

**The Influence of Body Composition and Energy Expenditure on
the Drive to Eat Under Conditions of Energy Balance and
During Dietary-Induced Weight Loss**

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The candidate confirms that the work submitted is his own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

Chapter 1 of the thesis was based in part on two jointly-authored publications:

- **Casanova, N.**, Beaulieu, K., Finlayson, G., & Hopkins, M. (2019). Metabolic adaptations during negative energy balance and their potential impact on appetite and food intake. *Proceedings of the Nutrition Society*, 1-11. doi: 10.1017/s0029665118002811
- **Casanova, N.**, Finlayson, G., Blundell, J. E., & Hopkins, M. (2019). Biopsychology of human appetite — understanding the excitatory and inhibitory mechanisms of homeostatic control. *Current Opinion in Physiology*, 12, 33-38. doi: 10.1016/j.cophys.2019.06.007

The candidate took a primary role in the writing of these two narrative reviews. Co-authors contributed to these publications in guiding and editing drafts of the manuscripts.

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- **Casanova, N.**, Beaulieu, K., Oustric, P., Gibbons, C., Finlayson, G. and Hopkins, M. (2019). The relationship between resting metabolic rate and free-living daily energy intake is moderated by body fat percentage in women with overweight and obesity. *Obesity Facts*. doi: 10.1159/000497797. European Congress on Obesity 2019, Glasgow, UK
- **Casanova, N.**, Beaulieu, K., Oustric, P., O'Connor, D., Gibbons, C., Finlayson, G. and Hopkins, M. (2020). The inhibitory effect of fat mass on energy intake is weakened by the accumulation of body fatness. *Journal of Nutrition*. Virtual ESPEN Congress 2020.
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- Beaulieu, K., **Casanova, N.**, Oustric, P., Hopkins, M., Varady, K., Finlayson, G., Gibbons, C. (2020). An exploratory investigation of the impact of 'fast' and 'feed' days during intermittent energy restriction on free-living energy balance behaviours and subjective states in women with overweight/obesity. *European Journal of Clinical Nutrition*. doi: 10.1038/s41430-020-00740-1

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Abstract

Background: Body composition (BC) and energy expenditure (EE) are implicated as determinants of the biological drive to eat, but it remains to be demonstrated how these components affect energy intake (EI) at differing levels of body fatness. During weight loss (WL), changes in BC and EE may influence appetite and EI, but it is unknown whether the WL method (intermittent [IER] or continuous [CER] energy restriction) affects compensatory responses.

Objectives: This thesis examined i) the associations between BC, EE and EI during energy balance and energy deficit across a spectrum of body fatness, and ii) whether changes in BC and EE during WL influenced compensatory changes in appetite or EI.

Methods: A series of studies examined BC, EE, physical activity (PA), appetite and EI under laboratory and free-living conditions. These included cross-sectional studies (2 experimental chapters) and a controlled-feeding WL RCT (IER vs CER; 3 experimental chapters).

Results: During energy balance, associations between fat-free mass (FFM), resting metabolic rate and total daily EE with free-living 24-hour EI were moderated by body fatness. Associations between fat mass (FM) and test meal EI were non-linear, with altered strength and direction at higher body fatness. High-metabolic-rate organs better explained the between-subject variability in hunger than FFM. During WL, IER was not superior to CER in attenuating FFM losses or compensatory responses. Adaptive thermogenesis during WL, but not changes in BC or PA, was associated with increased free-living daily appetite. Changes in PA were associated with the mean rate of WL.

Conclusions: The coupling between EE and EI, as well the proposed inhibitory influence of FM on EI, weaken at higher body fatness. Furthermore, IER was not superior to CER in preserving FFM or attenuating compensatory responses during WL, but changes in PA may represent a modifiable factor that influences the rate of WL.

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List of Abbreviations

AA – Amino acid

AEE – Activity energy expenditure

Anthro – Anthropometrics

AT – Adipose tissue

AUC – Area under the curve

BMI – Body mass index

BW – Body weight

CER – Continuous energy restriction

CHO – Carbohydrate

CCK – Cholecystokinin

CoEQ – Control of eating questionnaire

CV – Coefficient of variance

EE – Energy expenditure

EI – Energy Intake

FFA – Free-fatty acid

FFM – Fat-free mass

FFMI – Fat-free mass index

FM – Fat mass

FMI – Fat mass index

GLP-1 – Glucagon-like peptide 1

HMRO – High-metabolic rate organs

IER – Intermittent energy restriction

Lab – Laboratory

MVPA – Moderate-to-vigorous physical activity

MRI – Magnetic resonance imaging

NEAT – Non-exercise activity thermogenesis

NEPA – Non-exercise physical activity

P-ratio – Protein ratio

PA – Physical activity

PAEE – Physical activity energy expenditure

PAL – Physical activity levels

PFC – Prospective food consumption

PP – Pancreatic polypeptide

PYY – Peptide YY

QMR – Quantitative magnetic resonance

RMR – Resting metabolic rate

Sed – Sedentary time

SEM – Standard error of the mean

SD – Standard deviation

SWA – SenseWear Armband

TDEE – Total daily energy expenditure

TDEI – Total daily energy intake

TEF – Thermic effect of food

TFEQ – Three-Factor Eating Questionnaire

T PA – Total physical activity

VAS – Visual analogue scale

WL – Weight loss

Chapter 1 – General Introduction & Literature Review

Appetite is a complex biopsychological phenomenon that can be influenced by metabolic, behavioural, psychological and environmental factors. Although our understanding of the mechanisms influencing appetite has improved over the last 50 years, a lot of questions remain to be resolved as the obesity epidemic has been consistently rising mainly as a result of an increase in energy intake (EI) (Yancy et al., 2014), but also physical inactivity. Although obesity, resultant from a sustained positive energy balance, has been postulated to be a consequence of an 'obesogenic environment' comprised of high energy density foods with high palatability that facilitate passive overconsumption (Hall, 2018), research examining the mechanisms that contribute to this mismatch between EI and energy expenditure (EE) is warranted.

A negative energy balance will result in weight loss (WL) if sustained over time (Aragon et al., 2017, Hall and Guo, 2017). Despite the apparent simplicity of energy balance, which reflects the relationship between EI and EE, most WL and weight maintenance attempts are unsuccessful and recidivism is high (Wadden et al., 2011, Greaves et al., 2017, Dansinger et al., 2007, Dombrowski et al., 2014). While a lack of sustained WL can in part be explained by a failure to adhere to dietary and physical activity guidelines (Del Corral et al., 2011, Alhassan et al., 2008), compensatory metabolic, psychological and behavioural responses to a negative energy balance also act to undermine WL efforts and promote weight regain (King et al., 2008, Melby et al., 2017, Muller et al., 2016, Müller and Bosy-Westphal, 2019, Casanova et al., 2019). A better understanding of the relationships between EE and EI under conditions of both energy balance and energy deficit is needed if more effective long-term weight management strategies are to be developed. However, such strategies are complicated by the large inter-individual variability typically seen in response to WL interventions (Williams et al., 2008, Hopkins et al., 2014, Yancy et al., 2004, Gardner et al., 2018), and the lack of robust predictors of shorter-term and longer-term body weight and composition outcomes (Rosenbaum et al., 2018). Therefore, research

examining the relationships between EE and EI in different states of energy balance, as well how changes in body composition, EE and physical activity during dietary-induced WL influence the compensatory changes in appetite, EI or body weight are critical to develop effective strategies to improve obesity management success rates.

1.1 Energy Balance Regulation

Energy balance reflects the dynamic relationship between EE and EI, in which sustained energy imbalances result in weight change. It has been suggested that an energy deficit of 3500 kcal leads to the loss of 1 pound (~454 grams) of body weight (Wishnofsky, 1958), but this simplistic approach is known to overestimate WL (Hall et al., 2011). The '3500 kcal per pound' rule assumes that the composition of the weight lost would be 100% body fat during negative energy balance (based on the assumption that the energy value of 1 gram of fat is 9 kcal and adipocytes are composed of 85-90% of triglyceride), and fails to account for dynamic changes in the biological components of EE seen with WL. For a given energy deficit, it is also common to observe large variability in WL (and other physiological and behavioural responses) between individuals (Feig and Lowe, 2017, Gasteyer et al., 2010, King et al., 2007, Reinhardt et al., 2015). This heterogeneity is apparent following lifestyle (King et al., 2008, Gardner et al., 2018), pharmacological (Yancy et al., 2004) or surgical (de Hollanda et al., 2015) WL interventions, and while the statistical methods used to quantify such variability (Atkinson and Batterham, 2015) and its clinical significance (Williamson et al., 2018) have been debated, variability in treatment response appears to be the norm rather than the exception.

Although adherence to a WL intervention is likely to contribute to individual variability in outcomes measures (Alhassan et al., 2008, Del Corral et al., 2011), metabolic, behavioural and psychological compensatory responses will also underlie differences in treatment response. This is evidenced by the lower than expected WL typically observed in studies that predict changes in body weight based on 'static' mathematical models (e.g., the '3500 kcal per pound' rule), and it should not be assumed that a linear relationship exists between the initially prescribed energy deficit and actual WL. Rather, energy balance should be viewed as a dynamic regulatory system in which a perturbation to an individual

component may produce co-ordinated responses in other components that act to diminish the gap between EI and EE. For instance, greater than predicted decreases in resting metabolic rate (RMR) (Doucet et al., 2001, Bosy-Westphal et al., 2009), increased muscular efficiency (Leibel et al., 1995, Rosenbaum et al., 2003) and decreases in the amount of physical activity (Silva et al., 2018) have been observed in response to negative energy balance. Additionally, spontaneous increases in EI have also been reported after shorter- (Mars et al., 2006, O'Connor et al., 2016) and longer-term (Polidori et al., 2016) energy deficit. Thus, the apparent simplicity of energy balance belies a dense and complex network of inter-related biological, nutritional, behavioural and psychological determinants of EI and EE (Foresight, 2007), and multiple regulatory systems and feedback loops that operate concurrently to influence energy homeostasis (Figure 1.1).

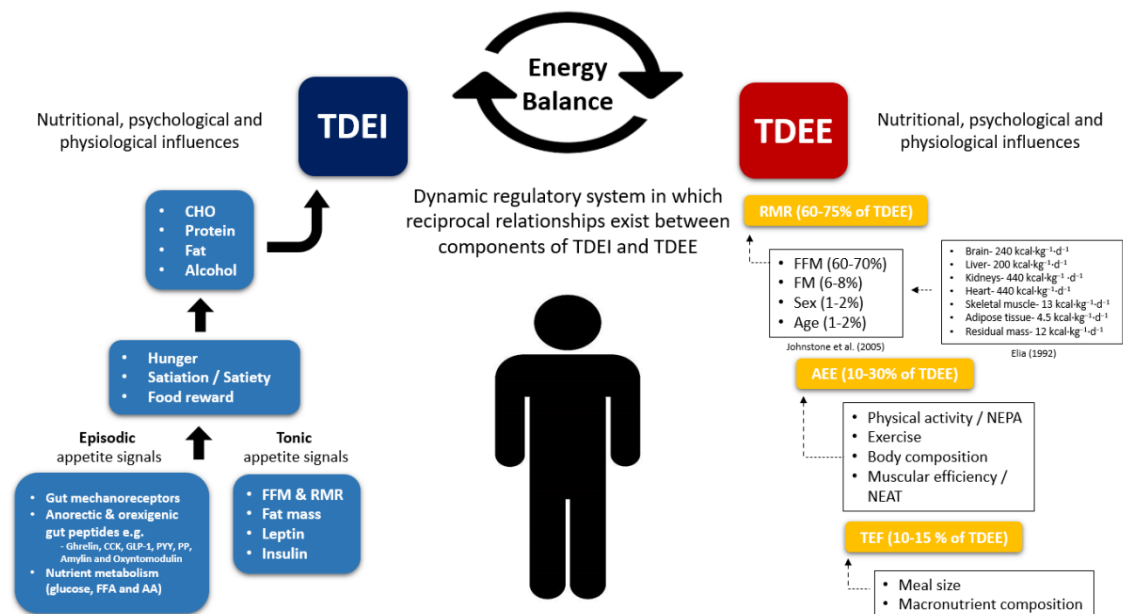


Figure 1.1 – Schematic overview of energy balance and the nutritional, psychological, behavioural and physiological influences on total daily energy intake and energy expenditure. TDEI, total daily energy intake. TDEE, total daily energy expenditure. CHO, carbohydrate. NEPA, Non-exercise physical activity. NEAT, non-exercise adaptive thermogenesis. CCK, cholecystinin. PP, pancreatic polypeptide. PYY, peptide YY. GLP-1, glucagon-like peptide-1. FFA, free-fatty acid. AA, amino acid. FFM, fat-free mass. RMR, resting metabolic rate. AEE, activity energy expenditure. TEF, thermic effect of food. From Casanova et al. (2019).

Figure 1.1 displays an overview of energy balance components. As depicted on the left side, EI is a result of the metabolizable energy consumed from the macronutrients carbohydrates, protein and fat. Energy intake is influenced by homeostatic factors such as hunger (that drives food intake), satiation (that leads to meal termination) and satiety (suppression of hunger between meals), although non-homeostatic factors (e.g., food reward) also have a critical role. Appetite-related signals can be divided into episodic (shorter-term) and tonic (longer-term). Episodic signals will not be deeply addressed in this thesis, but factors that could influence shorter-term EI are gut mechanoreceptors (sensitive to changes in gastric volume), anorexigenic (inhibit food intake) and orexigenic (stimulate food intake) appetite-related peptides and also nutrient metabolism and availability. On the right side of the figure, the determinants of total daily EE (TDEE) are shown. Resting metabolic rate usually accounts for 60-75% of TDEE and represents the energy needed to maintain vital functions. It is mainly determined by body composition (primarily fat-free mass but also fat mass; FFM and FM) although sex and age may also contribute to the variability between individuals (Johnstone et al., 2005). Of note, FFM is comprised of a heterogeneous group of tissues with different metabolic rates (Elia, 1992, Muller et al., 2013), and body composition analysis at the tissue-organ level better explains the variability in RMR between individuals than FFM as a uniform component (Gallagher et al., 1998). Depending on the individual's lifestyle, activity EE (AEE) usually accounts for 10-30% of TDEE and is mainly determined by the amount of physical activity/exercise, body size and composition (especially FFM), and muscular efficiency. Lastly, the thermic effect of food usually accounts for 10-15% of TDEE and is mainly determined by the energy content and macronutrient composition of the meal (Westterterp, 2004).

Taken together, it is important to recognise that albeit simple, energy balance represents a dynamic system comprised of multiple inter-related mechanisms. In the following sections (and throughout this thesis), a thorough description of the factors influencing EI (and appetite) and EE in both conditions of energy balance and energy deficit will be presented and discussed. A complete understanding of what influences energy balance regulation may provide critical insights into the aetiology of weight gain and obesity, permitting the development of strategies to improve success rates of weight management interventions.

1.2 Determinants of Appetite in the Context of Energy Balance

The processes through which appetite is controlled are best viewed within an energy balance framework as this allows the integration of biopsychological determinants of EI and EE alongside components of body composition. Nevertheless, food intake is not controlled solely as an outcome of energy homeostasis (Halford and Blundell, 2000), with non-homeostatic factors such as food hedonics and environmental contexts exerting important influence over eating behaviour (Finlayson, 2017). Models of homeostatic appetite control embody excitatory and inhibitory feedback signals that reflect acute (episodic) and long-term (tonic) energy availability. Tonic mechanisms exert a stable influence over appetite and provide a link between metabolic requirements, stored energy and day-to-day EI. This feedback has traditionally been centred around the inhibitory action of leptin (i.e., 'lipostatic theory'), but it is now recognised that the EE of metabolically active tissues also provides an enduring signal to eat (Blundell et al., 2015a). Episodic signals respond to the presence or absence of nutrients in the gastrointestinal tract and mechanical factors such as gastric distension that communicate with the brain to signal satiation and satiety. The classic satiety peptides cholecystokinin, glucagon-like peptide-1 and peptide tyrosine tyrosine, along with the orexigenic peptide ghrelin, supposedly act as physiological cues that influence appetite sensations (e.g., hunger, satiation and satiety) and the timing, type and amount of food consumed (Gibbons et al., 2013, Hopkins et al., 2017).

The central neural systems that underlie homeostatic eating are closely linked to those underpinning central reward pathways, with hormones such as leptin, insulin and ghrelin postulated to provide a molecular link between hypothalamic (homeostatic) and mesolimbic (reward related) systems (Berthoud et al., 2017). These control mechanisms can be conceptualised via the satiety cascade, which provides a theoretical framework that maps the underlying biological mechanisms of appetite onto the psychological experiences and behavioural events that influence EI (Blundell, 1991). A salient feature of the above appetite-related processes is their inherent inter-individual variability. Large variations in the individual profiles of appetite sensations and appetite-related peptides are seen following nutrient or exercise manipulations (Hopkins et al., 2013, King et al., 2017, Goltz et al., 2018, Gibbons et al., 2019). This variability may help account

for i) the diversity in eating behaviours between individuals, and ii) inter-individual variability in WL following lifestyle (Dansinger et al., 2005, King et al., 2008, Church et al., 2009, Yancy et al., 2010), pharmacological (Pi-Sunyer et al., 2015, Fujioka et al., 2016) and surgical (Courcoulas et al., 2013, Still et al., 2014, Courcoulas et al., 2015) interventions.

1.2.1 Fat-Free Mass and Resting Metabolic Rate as Drivers of Appetite and Energy Intake

Of note, body composition and EE have been recently recognised as strong determinants of appetite and EI (Weise et al., 2014, McNeil et al., 2015, Blundell et al., 2015a). A conceptual model highlighting a drive to eat based on energy needs has previously been proposed (Blundell et al., 2001), but only now are studies beginning to fully recognise body composition and EE as an important excitatory feature of homeostatic appetite control. In 1955, Edholm and colleagues suggested that “the differences in intakes of food must originate in the differences in energy expenditure” (p.297), but interestingly, his investigation failed to observe associations between TDEE and EI within one day (Edholm et al., 1955). However, the observation that some individuals were able to maintain a similar body weight over time suggested that EE should at least be partially coupled with EI. Indeed, in 1977, strong associations were observed between TDEE and EI when 1-week averages were examined (Edholm, 1977).

In 1989, a study examining the differences in misreporting between individuals with and without obesity observed a positive association between lean body mass and EI (Lissner et al., 1989). However, as this was not the main aim of that particular investigation, this finding was overlooked and was only brought to attention and replicated 20 years later, in which FFM, but not FM, was found to be positively associated with self-selected meal size and daily EI (Blundell et al., 2012). This finding has been replicated in the following years by several independent groups who have reported positive associations between FFM and EI (Weise et al., 2014, McNeil et al., 2015, Cameron et al., 2016, Basolo et al., 2018, Bi et al., 2019, Grannell et al., 2019a). For instance, Basolo et al. (2018) observed positive associations between FFM (measured through dual x-ray absorptiometry) and EI (3-day 24-hour *ad libitum* using computerised vending machines) in a group of 61 individuals with overweight. Taken together, this body

of work indicates that a greater amount of FFM is accompanied by an increased tonic drive to eat. Notably, this association has also been observed in adolescents (Cameron et al., 2016) and in disease states (Bétry et al., 2019), reinforcing the robustness of this biological orexigenic signal. Fat-free mass has also been found to be positively associated with daily hunger sensations (Cugini et al., 1998) and with self-reported EI (Vainik et al., 2016). Taken together, these data demonstrate that FFM exerts a strong orexigenic drive to eat.

In 2011, Blundell et al. suggested that “any orexigenic drive arising from FFM could be mediated via EE reflected by the RMR” (p.447). In the following years, it was demonstrated that the association between FFM and EI was mediated by RMR (Hopkins et al., 2016) and 24-hour EI (Piaggi et al., 2015), suggesting that the drive to eat may arise from the energetic demands created by these metabolically active tissues. This mediating influence of EE has been replicated in following publications (Hopkins et al., 2019, Hopkins et al., 2018), and positive associations have been observed between TDEE (Piaggi et al., 2015, Basolo et al., 2018) and RMR (Caudwell et al., 2013, McNeil et al., 2015, Bi et al., 2019) with appetite and EI. For instance, McNeil et al. (2015) observed positive associations between RMR with acute (one meal 3 hours after a standardised breakfast, n = 191) and daily EI (standardised breakfast, *ad libitum* test meal in the laboratory and intake from the containers that were taken home, n = 55). Interestingly, although these relationships between RMR and TDEE with EI have been consistently documented, the mechanisms that signal energy requirements to the brain creating a drive to eat remains unclear. The enzyme AMP-activated protein kinase has been proposed to be implicated in this translation of EE to motivation to eat by potentially altering the expression of orexigenic and anorexigenic peptides (Andersson et al., 2004, Kola, 2008), but studies investigating the relationships between EE, molecular pathways, and changes psychological / behavioural (e.g., appetite sensations and EI) determinants of food intake in humans are lacking. However, it is important to acknowledge that most components of FFM are also endocrine organs (e.g., skeletal muscle produces myokines while the liver produces hepatokines) and therefore the possible influence of specific signals arising from these tissues, independent from their energy requirements, on appetite and EI should not be discarded. It is also important to consider that sex and ethnicity may influence body composition. For instance, for a similar amount of total FM and FFM, African Americans have been

shown to present a lower absolute RMR than whites due to having a higher amount of skeletal muscle and lower amount of high-metabolic rate organs (Gallagher et al., 2006). Moreover, women generally have lower amounts of FFM and higher FM, which may lead to a lower absolute RMR (Johnstone et al., 2005) and potentially contribute to lower appetite sensations and EI. Indeed, men have been shown to present higher EI than women (Hagobian et al., 2013). Also, while men generally tend to present a higher fat deposition in the abdominal region, women tend to have a greater accumulation in the gluteofemoral area (Schorr et al., 2018). However, whether this influences appetite and/or EE remains unknown.

There are two limitations from these studies that should be acknowledged: 1) only assessing EI or appetite sensations instead of including both measurements in the same study; and 2) only examining EI or appetite sensations in laboratory or free-living conditions. Assessing appetite sensations alongside EI would be important as it would permit to combine psychological and behavioural measurements which have important roles in an energy balance framework. Additionally, although laboratory measurements of appetite could allow for a more objective assessment (e.g., providing test meals), the protocol may be limited by the available resources (e.g., food preparation for 1 meal vs meals for several days) and it may create a less ecologically valid scenario in which participants are restrained to certain food choices. Contrastingly, although free-living assessments may be more prone to misreporting (Ravelli and Schoeller, 2020), they are easier to implement and assess over longer periods of time (e.g., 1 week). Also, while appetite sensations measured in the laboratory are usually done in a fasted state or in response to specific meals, free-living assessments could provide important information regarding the overall daily motivation to eat. Therefore, assessing appetite sensations alongside EI in both laboratory and free-living conditions would allow for a better understanding of the factors influencing appetite.

1.2.2 Fat-Free Mass as a Heterogenous Energetic Component

Previous research has primarily examined the associations between FFM, appetite and EI using 2-compartment models of body composition, which only divides the body into FFM and FM. However, it is important to consider that FFM

is composed of an heterogeneous group of tissues with different metabolic rates (Elia, 1992). For instance, the RMR for skeletal muscle is ~13 kcal/kg/day, while for the kidneys and the heart is ~442 kcal/kg/day (Muller et al., 2013). Of note, high-metabolic rate organs (i.e., brain, liver, kidneys and heart) represent ~6-7% of total body weight, but ~60% of total RMR (possibly close to 80% if the lungs were included), while skeletal muscle only accounts for 20% of total RMR, but ~40% of total body weight (Elia, 1992, Gallagher et al., 1998). This means that for a certain amount of FFM, the EE associated to this group of tissues could be substantially different due to subtle changes in its composition. For example, a higher proportion of high-metabolic rate organs relative to skeletal muscle would originate a higher EE per kg of FFM.

Interestingly, it has been shown that including body composition analysis at the tissue-organ level better explains the variability in RMR between individuals (Gallagher et al., 1998) than 2-compartment models (Johnstone et al., 2005). However, there has been no attempt to date to incorporate the energetic demands of individual tissue-organs into models of appetite control. As RMR is a strong determinant of EI, it could be that including the specific components of FFM into the model could better explain the variability in appetite and EI between individuals. Interestingly, Cameron et al. (2016) observed that skeletal muscle was a predictor of EI in a group of adolescents (but not stronger than total FFM), but high-metabolic rate organs were not included in the analysis and therefore how these influence appetite and EI remains unknown.

