used to inform the study design utilised in Chapter 4, which led to the incorporation of CGM.

Further, in Chapter 4, this concept was challenged as no differences in AEE and interstitial glucose levels were observed between performing an exercise bout carbohydrate-fed (FEDex) or overnight fasted (FASTex). However, it is important to highlight that differences observed in exercise substrate utilisation rates during these two chapters can help to interpret these results. In Chapter 3, the average estimated carbohydrate utilisation during exercise was 2.04 g min^{-1} (153 g in 75 mins). While these values were comparable during the FASTex trial in Chapter 4 (2.12 g min^{-1} (159 grams per 75 mins)), the FEDex condition showed higher carbohydrates utilisation rates (2.72 g min^{-1} (204 grams in 75 mins), $p < 0.05$). Although in Chapter 3 it was suggested that increased carbohydrate availability after exercise could have favour higher AEE by replenishing carbohydrates stores (liver and muscle), the differences in substrate utilisation observed between these chapters indicate

that providing a carbohydrate drink before exercise could have increased carbohydrate utilisation beyond the quantity provided (i.e., 150 grams), which led both groups in Chapter 4 to finish the exercise with a negative feeding/utilisation carbohydrate and energy difference (FEDex -54 grams of CHO and -515 Kcal, and FASTex -159 grams of CHO and -1115 Kcal). Whether the increased carbohydrate utilisation came from endogenous or exogenous carbohydrates remains to be explored.

The higher utilisation of carbohydrates during the FEDex in Chapter 4 is not surprising, as this effect has been well described during exercise conditions after feeding carbohydrates in a healthy population (Goodpaster & Sparks, 2017; Hargreaves et al., 2004). Additionally, consuming carbohydrates before and during exercise spares liver glycogen (Gonzalez et al., 2015) by reducing endogenous glucose production (Jeukendrup et al., 1999; Wallis et al., 2006). Consequently, in Chapter 4, it was assumed that reductions in liver glycogen would be attenuated with the FEDex trial (Iwayama et al., 2023). However, in Chapter 4, besides the increased carbohydrate utilisation during exercise, reduced venous blood glucose concentrations were observed before exercising (FEDex 4.2 ± 0.8 vs FASTex 5.3 ± 0.4 mmol/L of glucose; $p < 0.05$), where 5 out of 10 participants expressed values below the ADA hypoglycaemia threshold (<3.9 mmol/L). This event possibly increased instead of attenuated the liver glycogen utilisation, influencing the effectivity of the carbohydrate/energy availability manipulation, and possibly explaining the absence of differences in the CGM metrics between conditions during the following days. Although the absence of CGM differences between conditions can also be due to the fact the glucose flux is increased after FEDex (i.e., glucose rate of disposal and rate of appearance) versus FASTex (Edinburgh et al., 2018), where liver metabolism is a key mediator in this process (Galassetti, Coker, et al.,

1999; Galassetti, Koyama, et al., 1999; Moore et al., 2017, 2018). Nevertheless, as the work done in the present thesis aimed to be exploratory, estimations of liver glycogen levels (by ^{13}C MRS), glucose flux, or glycogenolysis/gluconeogenesis rates were not obtained, leaving room for future work exploring these associations directly.

Within the context of this thesis, which included very physically active and high-level amateur athletes, it is possible that due to the imposed training schedules and controlled diets, reductions in carbohydrate oxidation rates or changes in their diet are less likely to serve as a compensation for the reduced carbohydrate availability. Consequently, the reduction of AEE by changing spontaneous physical activity could be an attempt of the body to preserve carbohydrates in this very specific context. However, whether these results are likely to happen when diet and physical activity patterns are allowed to vary freely (i.e., the full spectrum of physical activity levels and eating *ad libitum*) is still unknown.

Finally, the results obtained from Chapters 3 and 4 were further tested in Chapter 5, considering the differences in nutrient- (HCLF vs HCLC) and energy availability (FEDex vs FASTex) to investigate if habitual carbohydrate intake with or without considering daily energy intake predicted AEE in a free-living wider and heterogenous sample (UK Biobank cohort). Based on previous studies, associations between energy intake and physical activity levels have been drawn (Hopkins, Duarte, et al., 2019; Piaggi et al., 2015), but these effects have only been proposed to be unidirectional (i.e., AEE levels effects on EI). However, in this thesis, the results connecting carbohydrate availability with AEE suggest at least a bidirectional relationship. For this purpose, and after addressing collinearity, a random intercept model was fitted to test two models. One included carbohydrates and dietary

energy intake, and the second model only included carbohydrates. Here, it was observed that the model including only carbohydrates predicted AEE in the same magnitude (~2%) that the model including carbohydrates and dietary energy intake, suggesting that the results are at least partially related to the metabolic effects of carbohydrates more than only the daily energy intake, and that the relationship EI-AEE works bidirectionally. However, if these weak but significant effects are replicated in other populations (e.g., lifestyle-based weight loss interventions), this could help to develop practical nutrient/exercise counselling to favour higher levels of physical activity.