It is important to consider that other mechanisms apart from the mass-dependent EE influence of specific components of FFM over appetite and EI could exist. For instance, skeletal muscle (Grannell et al., 2019b) and the liver (Friedman, 2007, Gonzalez et al., 2019) have been proposed to exert an influence over appetite and EI independently of their energy requirements. Therefore, the hypothesis that factors apart from their energy requirements such as myokines secreted by skeletal muscle and liver energy status or glycogen availability could exert a specific signalling that modulates appetite should not be discarded.

This area of research could be critical as the examination of these relationships at the tissue-organ level may provide better models to explain the variability in appetite and EI between individuals, as well novel insight into pathophysiological mechanisms disrupting appetite with metabolic disease (e.g., whether visceral or

subcutaneous fat accumulation and their distribution differently influence appetite and EI).

1.2.3 The Influence of Fat Mass on Appetite and Energy Intake

While research has consistently reported that FFM is associated with a greater drive to eat, the strength and direction of the relationships between FM and EI remains inconclusive. Previous views of appetite focused on the inhibitory influence of FM on EI, especially after the discovery of the tonic anorexigenic hormone leptin (Zhang et al., 1994). However, although FFM and EE have consistently been found to be positively associated with appetite and EI, associations between FM and EI are inconsistent. For instance, Lissner et al. (1989) reported that FM was not associated with EI and this was corroborated by several studies published over the following years who failed to report associations between FM and EI (Blundell et al., 2012, Bi et al., 2019, Grannell et al., 2019a, Sanchez-Delgado et al., 2020). These findings were surprising as it has been postulated that increases in FM would lead to higher leptin levels and thus exert a greater tonic inhibition over appetite and EI to limit body weight and fat gain ('lipostatic theory') (Kennedy, 1957). A few studies have also reported negative associations between FM with daily hunger and EI (Cugini et al., 1998, Blundell et al., 2015a). Interestingly, these studies that reported negative associations between FM and EI were conducted in leaner individuals. For instance, an often-overlooked pair of studies by Cugini et al. (1998 and 1999) reported a negative association between FM and daily hunger sensations in clinically healthy individuals ($BMI = 21 \pm 2 \text{ kg/m}^2$), but not in a group of individuals with obesity ($32 \pm 7 \text{ kg/m}^2$). It was therefore hypothesised that higher levels of body fatness could lead to energy balance dysregulation by blunting the inhibitory influence of FM on appetite and EI. Interestingly, a similar idea had been suggested regarding FFM (Grannell et al., 2019a) and 'awake and fed thermogenesis' (Piaggi et al., 2015), in which their associations with EI were found to be weaker in individuals with higher levels of body fatness. However, whether the associations between body composition and EE with EI are weaker in individuals with overweight and obesity (in comparison to a group of lean participants) has never been statistically tested.

It is also relevant to highlight that weak positive associations between FM and EI through its smaller contribution to RMR (i.e., mediation) have been reported (Hopkins et al., 2019, Hopkins et al., 2018, Hopkins et al., 2016). For reference, FM has been shown to generally contribute to ~6% of RMR (Johnstone et al., 2005). This weak positive association between FM on EI could in fact strengthen at higher levels body fatness due to its higher contribution to RMR as it disproportionally increases relatively to FFM. Therefore, as the contribution of FM to RMR increases, it is possible that the tonic influence of FM over EI strengthens. However, the postulated inhibitory effect of FM on EI could be blunted and thus lead to energy balance dysregulation (i.e., uncoupling between EE and EI). This could potentially contribute to energy overconsumption. Alongside a higher RMR resulting from an increased body size, this greater tonic drive to eat concomitant with the absent inhibitory influence of FM on EI could substantially complicate weight management.

A weaker association between body composition and EE with appetite and EI at higher levels of body fatness would go in line with the concept of insulin and leptin resistance (Zhou and Rui, 2013, Koleva et al., 2013), in which the sensitivity of appetite-related feedback signalling is impaired. Appetite-related peptide secretion kinetics have also been found to be altered in individuals with overweight and obesity. Lower secretion of anorexigenic peptides (e.g., glucagon-like peptide 1 and peptide YY) and suppression of ghrelin in response to meals (Lean and Malkova, 2016) have been noted in individuals with overweight and obesity, which could compromise the ability to adjust EI to energy needs. However, it is important to recognise that other factors apart from physiology could potentially moderate and mediate the relationships between body composition and EE with appetite and EI. For instance, higher habitual levels of physical activity have been shown to be associated with a better coupling between EE and EI (Mayer et al., 1956, Beaulieu et al., 2016). For instance, Mayer et al. (1956) observed a J-shape relationship between EI and physical activity levels. In this study, a positive linear association was observed between daily EI and physical activity levels, but only in those with higher levels of daily physical activity ('regulated zone'). Furthermore, stronger negative associations between FM and EI have also been observed in individuals with higher habitual levels of physical activity (Beaulieu et al., 2018). This suggests that more active individuals may be more in tune with their energy needs. Interestingly, in the

narrative review conducted by Beaulieu et al (2018), a higher body weight was observed alongside a higher EI in the 'non-regulated zone' (i.e., lower levels of habitual physical activity alongside a higher EI). Considering that a higher body weight is associated with a greater RMR and this could lead to an increased drive to eat (i.e., higher EI), this raises the question of whether the higher EI observed in the 'non-regulated zone' was due to a dysregulation induced by lower levels of physical activity, or through the higher energy requirements arising from a larger body size.

Furthermore, the negative association between FM and EI has also been shown to be partially mediated by cognitive restraint (Hopkins et al., 2018). This means that the mechanism by which FM influences EI may in part be through differences in how much individuals exert restraint over EI, possibly to consciously (or unconsciously) control their body weight. For instance, leaner individuals have been shown to have higher levels of flexible restraint alongside lower disinhibition, which could allow for a greater control over EI and better weight management (Westenhoefer et al., 1999). Other factors such as body image concerns and emotional eating could also contribute to a greater cognitive restraint and self-regulation of EI (Megalakaki et al., 2013, Banna et al., 2018). When examining the associations between FM and EI, it is important to consider potential moderators and mediators of this association such as physical activity and eating behaviour traits.

Therefore, this thesis will focus on investigating the associations between body composition and EE with appetite and EI considering levels of body fatness, physical activity and eating behaviour traits as potential moderators and covariates. This will allow for a more complete understanding of the mechanisms influencing appetite in an energy balance framework in which metabolism, behaviour and psychology play key inter-related roles.

Interim Summary:

- Fat-free mass has consistently been shown to be positively associated with EI and this effect is mediated by its EE.
- Whether specific components of FFM (i.e., tissue-organ level) better explain the variability in appetite and EI between individuals remains unknown.
- Studies report equivocal findings regarding the associations between FM with appetite and EI.
- Although it has been suggested that levels of body fatness could weaken the associations between body composition, EE and EI, this has yet to be experimentally tested.
- For a complete understanding of the mechanisms influencing appetite, potential moderators and mediators such as physical activity and eating behaviour traits should not be overlooked.

1.3 Compensatory Mechanisms Resisting Weight Loss

As previously mentioned, energy balance is a dynamic system in which perturbations in one component will influence others in an attempt to reduce the energy gap between EE and EI (Melby et al., 2017). Interestingly, the forces that attempt to counteract the planned energy deficit seem to be stronger than those opposing energy surfeits (Figure 1.2). This suggests that compensatory responses to energy imbalance may be asymmetrical (Speakman, 2014) which in part explains the ease of gaining weight but difficulty in losing and maintaining a reduced body size.

The mechanisms that oppose a negative energy balance are inter-related and complex, individually subtle and often difficult to quantify (Melby et al., 2017, Rosenbaum et al., 2018). Metabolic and behavioural determinants of energy balance interact in a co-ordinated fashion during energy deficit (Figure 1.2), but the mechanisms through which physiology drives behaviour are rarely acknowledged in the context of WL and weight regain (Rosenbaum et al., 2018).

Methodological limitations associated with the measurement of EI and EE have long frustrated energy balance research and limit our understanding of the putative signals that link physiology to behaviour (Dhurandhar et al., 2015). These limitations have also contributed to the debate over the primary cause of weight gain and the secular trends in obesity prevalence (Ng et al., 2014). Given the fundamental relationships between components of EE, body composition and EI, it might also be further argued that successful WL and maintenance strategies will only be developed if the inter-relationships between physiology, behaviour and psychology are explicitly acknowledged and incorporated in their design. However, a fundamental weakness of current approaches is their failure to relate the metabolic and physiological components of energy balance to the psychological and behavioural mechanisms that undermine WL. Therefore, the following sections will be focused on describing the metabolic, behavioural and psychological compensatory responses that occur during periods of negative energy balance.

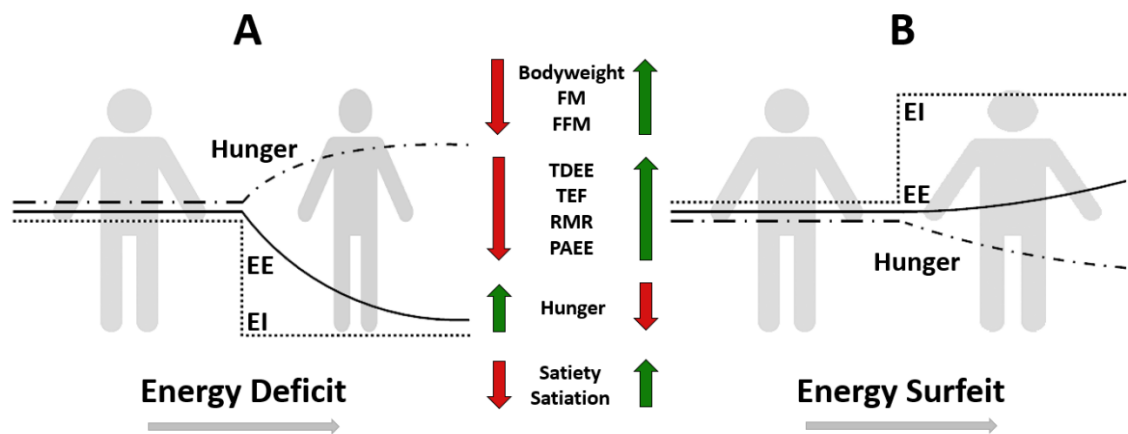


Figure 1.2 – Overview of physiological and behavioural responses during: A) energy deficit and B) energy surfeit. Asymmetrical response between periods of energy deficit (panel A) and surfeit (panel B) are displayed in which there is a greater force resisting weight loss than weight gain. Figure adapted from Melby et al. (2017). EI, energy intake. EE, energy expenditure. TDEE, total daily energy expenditure. RMR, resting metabolic rate. PAEE, physical activity energy expenditure. TEF, thermic effect of food. FFM, fat-free mass. FM, fat mass. From Casanova et al. (2019).

1.3.1 Metabolic Compensatory Responses

During periods of negative energy balance, several metabolic compensatory responses occur that attempt to attenuate the initially prescribed energy deficit (Melby et al., 2017). It is important to recognise that an obligatory decrease in all components of EE (e.g., RMR, AEE and TEF) is to be expected with WL. For instance, reductions in RMR and physical activity related EE are observed due to losses of both FM and FFM, decreasing the energy requirements in a resting state and the energy cost of physical movement (Leibel et al., 1995, Rosenbaum et al., 2003). Furthermore, TEF decreases as a consequence of a lower daily EI, reducing the amount of energy needed to metabolise and absorb the foods consumed (Westerterp, 2004).

Additionally, it has been observed that greater than predicted decreases in EE may occur in response to WL, a concept named 'adaptive thermogenesis' (Dulloo et al., 2012). Adaptive thermogenesis is usually considered as the difference between measured and predicted RMR after adjusting for changes in body composition during WL (Muller and Bosy-Westphal, 2013). For instance, a lower measured RMR (in comparison to the predicted value) would mean that adaptive thermogenesis is present. The evidence regarding the existence of adaptive thermogenesis in RMR is equivocal. While some studies demonstrate that it does indeed exist (Doucet et al., 2001, Bosy-Westphal et al., 2009, Johannsen et al., 2012, Tremblay et al., 2013), others (Ostendorf et al., 2018, Gomez-Arbelaes et al., 2018, Martins et al., 2020b), including a systematic review (Schwartz et al., 2012), suggest otherwise. Of note, it is important to highlight the large inter-individual variability observed in these studies, usually masked by the assessment of the changes at the mean level, which could in part be responsible for the discrepant findings. Moreover, periods of weight stabilisation have been found to almost completely reverse this greater than predicted decrease in RMR (Weinsier et al., 2000, Karl et al., 2015, Nymo et al., 2018, Martins et al., 2020b).

The conflicting findings could be due to a compounding effect of several methodological errors, as well discrepancy regarding the definition and assessment of adaptive thermogenesis between studies. Firstly, body composition measurements using 2-compartment models may lead to misestimation of RMR (i.e., inaccurate predicted RMR value) due to the heterogeneous nature of FFM. For instance, a study showed that including body

composition analysis at the tissue-organ level, measured via magnetic resonance imaging (MRI) in the RMR predictive equation better explained the variance in RMR between individuals, reducing the observed adaptive thermogenesis (Bosy-Westphal et al., 2009). However, due to the high cost and time investment, measures of body composition such as MRI that allow examination at the tissue-organ level are rarely used in clinical trials. Another potential reason for the equivocal results are the different equations used to calculate adaptive thermogenesis: 1) previously validated equations (e.g., Harris-Benedict); 2) equations created based on baseline data (linear regression); and 3) sum of the mass from the tissues collected using MRI (e.g., skeletal muscle, adipose tissue, high-metabolic rate organs) multiplied by their specific metabolic rate. Furthermore, many studies solely calculate the difference between predicted and measured values post-WL, but do not account for the differences present at baseline. For instance, it could be that an individual with a lower than predicted RMR post-WL also had it at baseline and thus, adaptive thermogenesis would be lower or non-existent.

Despite the equivocal findings between studies, the question that arises is whether adaptive thermogenesis, if it exists, has important implications for weight management. It has been postulated that a greater adaptive thermogenesis could undermine WL efforts (Major et al., 2007). However, some studies did not report associations between adaptive thermogenesis with WL or weight regain (Weinsier et al., 2000, Martins et al., 2020a). In fact, the study conducted on 'The Biggest Loser' contestants reported that participants that had the lowest weight regain after 6 years had the largest adaptive thermogenesis (Fothergill et al., 2016). Nonetheless, although it has been demonstrated that a greater decrease in 24-hour EE (which could be due to adaptive thermogenesis or changes in behaviour) in response to fasting (Reinhardt et al., 2015) or after 1 week of energy restriction (Heinitz et al., 2020) was associated with less WL at the end of the intervention, no study has ever examined the influence of adaptive thermogenesis in RMR on the rate of WL.

Lastly, decreases in physical activity EE may occur for two reasons: 1) changes in behaviour (see below); and 2) increases in muscular efficiency. Independently of the potential mechanisms, whether increases in muscular efficiency negatively affect WL remains unknown and alongside the thermic effect of food, these will

not be investigated throughout this thesis. Importantly, what may have a stronger impact in energy balance are changes in behaviour, which contrary to metabolic compensatory responses, are at least partially under voluntary control and are thoroughly described below.

1.3.2 Behavioural Compensatory Responses

Behaviour change in an energy balance framework can be mainly observed through changes in physical activity and eating behaviour. Physical activity has been mainly highlighted as an important strategy to facilitate weight maintenance (Ostendorf et al., 2019). However, interventions in which the energy deficit is matched, total WL is similar to diet-only interventions (Ross et al., 2000). Furthermore, interventions that combine diet and exercise usually lead to greater WL due to the accumulation of a higher energy deficit (Clark, 2015). Therefore, the role of exercise and physical activity during active WL should not be overlooked.

Although a large inter-individual variability is usually present, decreases in the amount of physical activity may be seen during WL, especially in diet-only interventions (Silva et al., 2018). Whether spontaneous (i.e., not prescribed) changes in physical activity throughout an intervention influence WL (amount or rate) remains unknown. It has been shown that weight gain and fat accumulation during 8 weeks of 1000-kcal overfeeding were strongly correlated with spontaneous changes in non-exercise activity thermogenesis (Levine et al., 1999), which are partially influenced by physical activity. Furthermore, a study demonstrated that the group of individuals that lost more weight during a dietary-induced WL (with physical activity recommendations) study (>10 kg) had higher levels of moderate-to-vigorous physical activity (Fazzino et al., 2017). Therefore, although evidence suggests that physical activity may be important during WL, it would be interesting to examine whether both adaptive thermogenesis and spontaneous changes in physical activity influence the amount or rate of WL. This information could be used to empower individuals as genetics, which is possibly the main determinant of adaptive thermogenesis since data suggests it is a reproducible trait (Muller et al., 2016), would not be as important as changes in physical activity behaviours, which are at least in part under voluntary control (Zhang and Speakman, 2019).

Spontaneous changes in EI in response to energy restriction have also been observed in shorter- (Mars et al., 2005) and longer-term interventions (Polidori et al., 2016). Importantly, changes in EI appear to have a stronger (~3 times more) influence over energy balance than EE (Polidori et al., 2016). This could be due to the fact that EI represents 100% behaviour (although it could be modulated by physiology, psychology and environmental factors) while the behavioural component of EE only represents ~30% of TDEE (i.e., physical activity), depending on the individual's lifestyle. Also, it is easier to consume a larger amount of energy in a shorter time period, while the energy equivalent in terms of EE requires large changes in body composition and/or increases in physical activity. In diet-only interventions, especially if foods are provided, it is hard to examine whether changes in EI occur. A tool that can be used but is often overlooked is the provision of *ad libitum* test meals which could be used to assess food intake in an objective manner by measuring changes in food intake in a laboratory setting alongside appetite sensations (discussed below). Unfortunately, studies examining changes in EI using such an approach are scarce. For instance, in response to a 12-week supervised exercise program (meal WL of -1.3 ± 2.7 kg), mean daily EI (4 *ad libitum* meals were provided before and after the intervention) decreased by 131 ± 297 kcal (Hopkins et al., 2014). Furthermore, in a drug and diet-induced WL intervention (-11.8 kg and -7.7 kg in men and women, respectively), *ad libitum* EI during 3 days in a whole-room calorimeter decreased by 503kcal and 293kcal in men and women, respectively (Doucet et al., 2003). However, how the EI during an *ad libitum* test meal changes after diet-only WL interventions, as well whether different nutritional strategies may lead to distinct outcomes, remains unknown.

It should be recognised that measuring energy balance behaviours remains an area of frustration, especially assessing EI in free-living conditions (Dhurandhar et al., 2015). Although several self-reporting tools have been used in weight management research, these are known to be prone to misreporting (Ravelli and Schoeller, 2020). Interestingly, with the observed accuracy improvement in physical activity monitors during the last few decades, some tools have been developed in an attempt to improve EI estimates in free-living conditions. For instance, the 'intake-balance method' uses changes in body energy stores (i.e., body weight or body composition) and TDEE (using a physical activity monitor or doubly labelled water) to calculate EI. Interestingly, this calculation has been

shown to provide accurate estimates (95% confidence interval of <300kcal/day), especially when used in the longer-term (i.e., more than 28 days) as smaller time frames may be influenced by unaccounted changes in body fluids (Hall and Chow, 2011). Thus, this tool could be important for 1) estimating EI in free-living conditions; and 2) assessing the plausibility of reporting when self-reporting methods are used.

1.3.3 Psychological Compensatory Responses

While metabolic and behavioural compensatory responses occurring during negative energy balance are important, understanding the changes in psychological states and traits should not be overlooked. Although many psychological factors can have a profound influence over weight management outcomes, this thesis will be mainly focused on appetite sensations and eating behaviour traits.

Measures of appetite sensations typically include questions relating to hunger and fullness, but also desire to eat and prospective food consumption. These sensations are often measured using visual analogue scales (VAS) and reflect the individual's interpretation of their motivation to eat (Stubbs et al., 2000). It is commonly suggested that hunger increases during and after WL (Melby et al., 2017, Hintze et al., 2017) and that this increase in hunger promotes weight regain. However, data regarding changes in appetite sensations during WL is equivocal. Several studies have reported increases in hunger sensations following shorter- (Mars et al., 2006, Clayton et al., 2016) and longer-term energy deficit (Doucet et al., 2000, Drapeau et al., 2007, Tremblay et al., 2015, Alajmi et al., 2016). For instance, a seminal study observed that hunger sensations and desire to eat increased after a 10-week very-low energy diet intervention (500-550 kcal/day), and remained elevated after 1 year despite substantial weight regain (Sumithran et al., 2011). However, others reported decreases in hunger sensations during WL (Nymo et al., 2017, Andriessen et al., 2018, Sayer et al., 2018) or no changes (Anton et al., 2009, Hoddy et al., 2016, Coutinho et al., 2018a). It has also been suggested that increases in hunger following WL may represent a normalisation towards a lower body size (DeBenedictis et al., 2020). These authors suggested that individuals with overweight or obesity would have lower levels of hunger in comparison to people that are lean, and therefore WL

would bring these perceptions to normal levels (i.e., increase). Therefore, it remains inconclusive how appetite sensations change during and after WL.

A potential reason for the underlying differences observed between studies may be the way appetite sensations are assessed (e.g., fasted or in response to meals or retrospective measures at the end of the day). Alongside the marked inter-individual variability that has been previously documented (Gibbons et al., 2019), this could partially explain the equivocal findings between studies. Therefore, for a more comprehensive understanding of how appetite sensations change in response to WL, a complete assessment including various methods should be utilised. As suggested in section 1.2.1, appetite sensations should be examined alongside measurements of EI, and these should be collected in laboratory and free-living conditions.

In this thesis, the main eating behaviour traits that will be examined are cognitive restraint and disinhibition. Restraint is defined as tendency to restrict food intake usually for body weight control, whereas disinhibition reflects a tendency to overeat in response to the presence of different contexts or emotional states (Westenhoefer, 1991). Several diet-induced WL interventions reported favourable changes, e.g., increases in restraint and decreases in disinhibition (Chaput et al., 2005, Svensson et al., 2014, Urbanek et al., 2015, Sanchez et al., 2017, Morin et al., 2018). Higher baseline levels of cognitive restraint have also been shown to be associated with WL throughout the intervention (Svensson et al., 2014). As a greater cognitive restraint (especially flexible restraint, characterised by a more balanced approach to eating and weight management in which foods are not labelled in a dichotomic way, e.g., as being 'good' or 'bad') and a lower disinhibition have been found to be associated with better weight management (Westenhoefer et al., 1999), it would be important to fully comprehend how eating behaviour traits change in response to diet-induced WL.

Interim Summary:

- Energy balance is a dynamic system in which compensatory changes occur in response to perturbation in any component of the system to reduce the gap between EE and EI, and these responses seem stronger during periods of energy deficit.
- Decreases in EE during WL are to be expected due to reductions in body size. The presence of adaptive thermogenesis, and its implications for weight management, remain inconclusive.
- Although changes in physical activity may occur during periods of energy deficit, how these influence WL remains to be comprehended.
- The effect of WL on appetite sensations is equivocal, possibly due to the heterogeneity in the methodology used when assessing appetite.

1.4 Alternative Dietary Strategies to Attenuate Compensatory Responses to Weight Loss

The previously described metabolic, behavioural and psychological compensatory responses have been typically reported in continuous energy restriction (CER) interventions (i.e., consistent daily energy deficit). In an attempt to attenuate some of these compensatory responses that hinder WL and weight maintenance efforts, intermittent energy restriction (IER) has been proposed as an alternative strategy. This approach involves the inclusion of 'feed' days in energy balance or surfeit alongside 'fast' days of severe energy restriction (75-100% energy deficit) (Varady, 2011, Anton et al., 2018). Several studies using IER have demonstrated greater WL (Byrne et al., 2018) and lower reductions in RMR (Davoodi et al., 2014, Byrne et al., 2018) compared to CER. However, the various methods of implementing IER make it difficult to consistently compare it with CER. One IER pattern that has been recently popularised is usually named 'alternate day fasting'. In this IER pattern, which has led to the implementation of several versions, a very-low energy day (i.e., fasting, ~500kcal/day or 25% of daily energy requirements) is alternated with a higher EI day (e.g., *ad libitum* feeding or ~125% of daily energy requirements). The original form of 'alternate

day fasting' involves the consumption of 25% of energy requirements in one meal (e.g., lunch or dinner) alternated with an *ad libitum* feeding day. Overall, the data suggests that IER is an effective WL dietary strategy (Harris et al., 2018, Alhamdan et al., 2016). However, data directly comparing CER to IER is limited.

Regarding changes in body composition, a meta-analysis of 10 studies reported that IER led to less WL, but a greater retention of FFM and relative loss of FM in comparison to CER (Alhamdan et al., 2016). This could be due to the fact that CER was comprised of interventions using very-low energy diets (< 800 kcal/day), which could lead to a greater loss of body weight per unit of time and thus FFM (Hall, 2007). However, other studies have failed to observe differences in body composition between CER and IER (Coutinho et al., 2018a, Trepanowski et al., 2018). Therefore, whether IER attenuates the losses of FFM remains to be fully elucidated. This is critical as losses of FFM have been found to be associated with increases in hunger (Turicchi et al., 2020) and weight regain (Vink et al., 2016). Of note, it has been demonstrated that the fraction of FFM lost during WL is lower when initial body fat percentage is higher, total WL is lower and rate of WL is slower (Forbes, 1987, Hall, 2007). However, these relationships were demonstrated under conditions of low-calorie diets or with large amounts of WL (i.e., >10kg), and always during CER. How these differ under conditions of a moderate energy deficit (e.g., 15-30% energy deficit) and between CER or IER remains unknown.

Studies comparing the changes in EE and physical activity during WL induced via IER and CER are limited and inconclusive. For instance, Coutinho et al. did not observe any statistically significant differences between IER (550-660 kcal on 'fasting days' and 100% energy requirements on 'feasting days') and CER (Coutinho et al., 2018a). Another study did not observe any differences regarding RMR between CER and IER (full fast alternated with 100% energy requirements) (Catenacci et al., 2016). Regarding physical activity, an increase in the number of steps per day in both CER and IER after 6 weeks has been reported, but a decrease in the following 6 weeks was found in CER only, and these changes were different between groups (Coutinho et al., 2018a). In this study, no other differences were found between groups regarding physical activity (i.e., minutes of sedentary, light, moderate and vigorous to very vigorous activity). Another study also observed an increase albeit not statistically significant (6931 ± 842 to

7648 ± 773 steps per day) in the number of steps per day following 6 months of IER (75% energy deficit alternated with 125% of energy requirements) (Kalam et al., 2019). However, a previous study reported no differences in the number of steps per day after 10 weeks of IER (75% energy deficit alternated with *ad libitum* feeding day) (Klempel et al., 2010). Lastly, how adaptive thermogenesis differs between these dietary patterns has never been examined. Overall, whether IER is superior to CER in maintaining a higher EE and physical activity levels remains unknown and will be examined in Chapter 6.

Regarding changes in appetite and eating behaviour traits between CER and IER, evidence is limited. For instance, Coutinho et al. (2018) reported no changes in fasting and postprandial appetite sensations after CER and IER. Hoddy et al. (2016) also reported no changes in postprandial hunger, but an increase in postprandial fullness, after 4% WL through IER (75% energy deficit alternated with *ad libitum* feeding day). These findings were corroborated by a recent study that showed no changes in hunger and fullness after a 5.5% WL through IER (600 kcal/day alternated with *ad libitum* feeding day) (Kalam et al., 2020). Interestingly, despite the difference in WL after 12 weeks (CER – 1.2kg vs IER – 4.0kg), Cai et al. observed a similar decrease in hunger between CER and IER (Cai et al., 2019). However, a study using a 5:2 approach observed greater increases in free-living hunger on IER than CER (Sundfør et al., 2018). As it has been proposed that increases in hunger occur during CER (Hintze et al., 2017), a complete examination of appetite responses during a matched WL through CER and IER is warranted.

Changes in eating behaviour traits (e.g., cognitive restraint and disinhibition) between CER and IER have never been compared. However, an increase in restrained eating and decrease in uncontrolled eating after 12 weeks (3kg WL) of IER has been reported (Bhutani et al., 2013), while another study showed no changes in restrained eating score after 12 months (Kroeger et al., 2018). Therefore, whether CER and IER lead to different changes in eating behaviour traits remains unknown.

An important limitation regarding comparisons between CER and IER interventions is that WL was not purposefully matched between groups and individuals. This is a critical but rarely acknowledged point as it has been shown that the composition of the weight lost (Hall, 2007), changes in EE (Nymo et al.,

2018) and appetite sensations (Nymo et al., 2017) differ depending on total WL. Therefore, matching total WL between groups and individuals (as conducted in Chapter 6) will allow for an accurate comparison between CER and IER and whether one better attenuates the compensatory responses observed during WL.

Interim Summary:

- Intermittent energy restriction has been proposed as an alternative dietary strategy to attenuate the losses of FFM and the compensatory responses observed with CER.
- Although some evidence suggests that IER may be beneficial, data directly comparing IER to CER with regard to changes in body composition, EE, physical activity, appetite and eating behaviour traits are limited.
- An important limitation from previous studies that will be addressed in this thesis is the absence of matching WL between groups and participants.

1.5 Influence of Changes in Body Composition and Energy Expenditure During Weight Loss on Appetite

In section 1.2.1, it was described that in conditions of approximate energy balance, FFM, RMR and TDEE are positively associated with appetite and EI, creating a tonic drive to eat to ensure daily energy demands are met. However, an important area of research that remains to be fully addressed is how these associations change during conditions of WL or gain. This is an important area of research since some evidence has demonstrated that hunger sensations may increase (Hintze et al., 2017). This appears contradictory when comparing to the associations between FFM, RMR and TDEE and appetite reported under conditions of approximate energy balance (i.e., greater body size and EE associated to a greater drive to eat). The first piece of evidence emerged after an analysis by Dulloo et al. (1997) of the Minnesota Semi-starvation experiment (1945). In this study, a group of 32 lean men went through 24 weeks of severe energy restriction (losing ~25% of their initial body weight), followed by 12 weeks

of controlled refeeding. Twelve of them also went through 8 weeks of *ad libitum* refeeding in the laboratory. Notably, it was observed that during the 8-week *ad libitum* refeeding, a hyperphagic response occurred that only ceased once FFM levels were restored to baseline (Dulloo et al., 1997). As FFM recovery was slower than for FM, this led to an overshoot in the levels of body fatness that surpassed baseline levels by ~70%, a concept named 'collateral fattening'. Interestingly, a similar finding was reported in a group of lean rangers (Nindl et al., 1997) that went through 8 weeks of energy deficit (1000 kcal/day) through dietary energy restriction, followed by 5 weeks of *ad libitum* feeding. However, it is important to consider that these two studies involved extreme WL conditions (i.e., semi-starvation).

More recently, after 5 weeks of a very-low calorie diet (500 kcal/day) or 12 weeks of a low-calorie diet (1250 kcal/day), Vink et al. observed a moderate positive association between percentage of FFM lost during WL with 9-month weight change (Vink et al., 2016). It was also observed that losses of FFM after a 12% WL through a low-calorie diet were associated with increases in hunger sensations in women, but not in men (Turicchi et al., 2020). In this study, it was postulated that this association was not observed in women due to their higher initial body fat percentage and smaller losses of FFM. Indeed, it has been observed that individuals with a higher body fat percentage lose a lower fraction of FFM during WL (Forbes, 1987, Hall, 2007). However, it remains unknown whether losses in FFM are associated with changes in appetite during more moderate WL interventions (e.g., 15-30% energy deficit) with smaller losses of FFM.

In attempt to explain the apparently contradictory findings regarding the associations between FFM and appetite during conditions of energy balance and energy deficit / WL, Dulloo et al. have suggested that FFM exerts 'passive' and 'active' influence over EI depending on the state of energy balance. While FFM exerts a passive tonic influence over EI through its energetic requirements under conditions of approximate energy balance (i.e., higher FFM associated with a greater EI), losses of FFM may act as an independent orexigenic signal that triggers a cascade of responses that increase hunger sensations and EI (i.e., active role) (Dulloo et al., 2017), which may have important implications for obesity management.

As previously mentioned, compensatory responses observed during WL occur in a co-ordinated fashion to reduce the prescribed energy deficit. Considering there is evidence suggesting that some individuals may be more susceptible to have a greater resistance to WL (Reinhardt et al., 2015), it would be plausible to suggest that larger decreases in EE could be associated with greater compensatory increases in appetite sensations. However, this does not necessarily mean that compensatory changes in EE and EI are casually linked, but that together they could be part of a phenotypic response that resists WL. For instance, an individual with a 'WL resistance' phenotype could have greater decreases in EE and increases in appetite sensations during periods of energy deficit. Interestingly, a supervised exercise intervention observed that a greater adaptive thermogenesis was associated with increases in daily EI (Hopkins et al., 2014). Furthermore, after a 12.4% WL following a supervised diet (700kcal daily energy deficit) and exercise intervention (aerobic exercise at 60-75% of VO_2 max for 20-30 minutes 2-3 times per week), a strong association ($r = 0.73$; $p < 0.05$) was observed between adaptive thermogenesis (during weight stabilisation) and changes in hunger from baseline to post-WL (Tremblay et al., 2013). This suggests that a greater than predicted decrease in RMR following WL may be accompanied by a greater drive to eat, potentially undermining WL and weight maintenance efforts.

Although the reasons underlying these associations remain unknown, Hopkins et al. (2014) observed an association between adaptive thermogenesis and leptin, in which individuals that had a greater than predicted decrease in RMR had lower leptin levels. Thus, it is possible that this decrease in leptin triggers a tonic compensatory drive to eat. Importantly, these associations have never been replicated in diet-induced moderate matched WL interventions. Therefore, the associations between adaptive thermogenesis and changes in appetite sensations after a moderate diet-induced WL intervention matched between individuals will be explored in this thesis.

1.6 Overall Summary

Appetite is influenced by several inter-related biological, psychological, behavioural and environmental factors. In the last few decades, FFM and EE have been demonstrated under conditions of energy balance to be strong determinants of EI to ensure energetic needs are met. On the other hand, how FM influences EI remains inconclusive. Additionally, whether the composition of FFM, or its inclusion in appetite models, better explains the variability in EI between individuals remains unknown. Importantly, a critical area of research is how the crosstalk between body composition, EE and EI change at different levels of body fatness and in different states of energy balance. This would provide important information regarding energy balance dysregulation commonly observed at higher levels of body fatness.

It is known that several metabolic, behavioural and psychological compensatory responses occur in response to negative energy balance, undermining WL and weight maintenance efforts. Thus, several nutritional patterns such as IER have been proposed as promising strategies to attenuate these compensatory responses that complicate weight management. However, although data comparing IER to CER is emerging, whether they differently influence appetite control in WL matched conditions remains unknown. This thesis will therefore contribute to a better understanding of the factors underlying energy balance dysregulation at higher levels of body fatness, as well comprehending the mechanisms resisting WL, how they differ between individuals and whether different dietary patterns better attenuate the compensatory responses occurring during periods of negative energy balance. This will contribute for the conceptualisation of strategies to improve the effectiveness of obesity management interventions.

Chapter 2 – PhD Aims

This thesis aims to improve the understanding of the relationships between body composition and EE with appetite and EI in energy balance and energy deficit conditions. It also aims to understand how different dietary patterns influence the compensatory responses occurring during WL. To achieve this, the experimental studies within this thesis employed a complete assessment of energy balance components including metabolic, behavioural and psychological aspects.

General Aim 1: Examine the associations between body composition and EE with EI and appetite in different states of energy balance and across a spectrum of body fatness.

- Objective 1: Examine the associations between body composition and EE with appetite and EI at different levels of body fatness (Chapter 4).
- Objective 2: Examine the associations between body composition at the tissue-organ level with appetite and EI (Chapter 5).
- Objective 3: Examine the influence of changes in body composition, EE and physical activity during dietary-induced WL on appetite and EI (Chapter 7).

General Aim 2: Examine the changes in body composition, EE and physical activity during dietary-induced WL and their impact on compensatory changes in appetite, EI and body weight.

- Objective 4: Investigate the differences in the metabolic, psychological and behavioural responses to CER and IER in individuals with overweight and obesity (Chapter 6).
- Objective 5: Examine the factors associated with the mean rate of WL and changes in body composition during diet-induced energy restriction in women with overweight and obesity (Chapter 8).

Chapter 3 – General Methods

3.1 Ethical Considerations

Ethical approval was obtained by the School of Psychology Research Ethics Committee, University of Leeds, for the studies in chapter 4 and chapters 6-8 (PSC-238, 10/01/2018; PSC-551, 12-12-18). Ethical approval was obtained from the Faculty of Mathematics and Physical Sciences Ethics Committee, University of Leeds (MEEC 18-021, 06/03/2019), for study 2 in chapter 5. Before taking part in the studies, the main objectives and procedures were explained to participants and written informed consent was obtained. To avoid influencing eating behaviours during the investigation, specific objectives were not completely disclosed until study completion. After participating in the study, participants were fully debriefed about the specific objectives of the investigation and given the opportunity to ask questions.

3.2 Participant Recruitment and Screening

All participants were recruited from the University of Leeds (Leeds, UK) and surrounding area via poster advertisements and mailing lists. A participant information sheet with all the details regarding the investigation was provided to interested participants and they were invited to complete an online screening questionnaire to assess their eligibility, which included questions pertaining to medical history, anthropometrics, diet and physical activity history, food allergies and intolerances, and food preferences. Specific inclusion and exclusion criteria are detailed in each experimental chapter. Exclusion criteria common to all experimental chapters are the following:

- Health conditions that could jeopardize participant's safety or compliance to the protocol
- Smoking (or ceased in the last 6 months)
- History of eating disorders
- Pregnant, thinking about becoming pregnant or breastfeeding

- Taking medication that could affect appetite or body weight during or the month before the study
- History of anaphylaxis to food
- Shift work
- Significant changes in body weight in the last 6 months (± 4 kg)
- Exercising >3 days per week, had significantly changed physical activity patterns in the past 6 months or intending to change them during the study

In the studies conducted in this thesis, except for study 1 in chapter 5, only women were recruited. This was mainly due to two reasons. Firstly, including both men and women would require a larger sample size, as sex could be a confounding factor. Furthermore, women tend to be more proactive in volunteering for WL studies (Franz et al., 2007). As the funding used to conduct the study presented in chapter 6 had a time limit, alongside the fact that choosing participants of both sexes would increase the required sample size, it was decided that only women would be recruited. Therefore, although the results described throughout this thesis should be similar in men, caution should be taken until these are replicated in men.

3.3 Behavioural Measurements

3.3.1 Energy Intake

Throughout this thesis, EI was assessed using two methods: 1) laboratory-based test meals; 2) self-reported using an online dietary record tool (www.myfood24.org).

3.3.1.1 Laboratory-based Energy Intake

Before taking part in the study, participants completed an eligibility questionnaire in order to assess their food preferences. In the case where they reported that they did not like some of the food items, they would be considered not eligible to participate in the study since it could influence their food choices and eating behaviours. All test meals were served in a feeding cubicle, in which no distractions were present, in the Human Appetite Research Unit, School of Psychology, University of Leeds (UK) and in the Energy Metabolism and Appetite Research Laboratory, School of Food Science & Nutrition, University of Leeds

(UK). Fixed test meals were designed to provide an EI equal to 25% of measured RMR, and participants were instructed to consume all the food and drink provided. During *ad libitum* test meals, food was provided in excess of expected consumption, and participants were instructed to eat as much or as little as they wanted until they felt comfortably full, and that more food was available upon request. All meals were weighed before and after consumption to the nearest 0.1g and macronutrient intake was calculated from the manufacturers' food labels. EI was calculated using metabolizable energy equivalents for protein, fat, and carbohydrate of 4, 9, and 3.75 kcal/g, respectively. Specific information about the composition of laboratory test meals is provided below, excepting for Chapter 5.

Fixed Breakfast Meals

In the studies providing a fixed breakfast (all except for Chapter 5), this meal was individually calibrated to 25% of measured RMR (65% carbohydrate, 15% protein, 20% fat). These were individually calibrated as it has been previously demonstrated that RMR is a strong determinant of self-selected meal size and 24-hour EI (Blundell et al., 2015a). Therefore, a fixed EI breakfast would not take into account the inter-individual differences in body size and composition that strongly influence appetite responses. The ingredients consisted of muesli base (Holland & Barret), raisins and sultanas (Holland & Barret), honey (Sainsbury's) and whole milk natural yogurt (Yeo Valley), and the quantity was adjusted to equate to 25% of measured RMR. 300 grams of coffee (Nescafe Gold), tea (Yorkshire Tea) or water were also provided, and 40 grams of milk (Sainsbury's) could be added to the drink or to the muesli (Figure 3.1). Participants were asked to eat and drink everything in order to standardise EI between participants. Food items were weighed before and after consumption to the nearest 0.1g.



Figure 3.1 – Fixed energy breakfast that provided 25% of participant’s resting metabolic rate.

***Ad Libitum* Test Meal**

In studies providing a fixed breakfast (all except for Chapter 5), an *ad libitum* test meal (lunch) was served after 3 hours. This comprised of risotto (Uncle Ben’s Tomato & Herb; 1.51 kcal/g), yoghurt (Yeo Valley Strawberry and MyProtein Maltodextrin; 1.48 kcal/g) served in excess of expected consumption (70% carbohydrate, 9% protein and 21% fat) and 300g of water (Figure 3.2). For this meal, participants were instructed to eat as much or as little as they wanted until they felt comfortably full and that more food was available if needed. Between breakfast and lunch, participants could leave the laboratory but were asked not to exercise, eat or drink, except for water provided. Food items were weighed before and after consumption and macronutrient intake was calculated from the manufacturers’ food labels EI was subsequently calculated using energy equivalents for protein, fat and carbohydrate of 4, 9 and 3.75 kcal/g, respectively.



Figure 3.2 – *Ad libitum* test meal (lunch) comprised of tomato & herb risotto and strawberry flavoured yoghurt.

3.3.1.2 Free-living 24-hour Energy Intake

In chapters 4-6, 24-hour EI was also measured in the free-living environment using Myfood24 (www.myfood24.org). This is an online self-administered 24-hour dietary record tool that has been previously validated (Albar et al., 2016, Wark et al., 2018). This tool has a detailed food search capability, alerts for commonly forgotten foods and supplements, and includes photographic images and portion suggestions in order to improve reporting precision and accuracy (Carter et al., 2015). After a familiarization session with the system, participants were asked to record all food items and drinks consumed over a 7-day period, keeping their eating habits as close as possible to their normal routine. A link was emailed every day at 7pm so participants could complete and submit the diary in the evening, or the following morning (Figure 3.3). In order to improve the precision of the food records, a logbook was provided in which participants could write all the food items consumed during the day. The completion of the food diary was verified every morning and a reminder would be sent in case it was not submitted. When an item was not present in the database, the nutritional information of those foods, provided on food packaging or from the manufacturer's website, was

manually entered by the researcher. Nutrient and energy content of foods were calculated based on the reference values of McCance and Widdowson's 6th Edition Composition of Foods UK Nutritional Dataset. To estimate 24-hour EI, a 7-day average was calculated and atypical days (self-reported by the participants on the website, e.g., illness) were excluded from the analyses.

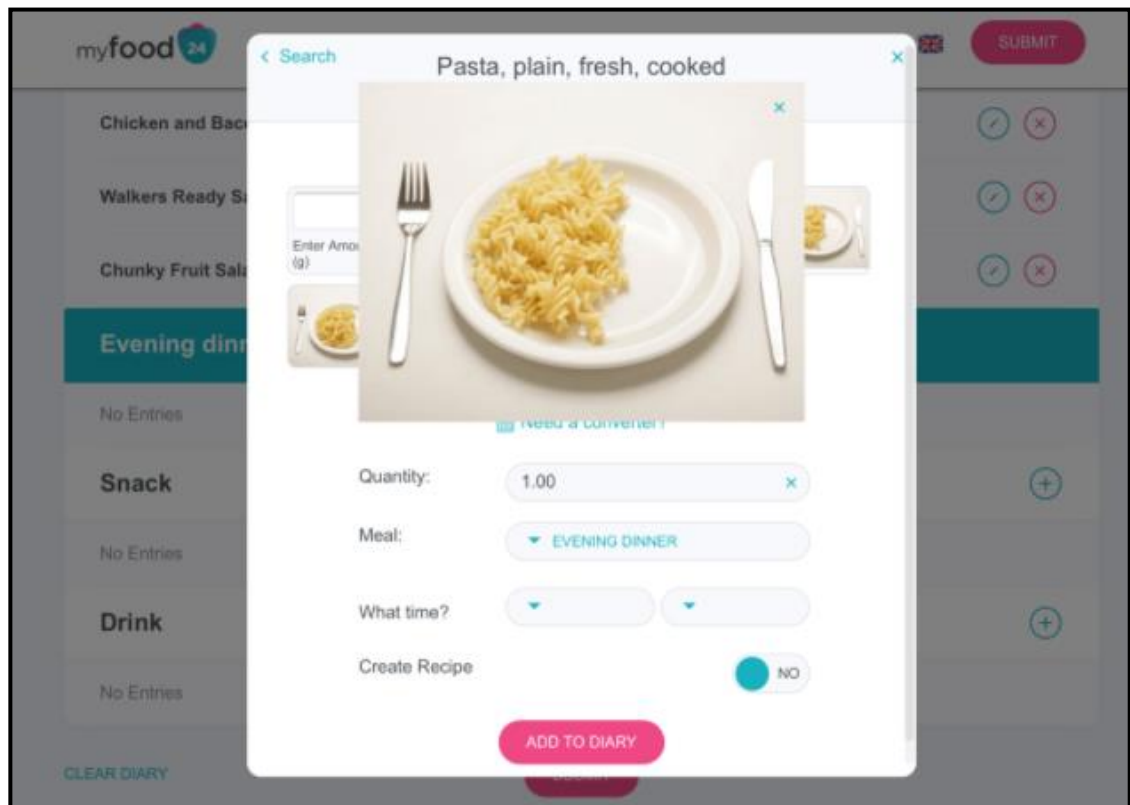


Figure 3.3 – Food searching page on Myfood24.

3.3.1.3 Calculated Energy Intake – ‘Intake-Balance Method’

Alongside the free-living self-reported assessment of EI using myfood24, EI was also estimated using the previously validated ‘intake-balance method’ (Hall and Chow, 2011, Racette et al., 2012, Shook et al., 2018). This method, which has been shown to provide valid estimates of EI, uses changes in body energy stores (i.e., body weight or body composition) alongside TDEE assessed using physical activity monitors (described below) or doubly labelled water. Two equations were used in this thesis:

If measurements of body composition were available:

$$\text{Mean Daily EI} = 9500 \frac{\Delta FM}{\Delta t} + 1020 \frac{\Delta FFM}{\Delta t} + TDEE$$

Where ΔFM and ΔFFM represent the changes in fat mass and fat-free mass (kg), while Δt represents the number of days between measurements, and TDEE the total daily energy expenditure. 9500 and 1020 represent the mean energy density of 1kg of FM and FFM, respectively (Shook et al., 2018).

When body composition was not available:

$$\text{Mean Daily EI} = 7400 \frac{\Delta BW}{\Delta t} + TDEE$$

Where ΔBW represents the changes in body weight during that time period. 7400 represents the mean energy density of 1kg of body weight (Racette et al., 2012).

These equations were used for 2 reasons: 1) to compare calculated and self-reported EI to assess the plausibility of reporting (Chapter 4); and 2) to compare the degree of energy deficit between dietary interventions (Chapter 6). These equations have been previously validated by several authors (Thomas et al., 2010, Shook et al., 2018) although there are a few assumptions that should be considered. Firstly, these equations assume that the energy density of FFM and FM remains constant throughout an intervention. This assumption is not necessarily true during shorter-term periods of WL (<28 days) due to unaccounted changes in body fluids which could lead to inaccurate estimations of EI (Hall and Chow, 2011). It has been suggested that measurements of at least 28 days help reducing this inaccuracy (Hall and Chow, 2011), which is the case of the intervention described in Chapter 6. The second assumption is that the TDEE used in the calculation of EI is constant throughout the intervention, which may not be true as suggested by Silva et al. (Silva et al., 2018).

3.3.2 Physical Activity

In the studies presented in chapters 4 and 6-8, participants wore a physical activity monitor (SenseWear Armband; BodyMedia, Inc., Pittsburgh, USA; Figure 3.4) to measure 7-day physical activity and sedentary behaviour and estimate TDEE and physical activity levels (PAL). Participants were instructed to wear the physical activity monitor halfway between their elbow and shoulder for at least 23 hours per day, only removing during activities that involved contact with water (e.g., shower and swimming). Compliance using the SenseWear Armband in this thesis was defined as having a minimum of 22 hours of verifiable time per day for at least 5 days (including one weekend day). Participants were told to keep their physical activity habits as close as possible to their normal routine.