6.2.2 Short-term appetite and energy intake responses to dietary manipulations around exercise.

Among the experimental chapters, dietary manipulations around exercise responses (i.e., HCLF vs HFLC in Chapter 3; FEDex vs FASTex in Chapter 4) were utilised to spare or replenish carbohydrate stores to assess the endocrine and feeding-related behaviours responses. While there is considerable interindividual variability in appetite and hormonal response to acute exercise(Douglas et al., 2017; Goltz et al., 2018; J. A. King et al., 2017), it is generally accepted that acute appetite and orexigenic hormone suppression, as well as increases in anorexigenic hormones, are typically observed in the first few hours after exercise (Thackray & Stensel, 2023). However, it is not well understood how acute nutritional manipulation before or after exercise can modulate the endocrine, appetite, and energy intake response in the following hours and days. This is especially true as an energy deficit created by exercising or dieting seems to produce opposite results.

In Chapter 3, an overnight-fasted exercise plus an isocaloric energy replacement using two different drinks showed no acute differences in appetite between conditions. Although both drinks stabilised appetite for the first two hours, appetite peaked at the end of the four hours. Similarly, in Chapter 4, when an isovolumetric flavour-matched carbohydrate-based or a placebo drink was used, no differences in appetite were observed between the two trials. However, when the appetite data was visually inspected, appetite seemed to increase across the next 2 hours and fall later after the *ad libitum* meal test. No differences in energy intake were observed during the *ad libitum* meal test (Chapter 4), the same day, or the following days for any of the experimental chapters. From these chapters, it is possible to recognise two distinctive characteristics of nutritional manipulation: first, the timing of food intake relative to exercise (i.e., before or after exercise) and post-exercise appetite measures, and second, the nutritional and energy content (Chapter 3, complete substrate/energy replacement; Chapter 4, incomplete substrate/energy provision).

Previously it has been highlighted that appetite and energy intake behave differently after acute diet- or exercise-based energy restriction, with food restriction showing a consistent increase in appetite and energy intake levels compared with exercise intervention (Cameron et al., 2016; J. A. King et al., 2011; Thivel et al., 2017, 2018, 2021). This suggests that, regardless of whether the FEDex and FASTex can produce different metabolic responses, the presence/absence of food in the oro-gastric region would mainly determine appetite and hormonal changes (Borer et al., 2005, 2009). The latter agrees with other studies showing that breakfast omission increases appetite response acutely (Clayton et al., 2016). In this line, exercising after skipping breakfast could be a good strategy to control appetite in the next 4-5 hours while producing a small energy deficit. Additionally, exercising before eating

could help to modulate appetite when meals are reduced in size or caloric/nutritional content while dieting. Although skipping breakfast before exercising has been suggested to help control appetite and energy intake acutely (i.e., hours), consistent benefits in terms of body mass have not been observed (Edinburgh et al., 2020; Gillen et al., 2013; Schoenfeld et al., 2014).

Another difference regarding the direction of appetite response after exercise between the two experimental chapters might involve the differences between exercise energy expenditure (ExEE) and the drinks' energy content. In chapter 3, despite the extreme differences in the nutritional content of the isocaloric drinks, no differences in appetite between conditions in the next four hours were observed. However, both drinks produced a transient stabilisation in appetite at hour 2 rising to a peak at hour 4. Conversely, in Chapter 4, post-exercise appetite followed an increasing pattern, regardless of the condition. When analysing appetite along with the endocrine responses for both chapters, higher levels of glucose, insulin, and lactate with carbohydrate intake were found, but they did not induce different appetite patterns as could be expected (Flint et al., 2007). When taking together the results from Chapters 3 and 4, it appears that the different patterns in acute appetite between both experiments may be due to factors such as the time between the drinks and post-exercise appetite measurements, the volume of the drinks consumed, or the matching between ExEE/energy intake. These factors may have had a greater impact on appetite than the metabolic effects of isolated nutrients or the anorexigenic effects of exercise. In Chapter 4, for example, participants consumed less volume (450 ml vs 900ml) and fewer calories (0-600 vs 1065 Kcal), and the post-exercise appetite measurements were taken at longer time intervals (165 vs 60 mins) compared to Chapter 3. Altogether, these differences can help to

explain the different patterns between Chapter 3 and 4 and the literature, according to the "Satiety Cascade" (Fig 6.1) (J. Blundell, 2010; J. Blundell et al., 2010b).