The SenseWear Armband has been previously validated, presenting good accuracy in detecting different intensities of physical activity and estimating free-living TDEE, especially at lower intensities (Calabr et al., 2014, Bhammar et al., 2016, Santos-Lozano et al., 2017). This is an important consideration as participants that were recruited to take part in the studies described in the following chapters were considered as 'inactive', which could allow for a more accurate estimation of TDEE. The SenseWear Armband measures minute-by-minute tri-axial accelerometry, galvanic skin response, skin temperature and heat flux. Total daily energy expenditure and minutes spend in sedentary (< 1.5 METs), light (1.5-2.0 METs), moderate (3.0-5.9 METs) and vigorous (≥ 6 METs) activities were calculated using proprietary algorithms presented in the device's accompanying software (version 8.0 professional). Basal metabolic rate was calculated by the software using the WHO equation including participants' information (height, body weight, age and sex) collected in the laboratory after an overnight fast. Additionally, the software calculates PAL as $TDEE / \text{basal metabolic rate}$.



Figure 3.4 – SenseWear Armband device.

3.4 Physiological Measurements

3.4.1 Energy Expenditure

Estimations of TDEE were obtained from the SenseWear Armband as described in the previous section. Activity energy expenditure was calculated using the following equation:

$$\text{Activity Energy Expenditure} = (TDEE - RMR) \times 0.9$$

In which TDEE refers to total daily energy expenditure estimated using the physical activity monitor, RMR measured using indirect calorimetry (see following section) and multiplying by 0.9 to account for the thermic effect of food that usually represents ~10% of daily energy requirements (Westterterp, 2004).

3.4.2 Resting Metabolic Rate

Resting metabolic rate was measured in the morning following an overnight fast (10-12 hours) with two different indirect calorimeters fitted with a ventilated hood (GEM, Nutren Technology Ltd – chapters 4 and 6-8; COSMED Quark RMR, COSMED srl, Rome, Italy – Chapter 5; Figure 3.5). Participants were asked to remain in a supine position for 40 minutes without moving, talking or falling asleep in order to obtain precise measurements of RMR. Before each measurement, an individual calibration process including adjustments in the fraction of carbon dioxide was performed. Room temperature was kept at a constant level (22°C)

and a ventilation system was present in order to avoid carbon dioxide accumulation which could influence the accuracy of the results.

The gas collection systems used were based on the air dilution technique. In this technique, a pump is set to draw ambient air at a constant rate through the ventilated canopy, and then exhaled air is diluted with room air and shunted to a mixing chamber for analysis. VO_2 and VCO_2 were calculated from O_2 and CO_2 concentration in inspired and expired air and averaged over 30-second intervals. Both the GEM (Kennedy et al., 2014) and COSMED Quark RMR (Blond et al., 2011) have been previously validated, presenting a high day-to-day reliability.



Figure 3.5 – COSMED Quark Resting Metabolic Rate

Resting metabolic rate was calculated using the 5-minute steady state method (McClave et al., 2003) which has been shown to provide the lowest value (Sanchez-Delgado et al., 2018). This is an important consideration as RMR represents the lowest basal energy requirements. After excluding the first 5 minutes of measurement, the coefficient of variance (CV) was calculated for VO_2 , VCO_2 , ventilation and respiratory exchange ratio in 5-minute intervals (e.g., from 6th to 10th minute, from 7th to 11th minute). Afterwards, the 5-minute interval that presented the lowest CV (at least lower than 10% for VO_2 , VCO_2 , ventilation and lower than 5% for respiratory exchange ratio) was chosen, and data was entered into the Weir equation (Weir, 1949):

$$RMR = (3.941 \times VO_2) + (0.85 \times 1.106 \times VO_2)$$

When the COSMED Quark was used, the abbreviated Weir equation was utilised:

$$RMR = (3.9 \times VO_2) + (1.1 \times VCO_2 \times 1.44)$$

3.5 Anthropometry

Height was measured without shoes to the nearest 0.1cm using a stadiometer (Leicester height measure, SECA, UK). Body mass was measured to the nearest 0.01kg using a weighing scale (BodPod, Life Measurement, Inc., Concord, USA). Body mass index was calculated using the following formula:

$$BMI (kg/m^2) = \frac{Body\ Mass\ (kg)}{Height\ (m^2)}$$

3.6 Body Composition

Measurements of body composition were collected whilst participants were wearing tight fitting clothing and a swimming cap between 7 and 9am following an overnight fast. Both FM and FFM were estimated to the nearest 0.01kg using air displacement plethysmography (BodPod, Life Measurement, Inc., Concord, USA; Figure 3.6), a 2-compartment model of body composition assessment that involves the measurement of air displacement within a dual-chambered fibreglass plethysmograph to determine body volume and calculate body density.



Figure 3.6 – Air displacement plethysmography (BodPod).

Air displacement plethysmography has been previously validated in individuals with normal weight and with overweight / obesity (Lowry and Tomiyama, 2015), presenting a similar accuracy to dual x-ray absorptiometry when tracking changes in body composition over time (Minderico et al., 2006). Studies have demonstrated that the BodPod generally has high test-retest coefficients (e.g., 0.99) (Vescovi et al., 2001) and small CV between days (2.0-2.3%) and intra-days (1.7-4.5%) (Fields et al., 2002). Manufacturer's instructions were followed and the Siri equation (Siri) was used to estimate body fat percentage:

$$\text{Body Density (kg/m}^3\text{)} = \text{Body Mass} / \text{Body Volume}$$

$$\text{Body Fat (\%)} = (4.95 / \text{Body Density} - 4.5) \times 100$$

Fat-free mass index (FFMI) and fat mass index (FMI) were also calculated using the following equations:

$$\text{FFMI (kg/m}^2\text{)} = \frac{\text{FFM}}{\text{Height}^2} \quad \text{FMI (kg/m}^2\text{)} = \frac{\text{FM}}{\text{Height}^2}$$

3.6.1 P-ratio

The protein ratio (p-ratio) is a calculation used to assess the fraction of FFM lost per body weight (e.g., a higher p-ratio during WL reflects a greater loss of FFM). Although p-ratio and fraction of FFM lost are slightly different (the p-ratio represents the fraction of an energy imbalance accounted for by changes of the body's protein stores), they will be used interchangeably throughout this thesis. The p-ratio depends on factors such as initial level of body fatness, degree of the energy deficit and inclusion of exercise (Forbes, 1987, Hall, 2007). Considering the potential influence of FFM on appetite, differences in p-ratio between IER and CER could be informative regarding WL and maintenance success, as well appetite control. P-ratio, or the fraction of FFM lost, was calculated using the following equation:

$$P - \text{Ratio} = \frac{\Delta \text{FFM}}{\Delta \text{BW}}$$

Where ΔFFM and ΔBW represent the changes in fat-free mass and body weight after WL, respectively.

3.7 Psychological Measurements

3.7.1 Appetite Sensations

Appetite sensations (i.e., hunger, fullness, desire to eat and prospective food consumption) were assessed using VAS, which have been shown to be valid and reproducible in assessing the motivation to eat (Stubbs et al., 2000). Each of the following questions were answered on a horizontal line anchored at each end by the words “Not at all” and “Extremely”, ranging between 0 and 100mm:

- “How hungry do you feel now?”
- “How full do you feel now?”
- “How strong is your desire to eat now?”
- “How much food do you think you could eat?”

During laboratory measures days, these questions were answered with a validated hand-held Electronic Appetite Rating System (Gibbons et al., 2011) (Figure 3.7). Area under the curve (AUC) was calculated using the trapezoid formula (Pruessner et al., 2003):

$$AUC = \frac{(m_2 + m_1) \times t_1}{2} + \frac{(m_3 + m_2) \times t_2}{2} + \dots$$

Where ‘*m*’ represents the individual appetite ratings and ‘*t*’ the time between both measurements.



Figure 3.7 – Electronic Appetite Rating System device.

Appetite sensations were also assessed in free-living conditions using the Control of Eating Questionnaire (CoEQ). The CoEQ (Hill et al., 1991) is a validated questionnaire comprised of 21 questions that retrospectively assess 24-hour hunger and fullness sensations, as well food cravings experienced although these were not examined in this thesis. This questionnaire was completed at the end of the day for 7 days and an average was calculated.

In order to have a uniform assessment of appetite sensations, a composite score was calculated using the following equation as suggested previously (Stubbs et al., 2000):

$$\text{Composite Scores (mm)} = \frac{\text{Hunger} + (100 - \text{Fullness}) + \text{PFC} + \text{Desire to Eat}}{4}$$

In which PFC represents prospective food consumption. For free-living assessments using CoEQ, only hunger and fullness were used to calculate the composite scores.

3.7.2 Three-Factor Eating Questionnaire

The Three-Factor Eating Questionnaire (Stunkard and Messick, 1985) is a validated 51-item scale that assesses three dimensions of eating behaviour: dietary restraint, disinhibition of control, and susceptibility to hunger (previously defined in section 1.3.3). Participants respond true or false to the first 36 items and choose one of four possible responses for the remaining questions depending on the level of agreement with each specific statement. Responses are scored for each of the three factors so that a higher score reflects a greater level of eating disturbances. In order to assess the type of dietary restraint, the restraint scale was subdivided in two subscales: rigid and flexible control (Westenhoefer, 1991, Shearin et al., 1994). Flexible restraint is characterised by a more balanced approach to eating and weight management in which foods are not labelled in a dichotomic way, e.g., as being 'good' or 'bad', while rigid restraint is characterised by a dichotomic 'all-or-nothing' approach to eating (Westenhoefer et al., 1999).

3.8 Statistical Approach

Throughout this thesis, data are reported as mean \pm standard deviation, with figures reporting mean \pm standard error of the mean. IBM SPSS for Windows (version 25; USA) was used for statistical analyses. Data were visually inspected for normality prior to statistical treatment using the Shapiro-Wilk test. PROCESS macro (version 3.1) was used in order to perform moderation analyses in Chapter 4 (Hayes and Rockwood, 2017). Further specific statistical procedures are explained within the methods section of each experimental chapter. Statistical significance was established at $p < 0.05$.

Chapter 4 – The Influence of Body Fatness on The Associations Between Body Composition and Energy Expenditure with Appetite and Energy Intake in Healthy Women

Chapter Aims:

- Examine whether the associations between EE and EI are moderated by body fatness.
- Investigate how the strength and direction of the association between FM and EI changes across a range of body fatness.

4.1 Introduction

A comprehensive understanding of energy balance and the factors that lead to its dysregulation is essential to understand the aetiology of obesity. Recent work has highlighted body composition and EE as potential tonic drivers of day-to-day EI (Blundell et al., 2012, Weise et al., 2014, McNeil et al., 2015), with associations between FFM, RMR and EI thought to reflect the energetic demand created by metabolically active tissues (Hopkins et al., 2018, Hopkins et al., 2016).

Resting metabolic rate has also been reported to be positively associated with hunger sensations (Caudwell et al., 2013). Earlier work by Cugini et al. reported a positive association between FFM and hunger, and a negative association between FM and hunger in healthy weight individuals ($BMI = 21 \pm 2 \text{ kg/m}^2$) (Cugini et al., 1998), but not in those with obesity ($BMI = 32 \pm 7 \text{ kg/m}^2$) (Cugini et al., 1999). The authors postulated that this may reflect disruption in the mechanisms that exert tonic control over daily hunger. Interestingly, Grannell et al. has recently reported a positive association between FFM and EI during an *ad libitum test* meal in 43 individuals with obesity, but the strength of this association was weaker at higher BMIs (Grannell et al., 2019a). Furthermore, Piaggi et al. observed a positive association between 'awake and fed thermogenesis' and

total daily EI in individuals with a BMI $\leq 29\text{kg/m}^2$ but not in those with a BMI $>29\text{kg/m}^2$ (Piaggi et al., 2015). Regarding the associations between FM and EI, equivocal findings have been reported. Negative associations between FM and EI have been observed in lean individuals (Blundell et al., 2015a), but studies in those with overweight/obesity often report no association between FM and EI (Lissner et al., 1989, Blundell et al., 2012, McNeil et al., 2015, Cameron et al., 2016). Overall, these studies suggest that the association between body composition and EE with appetite and EI may be weaker at higher levels of body fatness, but the potential mechanisms remain unclear and studies have yet to directly compare these associations between lean individuals and those with overweight/obesity.

It is also important to consider how factors such as eating behaviour traits and physical activity could influence these associations as they have been found to be associated with body composition and EI. For instance, a negative association between cognitive restraint and *ad libitum* EI over 3-days was found in 78 individuals with obesity (Graham et al., 2014). Additionally, disinhibition was positively associated with BMI in 116 women (Kruger et al., 2016). In this sample, women with higher disinhibition and lower restraint had higher levels of body fatness. Furthermore, a study reported that the negative association between FM and EI was partially mediated by cognitive restraint (Hopkins et al., 2018). Evidence also suggests that levels of physical activity may influence the coupling between EE and EI (Mayer et al., 1956, Beaulieu et al., 2016) and the associations between FM and meal size (Beaulieu et al., 2018). Therefore, although body fatness could be an important factor in the development of energy balance dysregulation, the possible influence that psychological and behavioural factors may have on appetite control should not be overlooked. Including these variables in models assessing the relationships between body composition and EE with appetite and EI could improve the understanding of the mechanisms influencing appetite control.

4.1.1 Objective & Hypotheses

The main aim of this chapter was to examine how body fatness influences the associations between body composition and EE with appetite and EI in order to test the following hypotheses:

1. FFM, RMR and TDEE would be positively associated with appetite and EI.
2. Body fat percentage would exert a moderating influence over the associations between RMR, TDEE and EI, in which a weaker relationship will be observed in participants with overweight/obesity.
3. The association between FM and EI would be influenced by the levels of body fatness, with a negative association being observed in leaner participants, but not in those with overweight/obesity.

4.2 Methods

4.2.1 Participants

Data presented in this chapter represent data from two separate studies with identical experimental procedures. The data from both studies were combined for analyses in this chapter to provide a comparison between lean individuals and those with overweight/obesity. The studies ran from February 2018 to September 2018 (overweight/obesity) and from February 2019 to October 2019 (lean). For study 1, participants with overweight and obesity were recruited to take part in a study examining 'the effects of a personalised weight loss meal plan on body composition and metabolism'. The data presented in the current chapter represent the baseline data before diet allocation. Details of the dietary intervention are provided in Chapter 6. For study 2, participants that were lean were recruited to investigate how the influence of body composition and EE on appetite and EI differed according to levels of body fatness. All participants were recruited from the University of Leeds and the surrounding area via posters and email lists. Volunteers were included if they were aged between 18-55 years and had a BMI between 18.5-24.9 kg/m² (study 2) or 25.0-34.9 kg/m² (study 1). Exclusion criteria are described in Chapter 3 and descriptive characteristics of all participants can be observed in Table 4.1.

Table 4.1 – Descriptive characteristics of all participants and separated by BMI categories.

	All (n = 93)		Lean (n = 45)		Overweight or Obesity (n = 48)	
	Mean	SD	Mean	SD	Mean	SD
Age (y)	35	10	35	10	35	10
Height (cm)	164.9	7.2	164.6	6.3	165.1	8.1
Body weight (kg) *	69.6	13.9	59.0	5.9	79.5	11.7
BMI (kg/m ²) *	25.5	4.2	21.8	1.5	29.0	2.5

BMI, body mass index. * Significant differences between participants that are lean and that have overweight or obesity ($p < 0.001$).

4.2.2 Screening

Following an online pre-screening questionnaire assessing general eligibility criteria, participants were invited to the laboratory for a full screening session where the protocol was thoroughly explained, eligibility was determined, and consent forms were signed. Participants were instructed not to change their physical activity and dietary habits for the duration of the study.

4.2.3 Procedure and Measurements

Participants completed a free-living week of measurements where body weight was measured fasted and nude each morning with a scale provided (Salter scale model 9206, UK) and an online dietary record tool (myfood24) was completed at the end of each of the 7 days. A physical activity monitor (SenseWear Armband) was worn continuously throughout the week (concurrent with the 7-day food diary) to assess minutes of physical activity and estimate TDEE. Upon completion of the week of measurements, participants attended the laboratory for a day of measurements.

All testing took place after a 10-12h overnight fast. Anthropometrics, body composition and RMR were assessed. This was followed by a fixed breakfast (25% of RMR measured with indirect calorimetry) and an *ad libitum* test meal 3

hours later (both meals are described in Chapter 3.3.1.1). Participants were then provided with paper versions of an eating behaviour questionnaire (Three-Factor Eating Questionnaire) to complete that evening at home. Appetite sensations were assessed using VAS in the morning, before and after food intake, and at 30-minute intervals between the breakfast and lunch meal (180 minutes). Anthropometrics, body composition, RMR, minutes of physical activity and TDEE, EI and eating behaviour traits (Three-Factor Eating Questionnaire) were assessed as previously described in Chapter 3.7.2. A representation of the protocol can be observed in Figure 4.1.

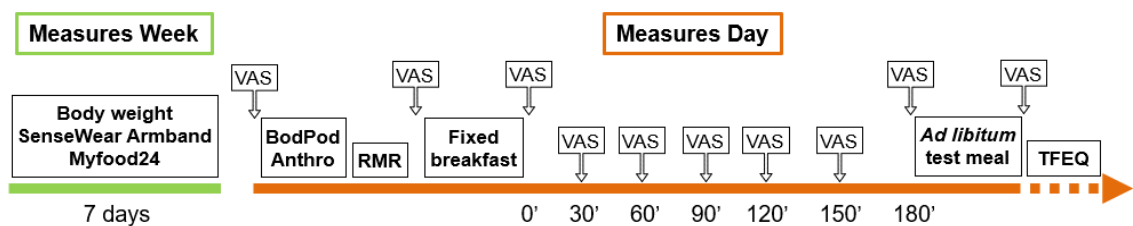


Figure 4.1 – Schematic representation of the protocol. Anthro, anthropometrics; RMR, resting metabolic rate; VAS, visual analogue scale; TFEQ, Three-Factor Eating Questionnaire.

4.2.3.1 Appetite Sensations

Appetite sensations were measured using VAS upon arrival to the laboratory, before and after food intake, and at 30-minute intervals between the breakfast and lunch meal (180 minutes) with a validated electronic system (Gibbons et al., 2011). Fasted and AUC appetite composite scores were calculated as presented in Chapter 3.7.1.

4.2.3.2 Plausibility of Reporting

As free-living 24-hour EI was assessed using a self-reported dietary record tool (myfood24), plausibility of reporting was examined using the ratio between EI and RMR (Goldberg et al., 1991) and this was compared between BMI categories.

Furthermore, the 'intake-balance method' was also used to compare reported and calculated EI. Energy imbalance was estimated multiplying the change in body weight during the 7 days by the energy coefficient 7400 kcal/kg (Racette et al., 2012). Mean daily EI was calculated (energy imbalance + TDEE), and this was compared to self-reported EI to examine the plausibility of reporting.

4.2.4 Statistical Analyses

Independent samples t-tests were conducted to analyse the differences between participants that were lean and with overweight/obesity, and between calculated and self-reported EI. Pearson's correlations were used to assess the associations between body composition, RMR and TDEE with test meal and mean 24-hour EI. Fisher Z-transformations were conducted to investigate the differences between correlations in individuals that were lean and those with overweight/obesity. Multiple linear regressions were used to examine the independent effects of body composition (FM and FFM), RMR and TDEE on test meal and mean 24-hour EI. These analyses were also repeated using FMI and FFMI to account for differences in height. To test for a non-linear association between FM and test meal EI, the quadratic term FM^2 was included alongside FFM and FM as predictor variables. The non-linear association was also tested using body fat percentage and FMI (Appendix), as FM is an unstandardized measure that is influenced by body weight and height.

To examine whether body fat percentage influenced the association between RMR and TDEE with mean 24-hour EI, moderation analyses were conducted using PROCESS macro for SPSS (version 3.1) (Hayes and Rockwood, 2017). Body fat percentage was used as the moderator as it has been proposed to be an important indicative of obesity-related risk factors (Macek et al., 2020). However, as body fat percentage has limitations (e.g., not accounting for height or total tissue mass), analyses were also repeated with BMI, FM and FMI as moderators (Appendix). Eating behaviour traits and physical activity were added as covariates in the non-linear regression model and moderation analyses if they were correlated to FM and body fat percentage, respectively. These analyses were also conducted adjusting for 'study'. However, no differences were observed, and 'study' was not a significant predictor in the models.

The study was powered to examine whether the associations between body composition (FM and FFM) and EE with EI were moderated by body fat percentage (primary outcome). Power calculations (G*Power v3.1) estimated that a sample size of 77 would be required to conduct a multiple regression with 3 independent variables, a medium effect size ($f^2=0.15$) with $\alpha=0.05$ and $1-\beta=0.8$.

4.3 Results

4.3.1 Participant's Characteristics

4.3.1.1 Body Composition

Body composition in all participants and differences between groups can be seen in Table 4.2. As can be observed, FM, FFM and body fat percentage were higher in the group of participants that had overweight or obesity (all $p < 0.001$).

Table 4.2 – Body composition of all participants and separated by BMI categories.

	All (n = 93)		Lean (n = 45)		Overweight or Obesity (n = 48)	
	Mean	SD	Mean	SD	Mean	SD
Fat mass (kg) *	24.9	10.6	16.3	3.6	32.9	8.4
Fat-free mass (kg) *	44.7	5.4	42.8	4.0	46.5	5.8
Body fat (%) *	34.4	8.6	27.3	4.6	41.0	5.6

* Significant differences between participants that are lean and that have overweight or obesity ($p < 0.001$).

4.3.1.2 Energy Expenditure and Physical Activity

Data on EE and physical activity can be seen in Table 4.3. Resting metabolic rate was not significantly different between groups ($p = 0.35$). However, despite presenting a higher TDEE, participants with overweight or obesity spent less time in physical activity behaviours (i.e., lower total / moderate-to-vigorous physical activity, and higher time in sedentary behaviours).

Table 4.3 – Energy expenditure and physical activity behaviours of all participants and separated by BMI category.

	All (n = 93)		Lean (n = 45)		Overweight or Obesity (n = 48)	
	Mean	SD	Mean	SD	Mean	SD
RMR (kcal/day)	1417	188	1398	155	1435	214
TDEE (kcal/day) ^{1 *}	2273	334	2141	277	2400	338
T PA (min/day) ^{1 *}	301	98	353	79	251	88
Sed (min/day) ^{1 *}	682	105	625	81	736	97
MVPA (min/day) ^{1 *}	94	43	114	43	74	33

RMR, resting metabolic rate; TDEE, total daily energy expenditure; T PA, total physical activity; Sed, sedentary time; MVPA, moderate-to-vigorous physical activity. ¹ All - n =92; Overweight/obesity - n=47; * Significant differences between participants that are lean and that have overweight or obesity (p < 0.001).

4.3.1.3 Energy Intake and Appetite Sensations

Data on test meal EI, free-living 24-hour EI, ratio between mean 24-hour EI and RMR, calculated EI ('intake-balance method') and appetite sensations can be observed in Table 4.4. There were no differences between groups regarding these variables (all p ≥ 0.53). Furthermore, self-reported EI was not significantly different from calculated EI in the whole sample (p = 0.18) or when examined by BMI categories (lean - p = 0.29; overweight/obesity - p = 0.39), suggesting that plausibility of reporting was similar between groups.

Table 4.4 – Energy intake and appetite sensations (composite scores) of all participants and separated by BMI category.

	All (n=93)		Lean (n=45)		Overweight or Obesity (n=48)	
	Mean	SD	Mean	SD	Mean	SD
Test meal EI (kcal)	862	245	863	230	861	260
24-hour EI (kcal/day)	1832	390	1811	379	1852	403
24-hour EI : RMR	1.30	0.25	1.30	0.28	1.30	0.22
Calculated EI (kcal/day)	1958	809	1931	728	1985	887
Fasted Appetite (mm)	54	21	54	23	54	20
AUC Appetite (mm*min)	6622	2389	6577	2217	6664	2563

EI, energy intake; 24-hour EI : RMR, ratio between mean free-living 24-hour energy intake and resting metabolic rate; AUC, area under the curve.

4.3.1.4 Eating Behaviour Traits

Data regarding eating behaviour traits and differences between groups can be seen in Table 4.5. No differences were observed for cognitive restraint, flexible and rigid restraint (all $p \geq 0.56$). However, participants with overweight or obesity presented higher levels of disinhibition and susceptibility to hunger (both $p < 0.001$).

Table 4.5 – Eating behaviour traits of all participants and divided by BMI categories.

	All (n = 87)		Lean (n = 40)		Overweight or Obesity (n = 47)	
	Mean	SD	Mean	SD	Mean	SD
Restraint ¹	7.8	4.5	7.7	5.4	7.9	3.8
Flexible Restraint ²	2.3	1.8	2.3	1.9	2.4	1.7
Rigid Restraint ²	2.5	1.8	2.7	1.9	2.4	1.7
Disinhibition ^{1**}	8.2	3.7	6.6 ^{**}	3.6	9.6	3.3
Hunger [*]	5.9	3.2	4.7 [*]	2.9	7.0	3.1

¹ All – 86 participants, Lean – 39 participants; ² All – 85 participants, Lean – 38 participants. * Significant differences between participants that are lean and that have overweight or obesity (p = 0.001); ** p < 0.001.

4.3.2 Associations Between Body Composition and Energy

Expenditure with Appetite and Energy Intake in the Whole Sample

4.3.2.1 Energy Intake

Associations between FM, FFM, RMR, and TDEE with EI (free-living mean 24-hour and test meal EI) can be seen in Figure 4.2 and Figure 4.3. As can be observed, FFM, RMR and TDEE were positively associated with mean 24-hour EI (all p < 0.001). However, FM was not associated with mean 24-hour EI (p = 0.11). Similar associations were observed between FMI (r = 0.10; p = 0.34) and FFMI (r = 0.27; 0.01) with mean 24-hour EI (Appendix, Figure S1). No associations were observed between components of body composition or EE and test meal EI (all p ≥ 0.45; Figure 4.3).

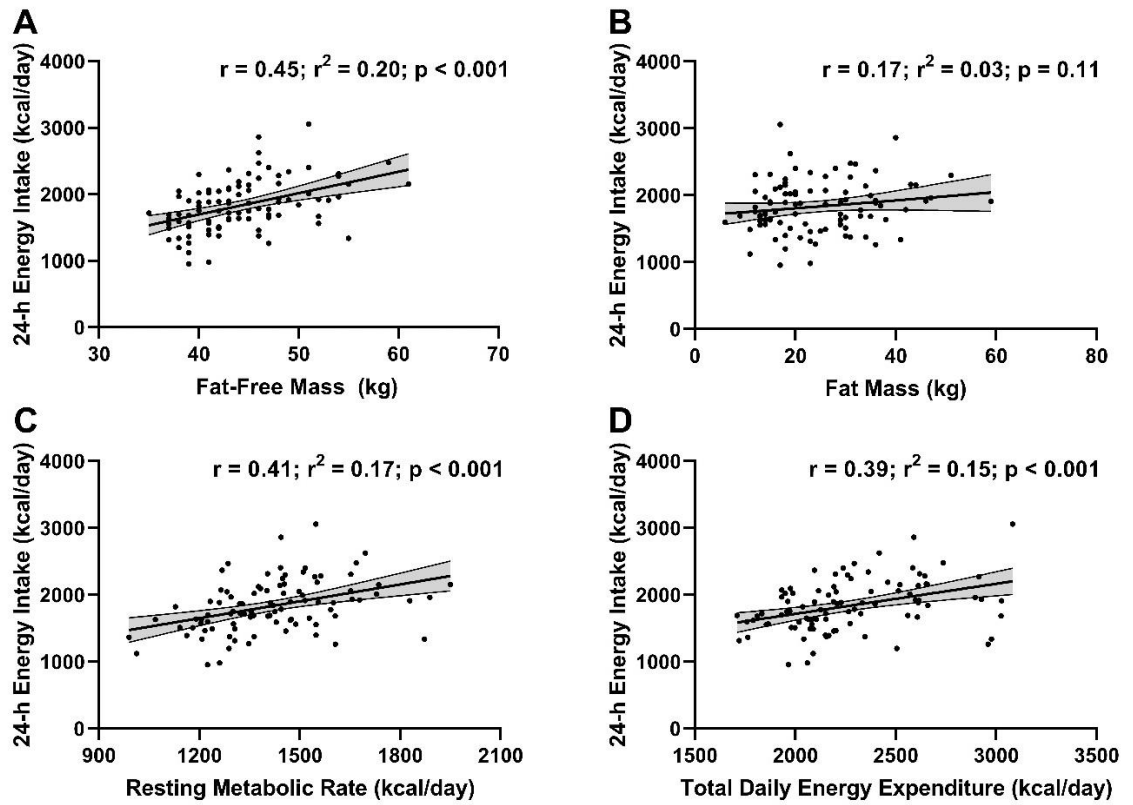


Figure 4.2 – Scatter plot for the whole sample showing the associations between mean 24-hour energy intake with A) fat-free mass B) fat mass C) resting metabolic rate D) total daily energy expenditure. Grey bands represent the 95% confidence intervals.

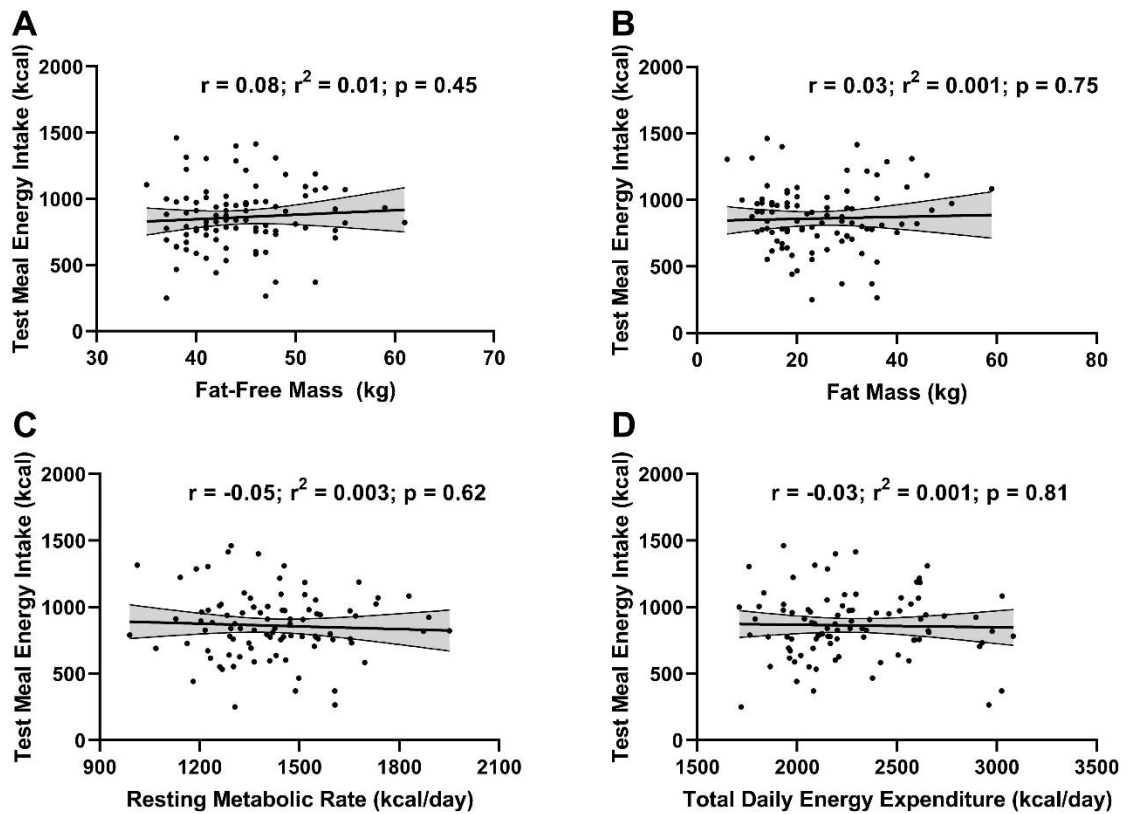


Figure 4.3 – Scatter plot for the whole sample showing the associations between test meal energy intake with A) fat-free mass B) fat mass C) resting metabolic rate D) total daily energy expenditure. Grey bands represent the 95% confidence intervals.

To examine the independent effects of body composition and EE on mean 24-hour EI, three multiple linear regressions were conducted in the whole sample (Table 4.6). In model 1, FM and FFM accounted for 19% of the variance in mean 24-hour EI [$F(2, 90) = 11.5, r^2 = 0.19, p < 0.001$], with FFM the only predictor ($\beta = 0.47; p < 0.001$). Adding RMR in model 2 [$F(3, 89) = 8.2, r^2 = 0.19, p < 0.001$], or TDEE in model 3 [$F(3, 88) = 7.9, r^2 = 0.18, p < 0.001$] did not increase the variance in mean 24-hour EI explained by the model. These analyses were replicated using FMI and FFMI and similar findings were observed (Appendix, Table S2). However, in models 2 and 3, both RMR ($\beta = 0.36; p = 0.01$) and TDEE ($\beta = 0.38; 0.001$) were significant predictors of mean 24-hour EI, respectively.

When models 1-3 were replicated with test meal EI as the dependent variable, none of the models explained the between-subject variance in test meal EI (all models $r^2 = 0.006-0.04; p = 0.42-0.78$).

Table 4.6 – Regression coefficients showing the effect of fat mass and fat-free mass (Model 1), fat mass, fat-free mass and resting metabolic rate (Model 2), and fat mass, fat-free mass, and total daily energy expenditure (Model 3) on mean 24-hour energy intake. N = 93.

Model 1				
	B	SE	β	P-value
Intercept	351.1	312.9		0.27
FM	-1.5	3.9	-0.04	0.68
FFM	34.0	7.6	0.5	< 0.001
Model 2				
	B	SE	β	P-value
Intercept	259.6	321.2		0.42
FM	-1.6	3.9	-0.04	0.68
FFM	25.1	10.6	0.3	0.02
RMR	0.3	0.3	0.2	0.23
Model 3				
	B	SE	β	P-value
Intercept	301.0	321.0		0.35
FM	-3.5	4.2	-0.1	0.40
FFM	24.9	9.9	0.3	0.01
TDEE	0.2	0.2	0.2	0.20

B, unstandardized beta coefficient; SE, standard error; β, standardised beta coefficient; FM, fat mass; FFM, fat-free mass; RMR, resting metabolic rate; TDEE, total daily energy expenditure.

4.3.2.2 Appetite Sensations

Associations between FM, FFM, RMR and TDEE with fasting and AUC appetite composite scores can be seen in

Table 4.7. No associations were observed between body composition, RMR or TDEE with appetite composite scores (all $p \geq 0.22$).

Table 4.7 – Associations between fat mass, fat-free mass, resting metabolic rate and total daily energy expenditure with fasting and AUC appetite composite scores (n = 93).

	Fasting Appetite			AUC Appetite		
	r	r ²	p-value	r	r ²	p-value
FM	-0.10	0.01	0.32	0.05	0.003	0.64
FMI	-0.13	0.02	0.22	0.03	< 0.001	0.77
FFM	0.03	< 0.001	0.75	0.07	0.004	0.52
FFMI	-0.07	0.004	0.52	-0.004	< 0.001	0.97
RMR	0.08	0.006	0.45	0.06	0.004	0.57
TDEE ¹	0.03	< 0.001	0.79	0.10	0.01	0.37

FM, fat mass; FMI, fat mass index; FFM, fat-free mass; FFMI, fat-free mass index; RMR, resting metabolic rate; TDEE, total daily energy expenditure. ¹ N = 92.

Multiple linear regressions were also conducted to examine the predictors of appetite sensations, but components of body composition and EE did not predict appetite ratings (all models $p \geq 0.51$).

Section Summary:

- Fat-free mass, RMR and TDEE were positively associated with mean 24-hour EI, but not with test meal EI.
- Fat mass was not associated with either 24-hour or test meal EI.
- Body composition, RMR and TDEE were not associated with appetite composite scores.

4.3.3 Differences Between Lean Individuals and Those with Overweight or Obesity

Scatter plots of the associations between body composition and EE with mean 24-hour EI within each BMI group (lean and overweight/obesity) can be observed in Figure 4.4. The associations between FFM, RMR and TDEE with 24-hour EI were stronger (i.e., higher r and r^2) in the group of participants that were lean. However, Fisher Z-transformations indicated that the differences in r values between BMI categories did not reach significance (FFM: $Z = 0.73$, $p = 0.23$; RMR: $Z = 1.27$, $p = 0.10$; TDEE: $Z = 1.54$, $p = 0.06$). No association was observed between FM and 24-hour EI in either group. These associations were replicated using FMI and FFMI and can be found in Table S3 (Appendix)

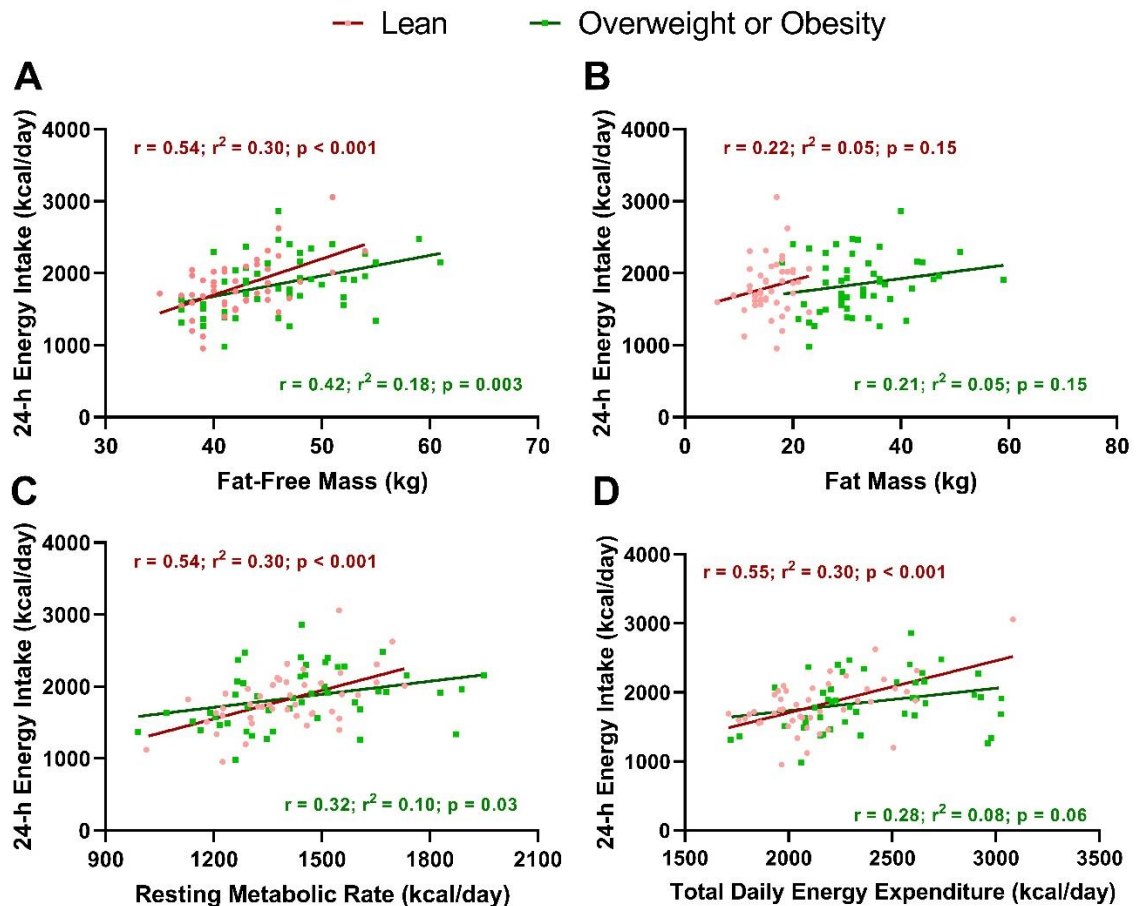


Figure 4.4 – Scatter plot showing the associations between mean 24-hour energy intake with A) fat-free mass, B) fat mass, C) resting metabolic rate and D) total daily energy expenditure in individuals that are lean and have overweight or obesity.

Separate multiple regressions were conducted to examine the predictors of mean 24-hour EI in participants that are lean and have overweight or obesity (Table 4.8). Fat-free mass was a predictor of 24-hour EI in model 1 in both groups, but not in models 2 and 3. Additionally, RMR was a predictor of mean 24-hour EI in model 2 in the group of participants that were lean, but not with overweight or obesity. Lastly, in model 3, there was a trend for TDEE to be a predictor of mean 24-hour EI in the group of lean participants ($p = 0.06$), but not in the ones with overweight or obesity. These differences suggest that body fatness may influence the coupling between EE and EI. These multiple regressions were replicated using FMI and FFMI and can be found in Table S4 (Appendix).

Table 4.8 – Regression coefficients showing the effect of fat mass and fat-free mass (Model 1), fat mass, fat-free mass and resting metabolic rate (Model 2), and fat mass, fat-free mass and total daily energy expenditure (Model 3) on mean 24-hour energy intake by BMI group.

Lean (n = 45)					Overweight or Obesity (n = 47)				
Model 1									
F(2, 42) = 9.5, r ² = 0.31, p < 0.001					F(2, 45) = 5.0, r ² = 0.18, p = 0.01				
	B	SE	β	P-value		B	SE	β	P-value
Intercept	-502.4	531.8		0.35	Intercept	468.9	443.0		0.30
FM	13.6	13.6	0.1	0.32	FM	3.9	6.9	0.08	0.57
FFM	48.9	12.2	0.5	< 0.001	FFM	26.9	9.9	0.4	0.009
Model 2									
F(3, 41) = 8.4, r ² = 0.38, p < 0.001					F(3, 44) = 3.4, r ² = 0.19, p = 0.03				
	B	SE	β	P-value		B	SE	β	P-value
Intercept	-809.3	530.9		0.14	Intercept	452.1	446.8		0.32
FM	7.8	13.4	0.07	0.56	FM	5.6	7.4	0.1	0.45
FFM	32.5	14.0	0.4	0.03	FFM	35.9	17.3	0.5	0.04
RMR	0.8	0.4	0.3	0.04	RMR	-0.3	0.5	-0.2	0.53
Model 3									
F(3, 41) = 8.1, r ² = 0.37, p < 0.001					F(3, 43) = 2.8, r ² = 0.16, p = 0.05				
	B	SE	β	P-value		B	SE	β	P-value
Intercept	-544.3	515.3		0.30	Intercept	583.1	465.3		0.22
FM	2.3	14.4	0.02	0.88	FM	4.0	7.6	0.08	0.61
FFM	31.2	14.9	0.3	0.04	FFM	27.8	13.5	0.4	0.05
TDEE	0.5	0.2	0.3	0.06	TDEE	-0.06	0.3	-0.05	0.81

B, unstandardized beta coefficient; SE, standard error; β, standardised beta coefficient; FM, fat mass; FFM, fat-free mass; RMR, resting metabolic rate; TDEE, total daily energy expenditure.

Associations between body composition and EE with test meal EI can be seen in Table 4.9. With the exception of the negative association between FM and FMI with EI in the group of participants that were lean (but not in the group with overweight or obesity), no other associations between body composition and EE with test meal EI existed in either BMI group (all $p \geq 0.11$).

Table 4.9 – Associations between fat mass, fat-free mass, resting metabolic rate and total energy expenditure with test meal energy intake by BMI category.

	Lean (n=44)			Overweight or Obesity (n=47)		
	r	r ²	P-value	r	r ²	P-value
FM	-0.43	0.18	0.004	0.23	0.06	0.11
FMI	-0.42	0.18	0.005	0.26	0.07	0.07
FFM	-0.02	0.0002	0.92	0.16	0.02	0.32
FFMI	0.17	0.03	0.28	0.20	0.04	0.17
RMR	-0.17	0.03	0.28	0.01	0.0002	0.93
TDEE	-0.14	0.02	0.38	0.04	0.002	0.77

FM, fat mass; FMI, fat mass index; FFM, fat-free mass; FFMI, fat-free mass index; RMR, resting metabolic rate; TDEE, total daily energy expenditure.

Scatter plots of the association between FM and test meal EI by BMI category can be observed in Figure 4.5 and Figure 4.6. A negative association between FM and test meal EI was observed in leaner participants ($r = -0.43$; $p = 0.004$), but not in those with overweight or obesity ($r = 0.23$; $p = 0.11$), and these r values were significantly different ($Z = -3.24$; $p = 0.001$). No other associations between body composition and EE with test meal EI existed in either group (all $p \geq 0.11$).

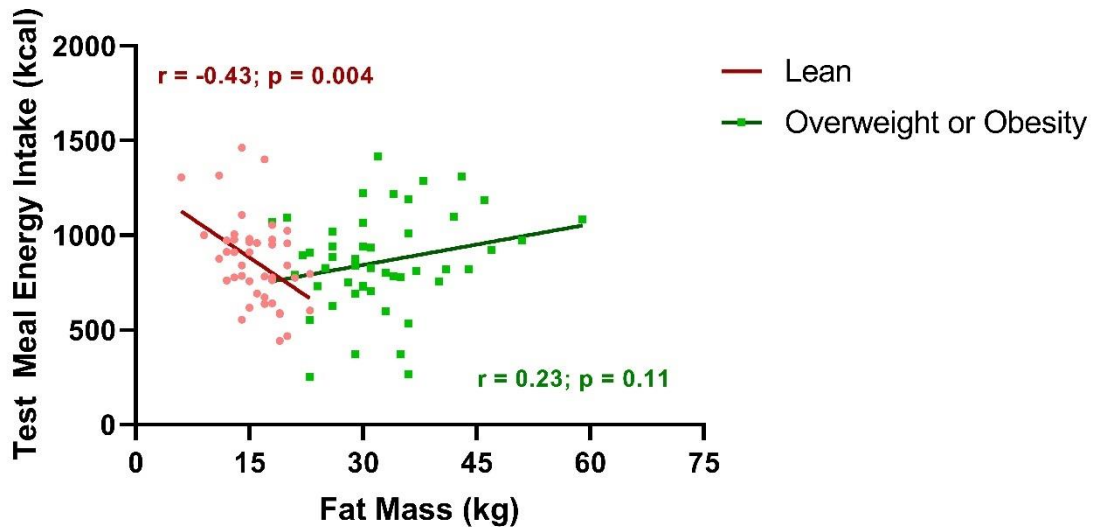


Figure 4.5 – Association between fat mass and test meal energy intake from the test meal in participants that are lean and have overweight or obesity.

After observing that the association between FM and test meal EI differed according to BMI status (Figure 4.5), those with overweight or obesity were also analysed separately (BMI = 25.0-29.9 kg/m² and BMI = 30.0-34.9 kg/m²). Descriptive characteristics of the three groups can be found in Table S1 (Appendix). As shown in Figure 4.6 there were no associations between FM and test meal EI in the group that had overweight, but a trend for a positive association emerged in those with obesity ($r = 0.41$, $p = 0.12$). Similar findings were observed with FMI (Appendix, Figure S2).

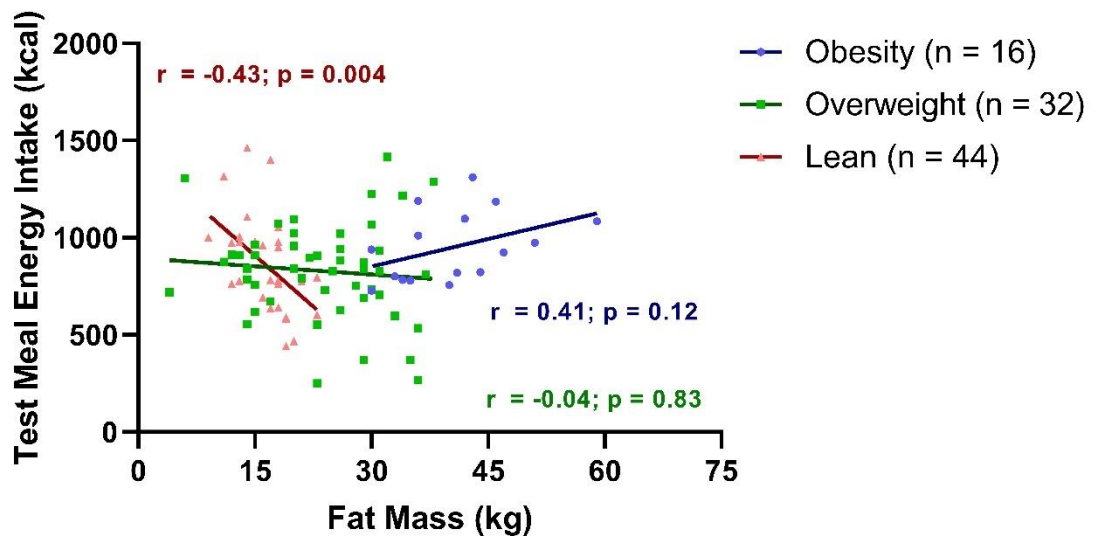


Figure 4.6 – Association between fat mass and test meal energy intake in participants that are lean, have overweight or obesity (3 groups).