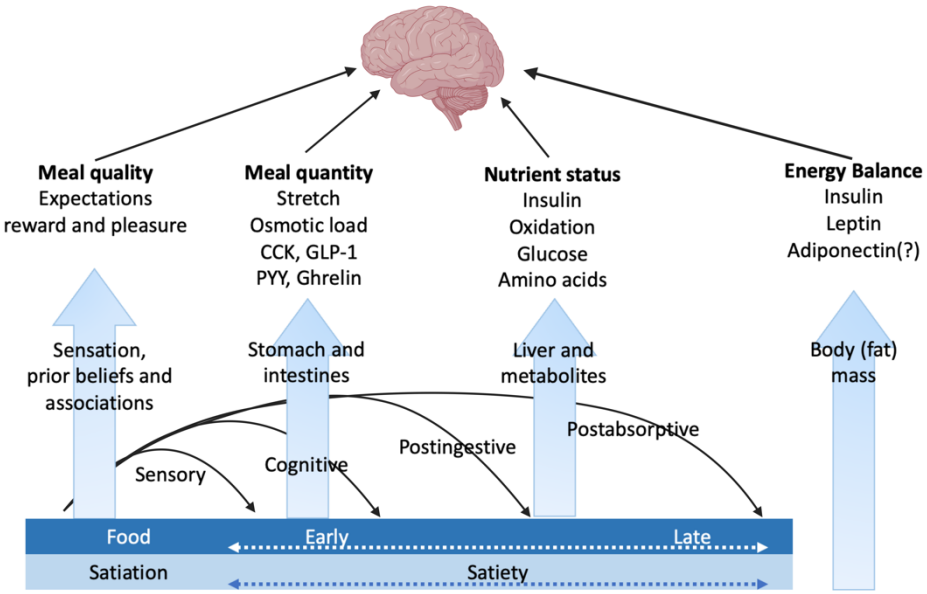

Figure 6.1. Satiety cascade. The diagram displays the satiety cascade which demonstrates the connection between satiation and satiety, along with the mediating psychological and physiological processes. It shows how the psychological and physiological stimuli caused by food consumption affect appetite sensations and eating patterns. Modified from Blundell, J. E. et al. Food Acceptance and Nutrition (Academic Press, London, 1987). Created in BioRender.com

Differences in appetite beyond 24 hours have rarely been reported after an acute bout of exercise. In the present thesis, in Chapter 3, appetite was reduced in the days after the HCLF vs. the HFLC energy replacement drink; however, the small differences did not produce any consummatory effects on energy intake. It is possible that this appetite suppressant effect could be related to the replenishment of the carbohydrate stores and its effects on glycaemic control. It has been reported that glucose dips can increase appetite and trigger food intake (Wyatt et al., 2021). Conversely, it is possible that the reduced appetite could be

a consequence of the differences observed in AEE levels without significant increases in TDEE. Where higher daily energy turnovers could have modulated appetite (Hägele et al., 2019b), leading to an overall condition effect for the HCLF trial. In Chapter 4, no differences in appetite on the following days were observed between conditions. It is speculated that the different responses between Chapters 3 and 4 could be associated with the replenishment or suppression of liver glycogen. In Chapter 4, the participants in the FEDex condition used more carbohydrates during exercise but also presented with hypoglycaemia before exercise in 5 out 10 of the participants included in the analyses, which may have led to similar liver glycogen utilisation between conditions, whereas in Chapter 3, post-exercise liver glycogen replenishment should have been enhanced by ingesting the HCLF drink (Casey et al., 2000). The latter aligns with several lines of investigation that have placed the liver as a key component in regulating appetite/hunger levels (Casanova et al., 2022; Russek, 1963, 1971, 1981). Indeed, Casanova et al, have shown that fasting hunger levels were related to liver mass and its role in covering the brain's energy demands (Hopkins & Blundell, 2023; Oltmanns et al., 2008), reinforcing the need to continue exploring these associations.