As the direction of the association between FM and test meal EI changed with increased body fatness, a non-linear (quadratic) regression was tested. While FM and FFM failed to explain the variance in test meal EI [$F(2,89) = 0.29$, $r^2 = 0.006$, $p = 0.75$], the addition of the quadratic term FM^2 ($\beta = 0.46$; $p < 0.001$) explained an additional 9% of the variance [$F(3, 88) = 3.0$, $r^2 = 0.092$, $p = 0.04$; Figure 4.7]. Disinhibition ($r = 0.42$; $p < 0.001$), total physical activity ($r = -0.55$; $p < 0.001$), sedentary time ($r = 0.56$; $p < 0.001$) and moderate-to-vigorous physical activity ($r = -0.49$; $p < 0.001$) were correlated to FM and therefore were added as covariates in the multiple regression. Their addition did not influence the β or statistical significance of the quadratic term and were not found to predict test meal EI (all $p \geq 0.12$). This non-linear association was similar when FMI or body fat percentage were used (Appendix, Table S5).

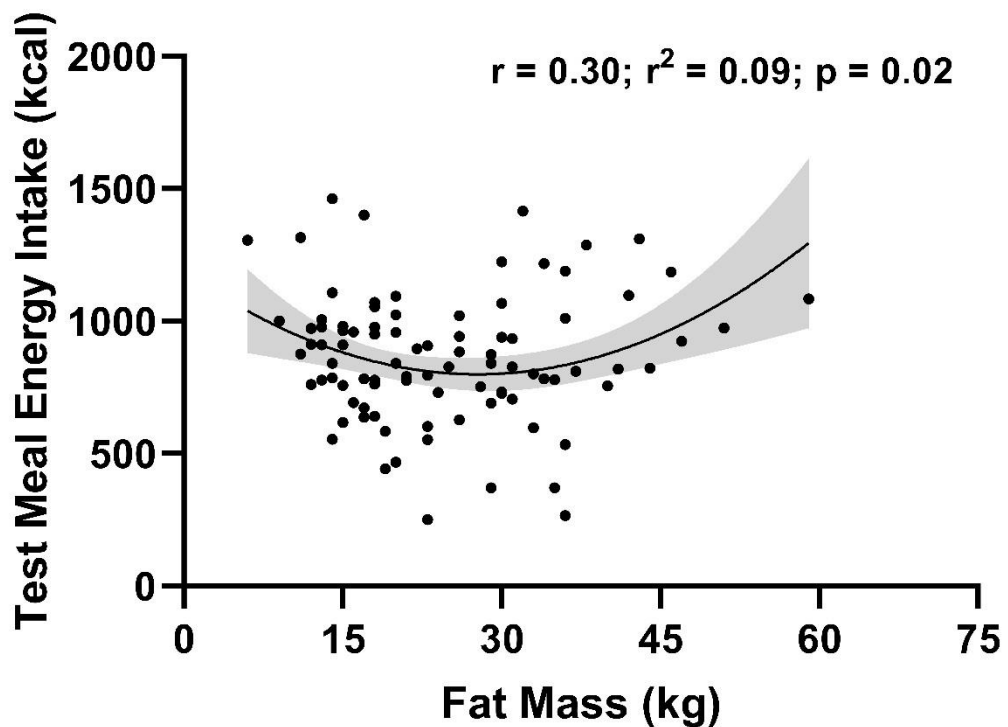


Figure 4.7 – Non-linear (quadratic) association between fat mass and test meal energy intake.

Associations between body composition and EE with fasting and AUC appetite composite scores can be seen in Table 4.10. As can be observed, only in lean participants FMI and RMR were negatively and positively associated with fasted appetite sensations, respectively. No other associations were observed in both groups.

Table 4.10 – Associations between body composition, resting metabolic rate and total energy expenditure with fasting and AUC appetite composite scores in participants that are lean (n = 45) and with overweight or obesity (n = 48).

Fasting Appetite						
	Lean			Overweight or Obesity		
	r	r²	P-value	r	r²	P-value
FM	-0.22	0.05	0.14	-0.17	0.03	0.24
FMI	-0.31	0.10	0.04	-0.22	0.05	0.14
FFM	0.26	0.07	0.08	-0.13	0.02	0.36
FFMI	0.11	0.01	0.49	-0.25	0.06	0.08
RMR	0.34	0.12	0.03	-0.12	0.01	0.43
TDEE	0.22	0.05	0.15	-0.14	0.02	0.36

AUC Appetite						
	Lean			Overweight or Obesity		
	r	r²	P-value	r	r²	P-value
FM	-0.17	0.03	0.24	0.14	0.02	0.3
FMI	-0.22	0.05	0.14	0.13	0.02	0.37
FFM	0.18	0.03	0.24	0.001	< 0.001	0.99
FFMI	0.13	0.02	0.39	-0.12	0.01	0.42
RMR	0.17	0.033	0.27	-0.007	< 0.001	0.96
TDEE	0.04	0.002	0.81	0.14	0.02	0.36

FM, fat mass; FMI, fat mass index; FFM, fat-free mass; FFMI, fat-free mass index; RMR, resting metabolic rate; TDEE, total daily energy expenditure.

Section Summary:

- Associations between FFM, RMR and TDEE and mean 24-hour EI were stronger in leaner participants than those with overweight and obesity.
- Fat mass was negatively associated fasting appetite sensations while RMR was positively associated with fasting appetite sensations in the participants that were lean, but not in those with overweight or obesity.
- In the whole sample, a non-linear (quadratic) association between FM and test meal EI was observed.
- A negative association between FM and test meal EI was observed in the participants that were lean, but not in those with overweight or obesity.

4.3.4 Are the Associations Between Energy Expenditure with 24-hour Energy Intake Moderated by Body Fat Percentage?

An interaction was found in which body fat percentage ($\beta = -1.88$; $p = 0.02$) moderated the association between RMR and mean 24-hour EI [$F(3, 89) = 8.1$, $r^2 = 0.21$, $p < 0.001$]. Body fat percentage was also found to moderate ($\beta = -1.91$; $p = 0.03$) the association between TDEE and mean 24-hour EI [$F(3, 88) = 6.8$, $r^2 = 0.19$, $p < 0.001$]. The multiple linear regression models can be found in Table 4.11. Participants with a lower body fat percentage (-1SD) presented stronger associations between RMR and TDEE with mean 24-hour EI and these weakened at higher levels of body fatness. These multiple regression models were replicated using BMI, FMI and FM and can be found in Table S6 (Appendix).

Table 4.11 – Regression coefficients for the moderation effect of body fat percentage on the association between resting metabolic rate (Model 1) and total daily energy expenditure (Model 2) with mean 24-hour energy intake (n = 93).

Model 1				
	B	SE	β	P-value
Intercept	-1941.1	1132.1		0.090
RMR	2.6	0.8	1.3	0.001
BFP	71.5	30.5	1.6	0.021
RMR x BFP	-0.05	0.02	-1.9	0.021
Model 2				
	B	SE	β	P-value
Intercept	-1308.9	1012.3		0.199
TDEE	1.5	0.5	1.3	0.002
BFP	57.5	28.6	1.3	0.047
TDEE x BFP	-0.03	0.01	-1.9	0.028

RMR, resting metabolic rate; BFP, body fat percentage; RMR x BFP, interaction between resting metabolic rate and body fat percentage; TDEE, total daily energy expenditure; TDEE x BFP, interaction between total daily energy expenditure and body fat percentage; B, unstandardized coefficient; SE, standard error; β, standardised coefficient.

A visual representation of the moderation effect of body fat percentage on the association between RMR and TDEE with mean 24-hour EI can be seen in Figure 4.8. In this figure, -1SD represents 1 standard deviation below the mean of body fat percentage (i.e., leaner participants) while +1SD represents 1 standard deviation above (i.e., participants with a higher body fatness). As can be observed, there is a decrease in the strength of the association (slope angle decreases) between EE and EI as body fatness increases.

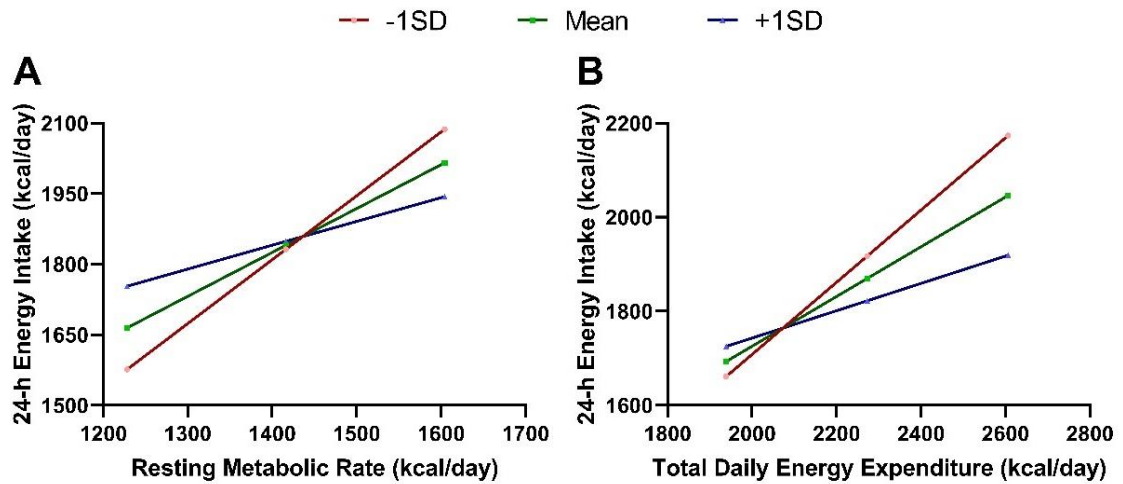


Figure 4.8 – Visual representation of the moderation effect of body fat percentage on the association between A) resting metabolic rate and B) total daily energy expenditure with mean 24-hour energy intake.

Disinhibition ($r = 0.41$; $p < 0.001$), total physical activity ($r = -0.57$; $p < 0.001$), sedentary time ($r = 0.59$; $p < 0.001$) and moderate-to-vigorous physical activity ($r = -0.49$; $p < 0.001$) were correlated with body fat percentage and were therefore added as covariates in the moderation analyses. This moderating effect remained significant after their inclusion and none of them predicted mean 24-hour EI (all $p \geq 0.08$). The moderation effect remained unchanged when BMI, FM and FMI were used as moderators.

Section Summary:

- The associations between RMR and TDEE with 24-hour EI were moderated by body fat percentage.
- This moderation effect of body fatness was not influenced by physical activity or eating behaviour traits.

4.4 Discussion

This chapter investigated if body fat percentage moderated the associations between EE and EI, and how the strength and direction of the association between FM and EI differed across a range of body fatness. Fat-free mass, RMR and TDEE were positively associated with mean 24-hour EI. Notably, body fat percentage moderated the associations between RMR and TDEE with mean 24-hour EI, in which participants with a lower body fat percentage presented stronger positive associations. A non-linear association between FM and objectively measured test meal EI was also demonstrated, with a negative association observed between FM and EI in participants that were lean but not in individuals with overweight/obesity. These data suggest that the associations between components of body composition and EE with EI may be weaker in those with higher body fatness.

4.4.1 Associations Between Fat-free Mass, Energy Expenditure and Energy Intake

Previous studies have reported positive associations between FFM, RMR and EI under conditions of approximate energy balance (Blundell et al., 2012, Weise et al., 2014, Blundell et al., 2015a, Cameron et al., 2016, Hopkins et al., 2016). These data suggest that energy requirements dictated by lean tissues are strong determinants of day-to-day EI. Those findings were replicated in the present chapter. For instance, it was observed that both FFM ($r = 0.45$) and RMR ($r = 0.41$) were positively associated with mean 24-hour EI. However, no associations were observed between FFM and RMR with test meal EI. It was also demonstrated that TDEE was positively associated with 24-hour EI ($r = 0.39$), which is in agreement with previous findings from Piaggi et al. that observed that 24-hour EE was an independent predictor of *ad libitum* EI during 3 days (Piaggi et al., 2015), and Basolo et al. that reported a positive association between 24-hour EI and *ad libitum* EI during 3 days (Basolo et al., 2018).

Importantly, data from the present chapter extend previous findings and suggest that the associations between body composition, EE and EI are moderated by body fatness. The strength of the associations between body composition, EE and EI was found to differ between lean and individuals with overweight or obesity. For instance, stronger associations between FFM, RMR, TDEE and 24-

hour EI were observed in leaner individuals. These findings were confirmed by a formal moderation analysis which, for the first time, demonstrated quantitatively that body fat percentage (and BMI, FM and FMI) moderated the association between RMR, TDEE and 24-hour EI. These data suggest that the associations between RMR, TDEE and EI may be weaker at higher levels of body fatness.

Notably, this moderating effect remained after accounting for potential covariates known to influence appetite control such as physical activity and eating behaviour traits. For instance, only disinhibition presented a positive correlation with body fat percentage. However, it did not predict mean 24-hour EI in the model when included as a covariate, suggesting that the observed moderation effect in these data was independent of eating behaviours traits. Furthermore, habitual levels of physical activity have also been shown to influence the coupling between EE and EI (Beaulieu et al., 2016). However, although minutes of sedentary time, total and moderate-to-vigorous physical activity were correlated with body fat percentage, they did not predict mean 24-hour EI in the model when included as covariates, suggesting that the moderation effect was also independent of physical activity levels. It is worth noting though that participants were excluded if they exercised more than 3 days per week. The absence of an effect of physical activity on appetite could therefore be attributed to the limited range of physical activity levels included in the present data.

The moderating effect of body fatness appears to weaken or impair the coupling between EE and EI, and may help explain energy balance dysregulation in individuals with a higher body fat percentage. These data are in line with previous research. For instance, Cugini et al. observed that while a positive association was observed between FFM and daily hunger in healthy weight individuals (Cugini et al., 1998), no associations were observed in participants with obesity (Cugini et al., 1999). Furthermore, Grannell et al. and Piaggi et al. observed that although FFM and 'awake and fed thermogenesis' were positively associated with EI, the strength of these associations weakened with increased body fatness (Piaggi et al., 2015, Grannell et al., 2019a). In the present study, an interaction between FFM and body fat percentage was also observed [$F(3, 89) = 9.7$, $r^2 = 0.25$, $p < 0.001$; interaction term – $\beta = -1.85$; $p = 0.03$].

It has previously been suggested that under conditions of approximate energy balance the associations between FFM, RMR and EI reflect a 'mass-dependent'

tonic drive to eat arising from the energy turnover of metabolically active tissues (Hopkins et al., 2019, Hopkins et al., 2018, Hopkins et al., 2016). In line with the present data, a weaker association between RMR and EI at higher body fatness might be expected given the disproportionate changes seen in FFM and FM with weight gain. As body weight increases, FM is disproportionately increased relative to FFM (Hall, 2007), thus making the relationship between RMR and body weight curvilinear (Heymsfield et al., 2019). This may lead to a non-linear relationship between RMR and EI in which RMR contributes less to the drive to eat at higher body fatness. This could in part explain the lack of significant differences in RMR between BMI groups despite the higher body weight in those with overweight/obesity. Of note, body composition was assessed in the present study using a 2-compartment model, which does not allow examination of specific components of FFM. As these tissues present different metabolic rates (Elia, 1992, Muller et al., 2013, Wang et al., 2012), future studies should examine how differences in the composition of FFM at higher levels of body fatness influence EI.

4.4.2 Associations Between Fat-Mass and Energy Intake

Previous research has demonstrated equivocal findings regarding the influence of FM on EI. It has been postulated that FM exerts a tonic inhibitory influence over appetite and EI, possibly through leptin's anorexigenic effect ('lipostatic model') (Kennedy, 1957). Indeed, negative associations have been observed between FM and EI in leaner individuals (Blundell et al., 2015a). In contrast, studies including individuals with overweight or obesity often report no associations between FM and EI (Blundell et al., 2012, Cameron et al., 2016) suggesting that this postulated inhibitory effect may be absent at higher levels of body fatness. In the current study, no associations were observed between FM and mean 24-hour EI although a weak positive association could be detected, possibly mediated by RMR due to the contribution of FM to energy requirements (Hopkins et al., 2019, Hopkins et al., 2018). Interestingly, while no associations were observed between FM and *ad libitum* test meal EI in the whole sample, these results changed when participants were separated by BMI categories. A negative association ($r = -0.42$) between FM and test meal EI emerged in leaner participants while no association was observed in the group with overweight or

obesity. These findings corroborate previous work (Cugini et al., 1999, Cugini et al., 1998, Blundell et al., 2012, Blundell et al., 2015a). Furthermore, after splitting the group with higher BMI in overweight (BMI = 25.0-29.9 kg/m²) and obesity (BMI = 30.0-34.9 kg/m²), a positive association between FM and test meal EI emerged in those with obesity. While it is acknowledged that the number of participants in the group with obesity was small (and likely underpowered to detect a significant association), regression analysis in the whole sample using the quadratic term FM² indicated that the association between FM and test meal EI was non-linear. These findings suggest that the associations between FM and EI may change from negative to positive at higher levels of body fatness. This is of importance since the weakening of the postulated inhibitory influence of FM on EI could lead to food overconsumption and weight gain.

It should also be noted that any effects of FM on EI may not be solely biologically mediated, with FM known to be associated with psychometric eating behaviours such as dietary restraint (Westenhoefer et al., 1999, Kruger et al., 2016). Indeed, cognitive restraint has been found to partially mediate the association between FM and EI (Hopkins et al., 2018). Minutes of moderate-to-vigorous physical activity have also previously been shown to influence the associations between FM and meal size (Beaulieu et al., 2018). However, when eating behaviour traits (cognitive restraint and disinhibition) and physical activity were added in the regression model to examine the non-linear association between FM and EI, they did not predict test meal EI nor affected the non-linear (quadratic) relationship. This suggests that this non-linear relationship between FM and test meal EI observed in the current study was independent of eating behaviour traits and physical activity. However, as previously mentioned, the absence of an influence from physical activity could be in part attributed to the study's inclusion criteria (\leq 3 days exercise per week).

Although appetite-related peptides were not measured in this study, these are potential mechanisms that could explain the weaker associations between body composition and EI with appetite and EI in individuals with overweight or obesity compared to participants who were lean. Adipose tissue was thought to be exclusively an energy store in which fatty acids would be metabolised according to energy needs. However, more recent views of adipose tissue's metabolism indicate that it can also serve as an endocrine organ (Trayhurn and Beattie, 2001)

that secretes hormones such as leptin, adiponectin and resistin that influence energy balance (Koleva et al., 2013). Previous theories of appetite control have focused on the inhibitory effects of FM and leptin on EI (i.e., 'adipocentric theory') (Kennedy, 1953). Theoretically, increases in FM would lead to concomitant increases of the anorexigenic hormone leptin, and in turn, lead to a reduction in EI. The absence of a negative association between FM and EI at higher levels of body fatness is in line with the notion of leptin and insulin resistance (Zhou and Rui, 2013, Koleva et al., 2013), which may alter central and peripheral sensitivity to appetite-related feedback signals (Schwartz et al., 2000, Badman and Flier, 2005, Lean and Malkova, 2016). Altered fasting and postprandial appetite-related peptide secretion has also been found in individuals with obesity compared to those who are lean (Lean and Malkova, 2016). For instance, a lower secretion of anorexigenic and suppression of orexigenic peptides has been observed in individuals with obesity compared to lean controls (Lean and Malkova, 2016). These changes in peptide concentrations in a fasted state and in response to a meal could lead to a lower strength of satiety in individuals with overweight or obesity, leading to energy overconsumption and therefore weight gain. Additionally, it has been observed that insulin resistance is present in the brain at higher levels of body fatness, namely in areas influencing appetite, possibly affecting the coupling between EE and EI in individuals with obesity (Anthony et al., 2006).

Differences in the association between FM and EI in lean individuals and those with overweight or obesity may also relate to changes in the proportional contribution of FM to RMR with weight gain. While the contribution of FM to RMR is smaller than of FFM (Johnstone et al., 2005), it would be expected that its contribution to RMR will become proportionally larger as FM increases with weight gain. Therefore, it could be hypothesised that differences in the strength (i.e., weaker) and direction (i.e., more positive) of the association between FM and EI at higher body fatness may reflect the increased contribution of FM to total body weight and RMR, alongside a blunting of its inhibitory influence on EI. However, the cross-sectional nature of the present and previous studies means that this hypothesis needs to be examined in studies where FM, FFM and EE are systemically manipulated. Indeed, the associations between components of body composition and EE may differ during WL (Dulloo et al., 2017, Stubbs et al., 2018, Turicchi et al., 2020). Further research is also needed to rule out potential

confounders of FM and EI relationships along the spectrum of BMI, such as body image concerns and self-regulation of EI which co-vary with adiposity and may be more evident in lean subjects. Physical activity or cognitive restraint did not influence the reported associations in the present study, but given the equivocal findings in studies examining the associations between FM and EI, these results need to be replicated and the potential physiological, behavioural and psychological mechanisms underlying any differences between lean individuals and those with overweight/obesity examined.

4.4.3 Associations Between Body Composition, Energy Expenditure and Appetite Sensations

The influence of body composition and EE on appetite sensations has also been previously examined. In the current study, no associations were observed between body composition, EE and appetite sensations in the whole sample. When dividing the groups according to BMI, FM was negatively associated while RMR was positively associated with fasting appetite composite scores in lean, but not in participants with overweight or obesity.

Considering the data regarding the influence of body composition and EE on appetite, the results of the current study are in agreement with previous findings by Caudwell et al. as RMR was positively associated with fasting appetite composite scores. Furthermore, these findings are also similar to the observations by Cugini et al. (1998 and 1999) regarding the negative association between FM and appetite sensations in the lean but not in participants with overweight or obesity. However, contrary to what Cugini et al. observed, no associations between FFM and hunger were present in the whole sample or by BMI groups.

4.4.4 Potential Implications

The present observation of an uncoupling between EE and EI, and the absence of the postulated inhibitory influence of FM on EI at higher levels of body fatness, is of importance to our understanding of energy balance dysregulation. Weight gain may lead to a greater drive to eat created by the increased body size.

Alongside the absence of the postulated inhibitory influence of FM on EI at higher levels of body fatness, this may facilitate energy overconsumption in an obesogenic environment and lead to further weight gain. Therefore, understanding the mechanisms behind this energy balance dysregulation at higher levels of body fatness remains a priority as it would allow for the development of strategies (e.g., pharmacological or lifestyle) to improve obesity management success rates.

4.4.5 Limitations

Total daily energy expenditure was assessed using accelerometry rather than doubly labelled water, but the device used here (SenseWear Armband) has been shown to provide valid estimates of TDEE (O'Driscoll et al., 2020) and minimum wearable time was set at 22 hours per day to improve estimates. Free-living 24-hour EI was assessed using a self-report tool, potentially leading to misreporting. This is an important consideration since increased levels of underreporting have been observed in individuals with overweight and obesity (Heitmann and Lissner, 1995, Ravelli and Schoeller, 2020), but in this study the 24-hour EI : RMR ratio and the difference between self-reported and calculated EI was similar between BMI categories, suggesting that the extent of misreporting did not differ between groups. The dietary tool used, myfood24, has been validated against urinary biomarkers (Wark et al., 2018), but the issue of energy and nutrient intake misreporting using self-report tools is well documented (Lichtman et al., 1992, Heitmann and Lissner, 1995, Lissner et al., 1989). The association between FFM and EI has previously been shown to be weaker with self-reported data in comparison to objectively measured EI, but the underlying association remained evident (Vainik et al., 2016). The strength of the association between FFM or RMR and EI in the present data ($r = 0.45$ and $r = 0.41$, respectively) were similar to the values observed in studies using laboratory-based measurements [$r = 0.22$ – 0.69 (Caudwell et al., 2013, Blundell et al., 2015a, McNeil et al., 2015)]. Of note, although these analyses may oversimplify the underlying biological mechanisms influencing energy balance, our data demonstrate that the inter-relationships between body composition, EE and EI are likely to be non-linear. These data are also cross-sectional in nature, and future studies should assess

how the strength and direction of the associations between components of body composition, EE and daily EI change with controlled WL or gain.

4.5 Conclusion

In conclusion, the findings from this study demonstrate that body fat percentage moderates the associations between RMR and TDEE with daily EI. Furthermore, a negative association between FM and test meal EI was observed in lean participants but not in those with overweight or obesity. These data suggest that the influence of body composition and EE on EI may be weaker in those with higher levels of body fatness. Therefore, higher levels of body fatness may be associated with energy balance dysregulation and a weaker coupling between EE and EI.

Chapter Summary:

- Fat-free mass, RMR and TDEE were positively associated with mean 24-hour EI, and these associations were stronger in leaner individuals.
- Body fat percentage and BMI moderated the association between EE and EI, suggesting that higher levels of body fatness may lead to a weaker coupling between EE and EI.
- A non-linear (quadratic) association was observed between FM and test meal EI, suggesting that the postulated inhibitory role of FM on EI may be absent with higher levels of body fatness.
- These data suggest greater FM may be associated with energy balance dysregulation and a weaker coupling between EE and EI.

Chapter 5 – Associations Between Body Composition at the Tissue-Organ Level, Energy Intake and Appetite Sensations

Chapter Aim:

- Examine the associations between body composition at the tissue-organ level with energy intake and appetite sensations.

5.1 Introduction

There is renewed interest in the influence of body composition on appetite and EI. In line with the findings from Chapter 4, evidence from independent laboratories have consistently reported positive associations between FFM and EI or appetite sensations (Lissner et al., 1989, Cugini et al., 1998, Blundell et al., 2012, Blundell et al., 2015a, Cameron et al., 2016). These associations between FFM and EI have been shown to be mediated by RMR, which has been interpreted to suggest that the energetic demand of metabolically active tissues exerts influence over appetite and EI (Hopkins et al., 2018, Hopkins et al., 2016).

Previous research investigating the associations between FFM and EI typically use 2-compartment models of body composition, which separate the body in FFM and FM. The heterogeneous nature of FFM is well recognised (Gallagher et al., 1998, Javed et al., 2010). While skeletal muscle represents ~40% of total body weight, it only accounts for ~20% of RMR (Elia, 1992, Gallagher et al., 1998). Furthermore, while high-metabolic rate organs (i.e., heart, kidneys, brain and liver) only account for ~5-7% of total body weight, their contribution to RMR reaches ~60% (Elia, 1992). Models including FM and FFM usually explain ~70% of the variance in RMR between subjects (Johnstone et al., 2005). Notably, the inclusion of body composition analyses at the tissue-organ level (i.e., high-metabolic rate organs, skeletal muscle, adipose tissue and residual mass) may increase the prediction capacity of such models to ~90% (Gallagher et al., 1998). This may occur in part due to the different scaling for each tissue with body size;

while skeletal muscle and the liver scale linearly with body size, the brain presents a lower exponential value and therefore, the energetic value per unit of total FFM (i.e., RMR/FFM ratio) decreases with increased body size (Heymsfield et al., 2019). In essence, the energy density of FFM decreases as body size increases, which in part explains why RMR scales non-linearly with body size (Heymsfield et al., 2019).

Given the aforementioned associations between FFM and EI, it could therefore be argued that analysing the associations between body composition and EI at the tissue-organ level may have a higher explanatory capacity for the variance in EI between subjects. However, there has been no attempt to date to incorporate the energetic demands of individual tissue-organs into models of appetite control. Until now, the only study partially assessing the relationships between FFM and EI at the tissue-organ level was an investigation conducted by Cameron et al. (2016) in which the strongest predictor of EI (3-day food record) in a group of 304 adolescents with obesity was skeletal muscle. However, skeletal muscle did not seem to be a stronger predictor than total FFM, possibly due to its smaller relative contribution to RMR (i.e., ~20%). Furthermore, as skeletal muscle was the only specific component of FFM assessed, how high-metabolic rate organs (i.e., brain, liver, kidneys and heart) influence EI and appetite sensations remains to be investigated. Increasing the ability to explain the variance in EI by including body composition analyses at the tissue-organ level could be an important precursor in understanding the fundamental biological mechanisms underpinning normal and dysregulated appetite and energy balance processes.

5.1.1 Objective & Hypotheses

The aim of this chapter was to examine the associations between body composition at the tissue-organ level with EI and appetite sensations. It was hypothesised that high-metabolic rate organs (i.e., brain, heart, liver and kidneys) would present a stronger association with EI and appetite sensations than FFM as a uniform component.

The analyses presented in this chapter were conducted using the data collected from two independent studies described below. Study 1 was a secondary analysis from a weight-cycling study conducted in Christian-Albrechts University (University of Kiel, Germany) in young men that were lean and had overweight.

This analysis served as a proof of concept to establish for the first time whether associations between body composition at the tissue-organ level and fasting appetite sensations existed. Study 2 was conducted at the University of Leeds in women that were lean and was designed to i) establish the MRI protocols and feasibility of MRI analyses at the University of Leeds, and ii) extend the findings from study 1 by including postprandial appetite sensations as well laboratory-based and free-living measurements of EI. However, due to the COVID-19 outbreak, research facilities were closed during data collection forcing this study to terminate early.

5.2 Study 1

5.2.1 Methods

Thirty-two healthy men (BMI = $23.5 \pm 2.0 \text{kg/m}^2$) were recruited for this study with the aim of assessing the physiological responses to energy balance perturbations during a 6-week subsequent overfeeding - caloric restriction - refeeding intervention (Müller et al., 2015). In the present secondary analysis, only baseline data from 24 participants that included measurements of organ mass and fasting hunger sensations were used to investigate the associations between body composition at the tissue-organ level (i.e., high-metabolic rate organs, skeletal muscle, adipose tissue and residual mass) and fasting hunger sensations.

Participants were non-smokers, weight stable ($\pm 2 \text{kg}$) during the preceding 12 months, did not use any medication, had no family history of diabetes, food allergies, contraindications for MRI, and were not athletes nor following a specific diet. Data was collected between February 2010 and September 2012 and the study was approved by the ethics committee of the Medical Faculty of the Christian-Albrechts University (Kiel) and conducted according to the principles of the Helsinki Declaration. All participants gave written consent after receiving oral and written information. This trial was registered at clinicaltrials.gov as NCT01737034.

5.2.1.1 Study Design

During 3 consecutive days in which participants remained in the institute (Christian-Albrechts University, Kiel, Germany), measurements of fasting hunger sensations (100-mm VAS), RMR (indirect calorimetry) and body composition (quantitative magnetic resonance) were collected. An average of the 3 measurements of fasting hunger, RMR and body composition was calculated. Data from the MRI was only collected once. All data were collected in close to energy balance conditions before the weight-cycling intervention started.

5.2.1.2 Procedures

Body Composition (Whole-Body)

Measurements of body composition (2-compartment model) were performed using quantitative magnetic resonance (ECHOMRI-AH; Echo Medical Systems). Fat mass was measured in kg, and FFM was calculated by subtracting FM from total body weight.

Body Composition (Tissue-Organ Level)

Magnetic Resonance Imaging Protocol

Body composition assessment at the tissue-organ level was conducted using whole-body MRI (Magnetom Avanto 1.5 T; Siemens Medical Systems). Magnetic resonance imaging is a safe and non-invasive technique that does not involve any ionizing radiation like x-rays and computerised tomography scans. Transversal images from the wrist to ankle were obtained using a continuous axial T1-weighted gradient-echo sequence (time to repeat: 157ms; time to echo: 4ms). Regarding the brain, the protocol comprised of continuous 4mm slices with 1mm inter-slice gaps (time to repeat: 313ms; time to echo: 14ms). All the other images were obtained with an 8mm slice thickness and 2mm inter-slice gap. Assessment of the thoracic / abdominal region was obtained using breath-hold, while heart mass was assessed using the breath-navigated and pulse-triggered T2-weighted half Fourier acquisition single-shot turbo spin-echo sequence (time to repeat: 700m; time to echo: 24mm). Lastly, liver fat was determined using the 2-point Dixon method with a volume interpolated breath-hold examination.

Organ Segmentation Protocol

The images collected using the MRI were manually segmented (Slice-O-Matic 4.3 software; TomoVision) by the same researcher (the intra-observer variance for repeated measurements was <2%). These analyses require a manual segmentation of each body composition component and, after an a priori calibration (e.g., defining that 1 pixel = 1mm), the software calculates the area per slice. Lastly, total organ volume was calculated from the sum of all areas multiplied by the slice thickness (and interslice gap if applicable). Due to having at least 150 images per participant (minimum of 1 slice per 1cm = ≥ 150 images for a 150cm individual), full body composition analyses for 1 subject may take up to 8-10 hours. Although the software is intuitive and provides tools that facilitate the segmentation (e.g., automatically detecting colours associated to each body component), it requires in-depth anatomical knowledge to be able to identify each tissue in each image.

Calculation of Organ Mass and Specific Metabolic Rate

Organ volume was converted to mass by multiplying the volume for its specific tissue density (Cameron, 1991):

- Liver = 1.06g/cm^3
- Heart = 1.06g/cm^3
- Kidneys = 1.05g/cm^3
- Brain = 1.036g/cm^3
- Skeletal Muscle = 1.04g/cm^3
- Adipose Tissue = 0.92g/cm^3

Tissue's metabolic rates (and contribution to RMR) were estimated using the specific RMR values (K_i) reported by Muller and Wang (Muller et al., 2013, Wang et al., 2012):

- Kidneys = $442\text{kcal}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$
- Heart = $442\text{kcal}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$
- Liver = $201\text{kcal}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$
- Brain = $241\text{kcal}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$
- Skeletal Muscle = $13\text{kcal}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$
- Adipose Tissue = $4.5\text{kcal}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$
- Residual = $12\text{kcal}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$

Resting Metabolic Rate

Resting metabolic rate was measured after an overnight fast using indirect calorimetry (Vmax Spectra 29n; SensorMedics; Viasys Healthcare, Biltoven, The Netherlands; software Vmax, version 12-1A; Cosmed Quark RMR, Cosmed srl, Rome, Italy). Measurements were collected for 30 minutes with participants lying down in a supine position, and the first 5-10 were discarded from the analyses. RMR was calculated using the 5-minute steady state method, described in Chapter 3.4.2.

Hunger Sensations

Hunger sensations were assessed using a 100-mm VAS (paper version) while participants were fasted. Each assessment was precisely measured with a ruler to the closest 1mm.

5.2.1.3 Statistical Analyses

A general description of the statistical approach used in this thesis can be found in Chapter 3.8. In this chapter, Spearman correlations were conducted to examine the associations between body composition (FM, FFM and specific components) and RMR with fasting hunger sensations. As multiple correlations were conducted, Bonferroni corrections were done by dividing 0.05 (p-value defined for statistical significance) by the number of tests performed (13). Therefore, statistical significance was defined as a $p < 0.004$. The contribution (%) of each tissue's mass to total body weight and specific metabolic rate to RMR was also calculated (e.g., heart's mass / total body weight x 100; heart's RMR / total RMR x 100). In these analyses, high-metabolic rate organs represent the sum of the brain, heart, kidneys and liver. Furthermore, residual mass was calculated as subtracting high-metabolic rate organs, skeletal muscle and adipose tissue from total body weight (Elia, 1992).

5.2.2 Results

5.2.2.1 Descriptive Characteristics

Descriptive characteristics, measurements of RMR, fasting hunger sensations and body composition (whole-body) can be observed in Table 5.1.

Table 5.1 – Descriptive characteristics, fasting hunger sensations, resting metabolic rate and body composition from the 24 participants (except liver fat n=22).

	Mean ± SD
Age (y)	25 ± 3
Height (cm)	182.0 ± 7.2
Body weight (kg)	77.8 ± 8.4
Body Mass Index (kg/m ²)	23.5 ± 2.1
Fasting Hunger (mm)	61 ± 16
Resting Metabolic Rate (kcal/day)	1891 ± 215
QMR Fat Mass (kg)	13.9 ± 5.2
QMR Body Fat (%)	17.8 ± 5.8
QMR Fat-free Mass (kg)	63.9 ± 7.8
Liver Fat (%)	6.5 ± 3.6

QMR, quantitative magnetic resonance.

Information regarding body composition at the tissue-organ level, as well the contribution of each component to total body weight and RMR can be seen in Table 5.2. While high-metabolic rate organs combined only accounted for ~5% of total body weight, their contribution to RMR was ~50%. Furthermore, while skeletal muscle represented ~39% of total body weight, it only contributed to ~21% of RMR.

Table 5.2 – Body composition at the tissue organ level and the contribution to total body weight and resting metabolic rate.

	Weight (kg)	% Body Weight	% RMR
High-Metabolic Rate Organs	3.7 ± 0.4	4.8 ± 0.4	49.7 ± 3.4
Liver	1.6 ± 0.3	2.1 ± 0.4	17.5 ± 2.6
Fat-Free Liver Mass	1.5 ± 0.2	1.9 ± 0.2	16.1 ± 2.0
Brain	1.6 ± 0.1	2.0 ± 0.2	20.3 ± 2.1
Kidneys	0.24 ± 0.04	0.3 ± 0.04	5.8 ± 0.7
Heart	0.26 ± 0.05	0.3 ± 0.04	6.2 ± 0.9
Skeletal Muscle	30.8 ± 3.2	39.0 ± 3.5	21.1 ± 1.6
Subcutaneous AT	13.2 ± 4.3	16.9 ± 5.6	3.2 ± 1.2
Visceral AT	1.1 ± 0.8	1.4 ± 1.0	0.3 ± 0.2
Residual	31.3 ± 8.5	38.3 ± 8.1	19.3 ± 4.6

AT, adipose tissue. High-metabolic rate organs, n = 23; skeletal muscle, n = 16; subcutaneous and visceral fat, n = 20; residual, n = 14.

5.2.2.2 Associations Between Resting Metabolic Rate and Body Composition with Fasting Hunger Sensations

Associations between RMR and specific components of body composition with fasting hunger sensations can be observed in Table 5.3. High-metabolic rate organs combined ($r_s = 0.56$; $p = 0.005$), and the liver ($r_s = 0.57$, $p = 0.005$; fat-free liver mass, $r_s = 0.50$, $p = 0.02$), were positively associated with fasting hunger sensations although they did not reach statistical significance. Although not statistically significant, RMR ($r_s = 0.44$; $p = 0.04$) and FFM ($r_s = 0.44$; $p = 0.03$) were also positively associated with fasting hunger sensations, but presented a smaller correlation coefficient than high-metabolic rate organs combined. A visual representation of these associations can be seen in Figure 5.1. No other associations were observed between components of body composition and fasting hunger sensations.

Table 5.3 – Correlations between resting metabolic rate and body composition components with fasting hunger sensations.

	Fasting Hunger Sensations (mm)	
RMR (kcal/day)	r_s	0.44
	p	0.04
QMR FM (kg)	r_s	0.05
	p	0.82
QMR FFM (kg)	r_s	0.44
	p	0.03
HMRO (kg)	r_s	0.56
	p	0.005
Brain (kg)	r_s	0.03
	p	0.88
Kidneys (kg)	r_s	0.29
	p	0.18
Heart (kg)	r_s	0.18
	p	0.42
Liver (kg)	r_s	0.57
	p	0.005
Fat-free liver mass (kg)	r_s	0.50
	p	0.02
Skeletal Muscle (kg)	r_s	0.18
	p	0.51
Subcutaneous AT (kg)	r_s	-0.10
	p	0.69
Visceral AT (kg)	r_s	-0.25
	p	0.29
Residual (kg)	r_s	0.52
	p	0.06

RMR, resting metabolic rate; QMR, quantitative magnetic resonance; FM, fat mass; FFM, fat-free mass; HMRO, high-metabolic rate organs; AT, adipose tissue.

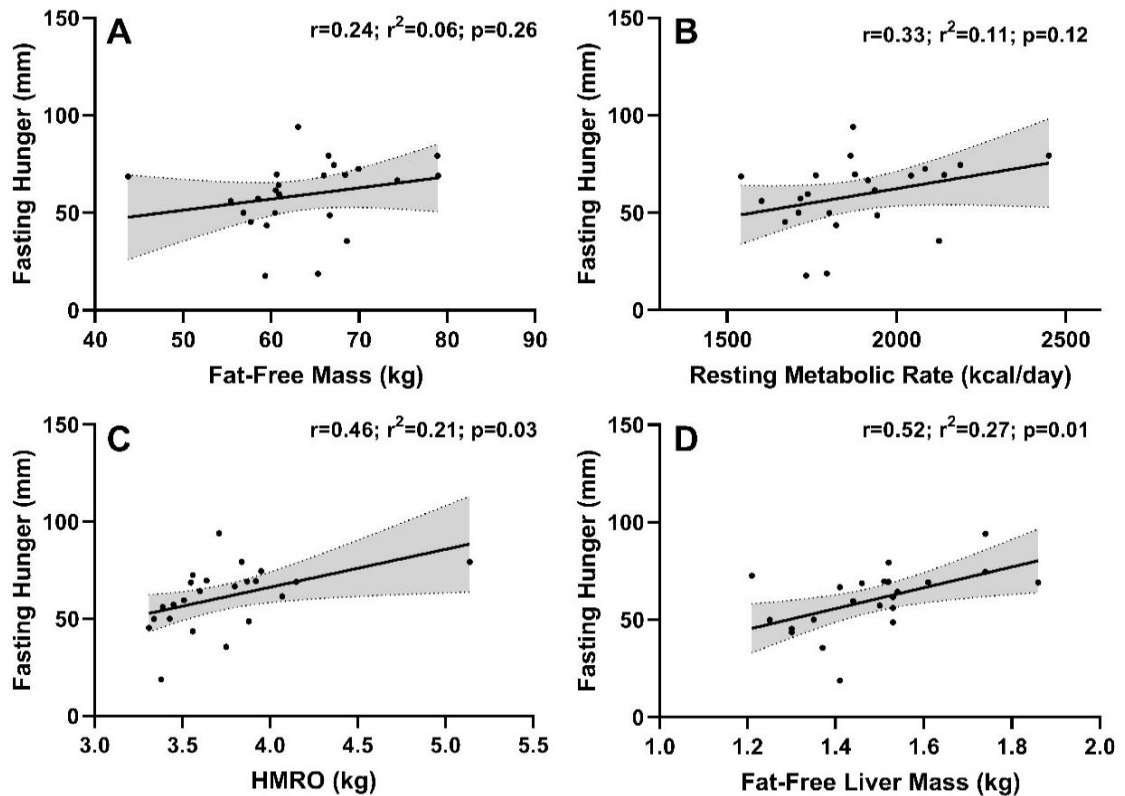


Figure 5.1 – Scatter plots illustrating the associations between fasting hunger and A) fat-free mass; B) resting metabolic rate; C) high-metabolic rate organs (HMRO); and D) liver. Grey bands represent the 95% confidence intervals. Excluding the participant in the far right of panels C and D (potential outliers) did not influence the results.

Section Summary:

- Only high-metabolic rate organs combined were positively associated with fasting hunger sensations.
- Although RMR and FFM were also positively associated with fasting hunger sensations, these were not statistically significant, and the correlation coefficients were smaller than when using high-metabolic rate organs.

5.3 Study 2

5.3.1 Methods

A sub-sample of participants ($n = 6$) enrolled in a larger study examining the influence of FFM and RMR as drivers of appetite and EI in women that were lean or had overweight/obesity ($n = 93$; described in Chapter 4) were recruited. Based on the power calculations shown in section 5.3.1.3, 15 participants were due to be recruited but the study had to be terminated early due to COVID-19. The main aim of this study was to examine the cross-sectional associations between body composition at the tissue-organ level with EI (free-living and objectively measured in the laboratory) and appetite sensations. Healthy women aged 18-55 years with a BMI between 18.5-25.0kg/m² were recruited. Participants were excluded if they had contraindications to MRI [e.g., metallic implants and claustrophobia (Dill, 2008)]. The remaining inclusion and exclusion criteria are described in Chapter 3.2. All participants gave written consent after receiving oral and written information. This study was approved by the University of Leeds MAPS Ethics Committee (MEEC 18-021, 06/03/2019).

5.3.1.1 Study Design

Free-living Measures Procedure

During a free-living measures week, participants completed a 24-hour online food diary (myfood24.org) at the end of each day for a period of 7 days. To get an estimate of 24-hour EI, an average of the 7 days was calculated. A complete description of the assessment of free-living 24-hour EI using myfood24 can be found in chapter 3.3.1.2.

Laboratory Measures Procedure

Upon completion of the free-living measures week, body composition (air-displacement plethysmography and MRI) was assessed after a 12-hour overnight fast. On the next day, RMR (indirect calorimetry; Cosmed Quark RMR, described in Chapter 3) was assessed and was followed by 3 *ad libitum* meals every 4 hours and a snack box to take home (objectively measured 24-hour EI). Appetite sensations were assessed using a 100-mm VAS in the fasting state, before and after each meal and at 60-minute intervals. A description of the assessment of

body composition (air-displacement plethysmography), RMR (indirect calorimetry) and appetite sensations (VAS) can be found in Chapter 3.

5.3.1.2 Procedures

Body Composition at the Tissue-Organ Level and Specific Metabolic Rate Magnetic Resonance Imaging Protocol

Body composition at the tissue-organ level was assessed using non-contrast whole-body MRI (Siemens Prisma 3T MRI scanner). As it was the first time conducting body composition analyses at the tissue-organ level at the University of Leeds, the protocol was mimicked from the one presented in study 1. Participants were asked to remain in a supine position with minimal clothing for the duration of the measurement (1 hour) while following the technicians' instructions for each measurement. Liver fat was not assessed in this study as the protocol would require a substantial extra time investment for the MRI measurement. However, this should not be seen as a limitation as participants were lean and healthy as in study 1 (meaning that the amount of liver fat would be small) and the adjustment of liver mass for its fat content did not influence the results of that study (i.e., associations between total liver mass or fat-free liver mass with fasting hunger in study 1 were similar). Due to the heart's contractions, a specialist collaborator was supposed to measure its volume, but due to COVID-19 outbreak, it was not possible to measure its volume and thus calculate its mass. Therefore, the heart was considered as a component of residual mass.

Organ Segmentation Protocol

The protocol to analyse the images collected using MRI was the same as for study 1. Organ volumes were measured via whole-body manual image segmentation (Slice-O-Matic 4.3 software; TomoVision) conducted by the same researcher (the PhD candidate). The intra-observer variance of repeated measurements (coefficient of variation of 3 blinded measurements separated by at least 2 days) was 1.0% for the brain, 0.9% for the liver and 1.6% for the kidneys. This value was not calculated for skeletal muscle or adipose tissue but previous research has reported values of ~2% (Müller et al., 2015, Gallagher et al., 2017).

Calculation of Organ Mass and Specific Metabolic Rate

Total organ volume was calculated from the sum of all areas multiplied by the slice thickness (and interslice gap if applicable). Organ volume was converted to mass by multiplying for its specific tissue density and tissue's metabolic rates (and contribution to RMR) were estimated as presented for study 1. Residual mass was calculated by subtracting high-metabolic rate organs (except the heart), skeletal muscle and adipose tissue from total body weight (Elia, 1992).

Objectively Measured 24-hour Energy Intake

After measuring RMR, 3 *ad libitum* meals separated by 4 hours and a snack box to consume at home were provided to assess 24-hour EI. All foods were provided in excess of expected consumption and participants were informed that they could request more if needed. Regarding the snack box, participants were asked to eat during that day and to bring the leftovers the next morning.

Breakfast consisted of whole grain bran cereal, wholegrain malties cereal, sliced banana, semi-skimmed milk and coffee / tea. Unless specified, the foods' brand was Sainsbury's. Lunch consisted of risotto (Uncle Ben's Tomato & Herb), strawberry yogurt (Yeo Valley) and water. Dinner consisted of vegetable chilli, basmati rice, medium grated cheese, cucumber, cherry tomato, chocolate brownies (Thorntons) and water. The snack box to bring home consisted of grapes, pineapple, honey flavoured greek yoghurt, almonds, cheese crackers (Jacob's) and raisin & hazelnut cereal bars (Jordans Frusli). A visual representation of the 4 meals can be seen in Figure 5.2.