6.3 Limitations of the thesis

Where relevant, specific limitations to various aspects within this thesis have been discussed, including methodological limitations, which were also specifically described in the General Methods chapter. However, some limitations were shared across the experimental chapters, which are addressed here. Sample sizes of Chapters 3 and 4 are relatively small (n=12). In Chapter 3, this was related to its explorative nature and to be comparable with previous studies (Edinburgh et al., 2019; Hopkins et al., 2014b), as it was not clear whether differences between conditions for the main outcomes (i.e., energy intake, appetite, and

energy expenditure) would be observed. For Chapter 4, the sample size was selected based on the results obtained from Chapter 3 to ensure that a difference in AEE levels could be detected, leading to n = 12.

The control of diets before the experimental visits was also a limitation in Chapters 3 and 4. The provision of food to participants in the preceding days would have avoided the possibility of differences in diet impacting exercise metabolism, appetite, energy intake or energy expenditure. However, the baseline measurements of appetite and RMR were comparable on day 1 between conditions in Chapters 3 and 4, suggesting that food intake was similar. Additionally, the control of physical activity levels before starting each experimental trial could have been improved. Although participants were requested to refrain from practising any strenuous physical activities the previous 48 hours, no objective data was obtained to confirm compliance. This was thought as a measure to reduce the burden on our participants, as they were asked to wear 24 hours a day the combined heart rate and accelerometry sensor in Chapter 3, and additionally in Chapter 4, a CGM device.

Finally, it is important to note that the lack of measurements of muscle and liver glycogen, as well as the estimation of glucose flux during and after exercise, restricted the possible conclusions that could be drawn from the current findings. The decision not to include these methodologies in the present thesis was due to the exploratory nature of the research and the available resources.

6.4 Suggestion of future work and conclusion

The research conducted in Chapters 3-5 presents a plethora of opportunities for future work and exploration. Although metabolic impacts of acute carbohydrate availability manipulations have been tested previously, the specific effects of modifying liver glycogen content by exercise and diet on energy balance components remain to be explored utilising more sophisticated methodologies (muscle biopsies, 13C MRS, and tracers). This should be investigated in populations living with metabolic diseases, as during this thesis, it has been suggested that the effects of carbohydrate availability could be mediated by how well carbohydrates are handled within the body, which can be affected in conditions like insulin resistance, dyslipidaemia, metabolic syndrome or with the use of medicine. Similarly, if the results observed for AEE are replicated in other populations, mechanistic approaches should be applied to understand how carbohydrate availability is sensed by the central nervous system (neural, endocrine, or humoral afferents) to favour higher AEE in the context of acute manipulations of the diet and exercise.

Additionally, future work should explore the influences of energy balance (i.e., positive or negative energy balance) on the observed responses (Stubbs et al., 2018), as within restrained dietary patterns (e.g., time-restricted feeding or intermittent fasting (Templeman et al., 2021)) or weight loss medicine (e.g., GLP-1 agonist or SGLT-2 inhibitors (Yates et al., 2022)) the exercise and carbohydrate timing could be even more relevant. The present results also highlight the need to study the independent effects of modifying energy- or carbohydrate availability in athletes, as in the context of high carbohydrate- and energy flux, the present results could be useful for developing tailored nutritional advice for health and performance (Areta et al., 2020; Hammond et al., 2019).

To conclude, the broad aim of the thesis was to understand the effects of exercise and carbohydrate intake interactions on the short-term energy balance components.
The overarching aim has been met by addressing the three specific aims of the thesis. Chapter 3 showed that a high carbohydrate energy replacement post-exercise can modulate appetite and AEE better than an isocaloric high-fat drink. Chapter 4 aimed to further elucidate the impact of energy/carbohydrate availability before exercise, showing no differences between conditions and revealing that FASTex produces comparable effects on the components of energy balance. Chapter 5 showed that habitual carbohydrate intake can predict AEE similarly to more complex models, suggesting that the influence of habitual carbohydrate intake in AEE are not only due to increasing energy availability but also metabolic effects —showing that the relationship between diet and AEE work in a bidirectional way.

Overall, this thesis has provided new data suggesting that carbohydrate availability/metabolism around exercise/physical activity can modulate some components of energy balance (e.g., AEE and appetite) differently. These results fit some features of previously stated theories (e.g., glucostatic, glycogenostatic, and hepatostatic), although the observed responses are less robust than previously believed. Furthermore, while carbohydrate availability around exercise could modulate some energy balance components in the short term, their effects may be mediated by the simultaneous changes in the utilisation/sparing of the energy sources (e.g., muscle glycogen, liver glycogen, and adipose tissue) and their impact in other physiological functions, depending on the feeding state and exercise characteristics. If the present results are replicated, future work should aim to find how the signals from peripheral energy sources interact with the central nervous system.

6.5 References

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