Food items were weighed before and after consumption, macronutrient intake was calculated from the manufacturers' food labels, and 24-hour EI was calculated using metabolizable energy equivalents for protein, fat and carbohydrate of 4, 9 and 3.75 kcal/g, respectively.



Figure 5.2 – *Ad libitum* meals provided during the measures day. A) breakfast; B) lunch; C) dinner; and D) snack-box.

5.3.1.3 Statistical Analyses

The sample size for this study was determined using G*Power statistical program (version 3.1.9.2) based on previous research that reported positive correlations between FFM and EI ($r = 0.63$) (Blundell et al., 2015a), a statistical power of 80% and a level of significance of 5% (required sample size = 14). Therefore, it was planned that 15 participants were going to be recruited, but due to COVID-19 outbreak, only data from 6 participants were available for analyses (5 including organ mass).

Considering the small sample size, a descriptive statistical approach was used for this study rather than relying on statistical significance. Spearman correlations were conducted to describe the associations between body composition (FM, FFM and specific components) and RMR with EI (free-living and laboratory measurements) and appetite sensations (composite scores). A correlation

coefficient <0.30 was considered as weak, 0.30-0.50 as moderate and >0.50 as strong (Cohen, 1988). The contribution of each tissue's mass to total body weight and specific metabolic rate to RMR was also calculated (i.e., percentage of total body weight and RMR). In these analyses, high-metabolic rate organs represent the sum of the brain, kidneys and liver while the heart was included in residual mass.

5.3.2 Results

5.3.2.1 Descriptive Characteristics

Descriptive characteristics, measurements of RMR, body composition (2-compartment model), EI (free-living and laboratory measurements) and appetite composite scores can be observed in Table 5.4.

Table 5.4 – Descriptive characteristics, resting metabolic rate, body composition, energy intake and appetite sensations. N = 6.

	Mean ± SD
Age (y)	29 ± 5
Height (cm)	165.5 ± 8.7
Body Weight (kg)	57.5 ± 7.9
Body Mass Index (kg/m ²)	20.9 ± 1.7
Body Fat (%)	24.0 ± 6.7
Fat Mass (kg)	14.1 ± 5.2
Fat-free Mass (kg)	43.4 ± 3.9
Resting Metabolic Rate (kcal/day)	1278 ± 163
Free-living 24-hour Energy Intake (kcal/day)	1697 ± 341
Laboratory 24-hour Energy Intake (kcal/day)	2637 ± 566
Fasting Appetite Sensations (mm)	76 ± 12
AUC Appetite Sensations (mm * min)	20388 ± 2914

AUC, area under the curve.

Information regarding body composition at the tissue-organ level, as well the contribution of each component to total body weight and RMR can be seen in Table 5.5. While high-metabolic rate organs combined only represented ~5% of total body weight, their contribution to RMR was around ~56%. This value could possibly reach the previously mentioned 60% if heart mass was included. Furthermore, while skeletal muscle represented ~33% of total body weight, it only contributed to ~19% of RMR.

Table 5.5 – Body composition at the tissue organ level and their contribution to total body weight and resting metabolic rate.

	Weight (kg)	% Body Weight	% RMR
High-Metabolic Rate Organs	3.7 ± 0.4	5.2 ± 0.4	56.6 ± 2.6
Liver	1.3 ± 0.2	2.3 ± 0.2	20.8 ± 2.8
Brain	1.4 ± 0.2	2.5 ± 0.5	27.3 ± 2.2
Kidneys	0.23 ± 0.02	0.4 ± 0.04	8.4 ± 1.0
Skeletal Muscle	18.3 ± 3.4	33.0 ± 4.4	19.4 ± 2.0
Adipose Tissue	15.6 ± 3.2	28.1 ± 3.7	5.8 ± 1.3
Residual	18.3 ± 1.7	33.6 ± 2.6	18.4 ± 2.6

5.3.2.2 Associations Between Resting Metabolic Rate and Body Composition at the Tissue-Organ Level with Energy Intake and Appetite Sensations

Resting metabolic rate and body composition were weakly associated with 24-hour EI measured in the laboratory (all $r_s \leq 0.31$; $p \geq 0.62$) except for FM ($r_s = -0.43$; $p = 0.40$) and kidney mass ($r_s = -0.50$; $p = 0.39$). Kidney ($r_s = 0.40$; $p = 0.51$) and residual mass ($r_s = 0.50$; $p = 0.39$) were moderately associated with free-living 24-hour EI, while the brain was not associated. Furthermore, RMR ($r_s = 0.77$; $p = 0.07$) fat-free mass ($r_s = 0.77$; $p = 0.07$), high-metabolic rate organs combined ($r_s = 0.70$; $p = 0.19$), skeletal muscle ($r_s = 0.70$; $p = 0.19$), adipose tissue ($r_s = 0.80$; $p = 0.10$) but particularly the liver ($r_s = 0.90$; $p = 0.04$), were strongly associated with free-living 24-hour EI. A visual representation of these associations can be seen in Figure 5.3.

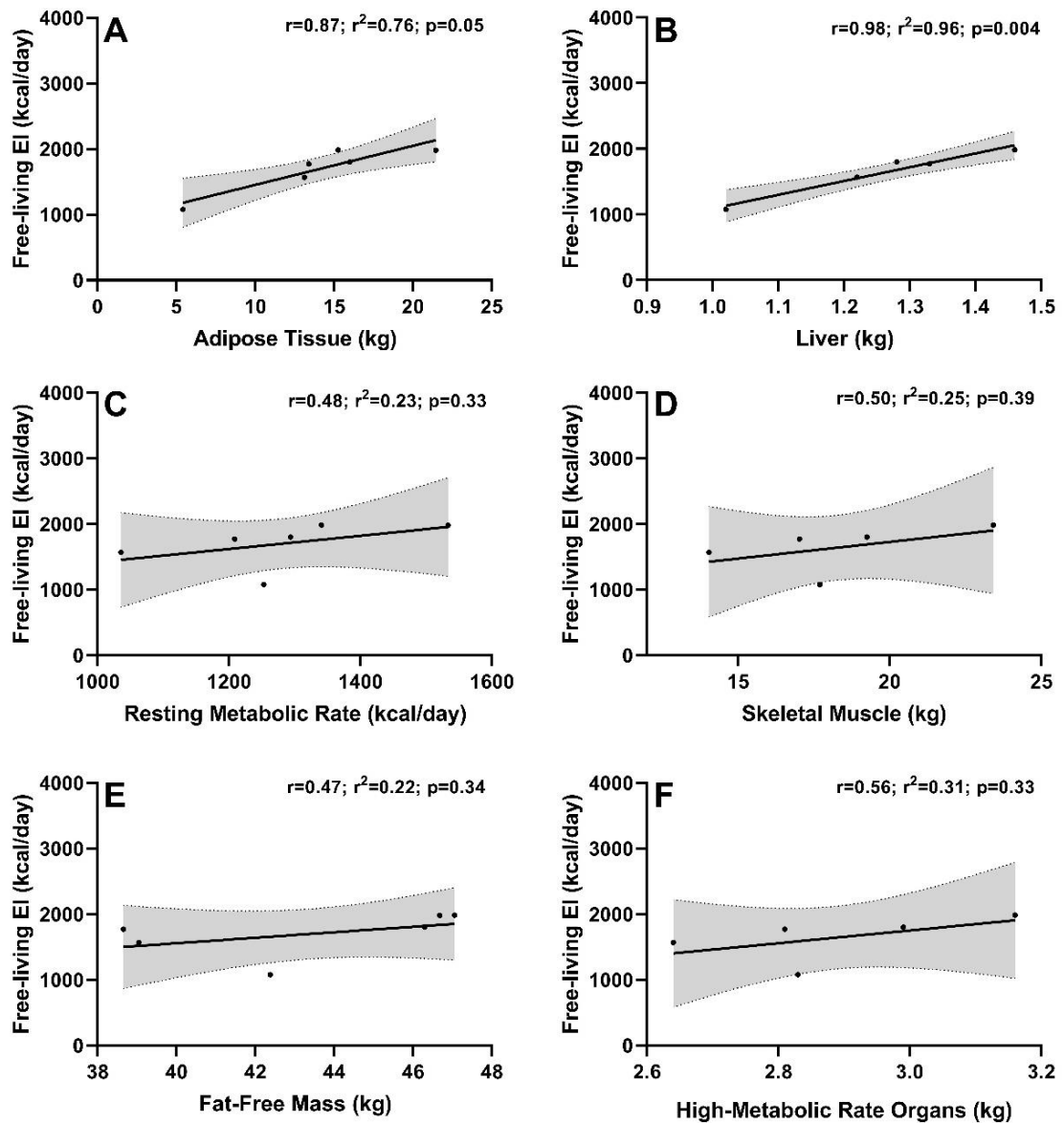


Figure 5.3 – Scatter plot illustrating the associations between free-living 24-hour EI with A) adipose tissue; B) liver; C) resting metabolic rate; D) skeletal muscle; E) fat-free mass; and F) high-metabolic rate organs combined. Grey bands represent the 95% confidence intervals.

Fat-free mass ($r_s = 0.70$; $p = 0.13$), RMR ($r_s = 0.58$; $p = 0.23$), high-metabolic rate organs combined ($r_s = 0.87$; $p = 0.05$), brain ($r_s = 0.56$; $p = 0.32$) and skeletal muscle ($r_s = 0.87$; $p = 0.05$) were strongly associated with fasting appetite composite scores. The remaining components were weakly to moderately associated with fasting appetite composite scores (all $r_s \leq 0.41$; $p \geq 0.49$). A visual representation of these associations can be observed in Figure 5.4.

Fat-free mass ($r_s = 0.77$; $p = 0.07$), RMR ($r_s = 0.71$; $p = 0.11$), high-metabolic rate organs combined ($r_s = 0.50$; $p = 0.39$), brain ($r_s = 0.60$; $p = 0.29$), kidneys ($r_s = -0.80$; $p = 0.10$) and skeletal muscle ($r_s = 0.50$; $p = 0.39$) were strongly associated with AUC appetite composite scores, while the remaining components were weakly associated with AUC appetite composite scores (all $r_s \leq 0.30$; $p \geq 0.54$).

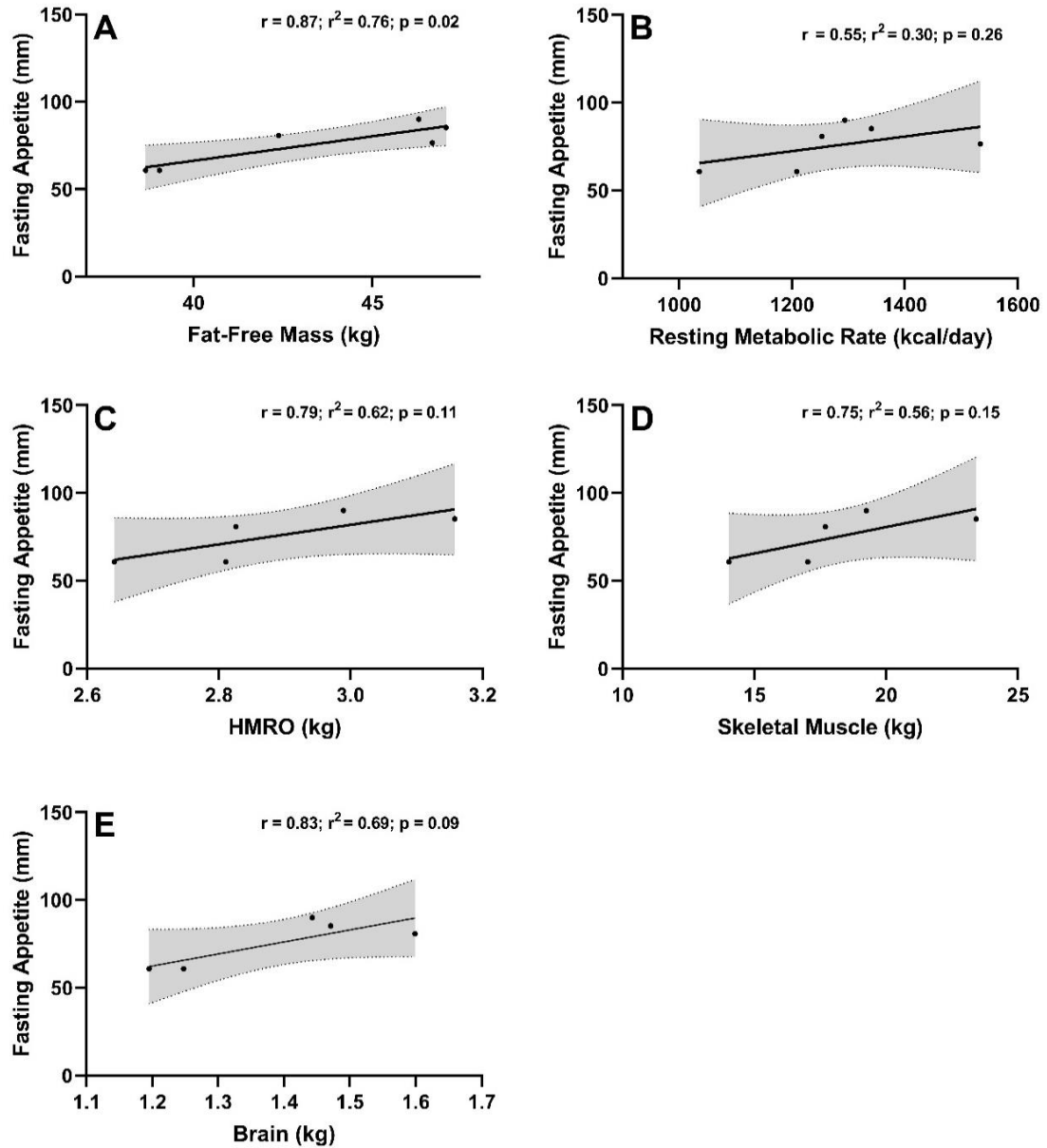


Figure 5.4 – Scatter plot illustrating the associations between fasting appetite composite scores with A) fat-free mass; B) resting metabolic rate; C) high-metabolic rate organs (HMRO) combined; D) skeletal muscle; and E) brain mass. Grey bands represent the 95% confidence intervals.

Section Summary:

- Weak associations were observed between body composition and RMR with laboratory objectively measured 24-hour EI.
- Positive moderate associations were observed between FFM and RMR with free-living 24-hour EI.
- The associations between high-metabolic rate organs combined and the liver with free-living 24-hour EI were stronger than with total FFM.
- FFM, RMR, high-metabolic rate organs combined, brain and skeletal muscle were strongly associated with fasting appetite composite scores.
- FFM, RMR, high-metabolic rate organs combined, brain, kidneys and skeletal muscle were strongly associated with AUC appetite composite scores.

5.4 Discussion

The aim of this chapter was to examine for the first time the associations between body composition at the tissue-organ level, EI and appetite sensations. It was hypothesised that high-metabolic rate organs would present stronger associations with markers of appetite in comparison to FFM as a uniform component. The findings from this chapter support this hypothesis by demonstrating for the first time that the associations between high-metabolically active organs and markers of appetite may be stronger than with total FFM (i.e., higher correlation coefficients).

5.4.1 Associations Between Body Composition at the Tissue-Organ Level, Energy Intake and Appetite Sensations

Study 1

The analyses conducted in study 1 were the first to examine the associations between body composition at the tissue-organ level and appetite sensations. In this study, FFM and RMR were weakly-to-moderately positively associated (albeit not statistically significantly) with fasting hunger sensations. Notably, moderate associations between high-metabolic rate organs combined and the liver with fasting hunger sensations were found and these were stronger than when using total FFM. These findings support the proposed hypothesis and highlight the importance of examining the influence of body composition at the tissue-organ level on appetite. Unfortunately, these analyses only included the assessment of fasting hunger sensations, but served as an important proof-of-concept to design a follow-up study assessing these relationships using a more complete assessment of appetite and eating behaviours (study 2).

Study 2

This investigation aimed to expand the findings from study 1 by examining the associations between body composition at the tissue-organ level and a more thorough assessment of appetite and eating behaviours (i.e., free-living and objectively measured EI, and postprandial appetite sensations). In this study, positive moderate associations were observed between FFM and RMR with free-living 24-hour EI, but those with laboratory measured EI were weak. The weak associations with objectively measured EI were surprising as previous studies using similar protocols (i.e., provision of one to several *ad libitum* meals objectively measured in the laboratory) have observed associations between body composition and EI (Blundell et al., 2012, McNeil et al., 2015, Grannell et al., 2019a). This was likely due to the fact that only six participants were included in the analyses as the COVID-19 outbreak halted data collection early. Interestingly, as in study 1, the correlation coefficients were higher when the associations between body composition and free-living 24-hour EI were examined at the tissue-organ level in comparison to total FFM. Regarding fasting appetite sensations, strong associations were observed between both total FFM

and high-metabolic rate organs combined with fasting appetite composite scores. Interestingly, while total FFM was strongly associated with AUC appetite composite scores, high-metabolic rate organs were only moderately associated. Overall, these findings partially support the ones from study 1. Unfortunately, due to COVID-19, the sample size for this study was limited to 5-6 participants and therefore, it is not possible to produce strong conclusions with this dataset. Thus, it remains unknown how different components of FFM influence markers of appetite and whether these contribute differently to appetite sensations and food intake. However, this study, alongside study 2, provide important proof of concept data demonstrating the relevance of examining the associations between body composition at the tissue-organ level and EI / appetite to fully understand the biological signals influencing appetite and food intake.

5.4.2 Potential Mechanisms

An interesting finding emerging in both studies was the stronger association between high-metabolic rate organs combined and markers of appetite (study 1 – fasting hunger; study 2 – free-living 24-hour EI), than when using total FFM. Interestingly, the associations between the liver with fasting hunger (study 1) and free-living 24-hour EI (study 2) were stronger than with other body composition components. Altogether, these findings could be partially explained by 2 reasons: 1) high-metabolic rate organs accounted for 50-60% of total RMR and therefore could exert a stronger influence on EI and appetite through its energetic demands; 2) the liver not only strongly contributes to RMR (~20%), but scales linearly with height and body size (Heymsfield et al., 2019). For example, while the liver scales with height with a power of 2, the brain scales with an exponential lower than 1. This means that the contribution of the brain to RMR decreases with increased body size. In the current study, the contribution of the brain to RMR (EE as a percentage of RMR) seemed to lessen at a higher total FFM ($r = -0.56$; $p = 0.007$), while the contribution of the liver ($r = -0.02$; $p = 0.93$) and skeletal muscle ($r = -0.08$; $p = 0.78$) does not seem to be associated with total FFM. These findings suggest that while the contribution of the brain to RMR decreases at increased body size, the contribution of the liver and skeletal muscle does not change. This could be due to the fact that the liver and skeletal muscle scale linearly with body size (Heymsfield et al., 2019). This change in the composition

of FFM with increased body size also explains why the RMR/FFM ratio decreases at higher levels of FFM and why RMR scales non-linearly with body size (power of ~ 1.5) (Heymsfield et al., 2019).

Of note, as tissues such as skeletal muscle, kidneys and the heart seem to scale with body size with the same exponential power as the liver (Heymsfield et al., 2019), their association with markers of appetite could be postulated to be similar. However, the absence of or weaker associations with these tissues in the present data could be for several reasons: 1) a lower percentage of contribution to RMR (kidneys and heart = 6% each vs $\sim 20\%$ for the liver); 2) lower EE per unit of mass (skeletal muscle = ~ 13.1 kcal/kg/day vs 241 kcal/kg/day for the liver).

Due to the nature of these analyses (i.e., cross-sectional correlations), it is not possible to pinpoint specific mechanisms regarding these associations. However, previous studies have suggested that individual organs may play specific roles in appetite control. For instance, a potential role of the liver in appetite control has been previously suggested (Fam et al., 2012), in which a lower production of ATP in hepatocytes can trigger an increase in appetite by communicating with the brain via vagal afferent neurons e.g., the 'energostatic' theory of appetite (Friedman, 1995, Friedman, 2007). Furthermore, hepatic glycogen availability has also been suggested to influence appetite, with fibroblast growth factor 21, reported to be responsive to carbohydrate availability and to regulate nutrient intake, providing a vagal-mediated communication pathway between the liver and the brain (Gonzalez et al., 2019). However, apart from a drive to eat created by energy requirements, whether specific metabolic and molecular pathways from specific tissues influence appetite remains unknown. For a complete understanding of the biological signals that influence appetite, future studies should conduct concomitant assessments of body composition (at the tissue-organ level), EE, food intake, markers of appetite and potential mediators / moderators (e.g., appetite-related peptides and myokines).

The findings from these analyses are of importance as they expand the understanding of the mechanisms regarding appetite control by highlighting for the first time a possible specific influence arising from high-metabolic active organs. Furthermore, they highlight the importance of including assessments of body composition at the tissue-organ level in future appetite-related research and weight-change interventions. For instance, greater losses of FFM have been

found to be associated with a hyperphagic response (Dulloo et al., 1997), increases in hunger in men (Turicchi et al., 2020), and weight regain (Vink et al., 2016, Turicchi et al., 2019). However, whether this is due to losses of skeletal muscle, high-metabolic rate organs or other components of FFM remains unknown. This is a crucial area of research because if the component responsible for this hyperphagic response is skeletal muscle, this would reinforce the importance of exercise, especially resistance training, for longer-term appetite control, energy balance regulation and weight management.

5.4.3 Limitations

Some limitations in the current chapter need to be acknowledged. Firstly, the small sample size in study 2 (due to COVID-19) significantly limits the statistical power of that dataset. However, results regarding the associations between body composition and free-living 24-hour EI were similar to the ones observed in study 1, with a greater sample size. Therefore, these findings should be viewed as a proof of concept for future research regarding this topic. Secondly, in study 2, the heart could not be measured and therefore it was included in the residual component. However, as findings between study 1 and 2 were similar, this suggests that not including the heart in the group of high-metabolic rate organs did not substantially influence the findings, although it usually accounts for 8-9% of RMR (Elia, 1992).

Considering the previously mentioned limitations, it is important to recognise that an important aim of study 2 was to establish the feasibility of conducting body composition analyses at the tissue-organ level in the University of Leeds. This was successfully done for the first time and will allow for future research to be conducted in the areas of appetite and energy balance regulation. Although several challenges were encountered during the process, which are described in detail in Chapter 9.7 (methodological considerations), the PhD candidate acquired the necessary competences that will allow him to increase the sample size of this study during his ISSF fellowship.

5.5 Conclusion

Consistent with previous research, FFM and RMR were positively associated with EI and appetite sensations. However, the current findings further suggest that the associations between FFM and markers of appetite may be stronger when body composition analysis is conducted at the tissue-organ level. Although it remains unknown whether high-metabolic rate organs present specific metabolic or molecular characteristics that influence appetite, their greater energetic demands and contribution to RMR may explain why their associations with free-living 24-hour EI and fasting appetite sensations were stronger than when using FFM as a uniform component.

Chapter Summary:

- RMR and FFM were positively associated with EI and appetite sensations.
- Examining the associations between body composition and markers of appetite at the tissue-organ level may lead to an improvement in the explanation capacity of models investigating the predictors of between subject variability in appetite sensations and EI.

Chapter 6 – The Impact of Weight Loss Through Continuous and Intermittent Energy Restriction on Metabolic, Behavioural and Psychological Compensatory Responses

Chapter Aim:

- Examine whether matched WL through continuous or intermittent energy restriction lead to different metabolic, psychological and behavioural compensatory responses.

6.1 Introduction

In an attempt to attenuate the compensatory responses occurring during WL described in Chapter 1.3, IER has been proposed as an alternative to CER (Sainsbury et al., 2018, Peos et al., 2019). It could be possible that including days in energy balance or surfeit throughout the week could attenuate these compensatory responses that undermine WL and weight maintenance efforts. Indeed, some IER patterns have been shown to be superior to CER in attenuating the losses of FFM (Campbell et al., 2020) and reductions in RMR (Davoodi et al., 2014, Byrne et al., 2018) during WL. However, data is limited and the variability between dietary patterns make it hard to make comparisons between interventions.

A systematic review and meta-analysis comparing alternate day fasting with continuous very-low energy diets observed that the former presented a greater relative reduction in FM and smaller loss of FFM (Alhamdan et al., 2016). However, this could be due to the fact that very-low energy diets presented a larger energy deficit and therefore greater total and rate WL, potentially increasing the losses of FFM (Hall, 2007). If IER better retains FFM during WL, it could potentially attenuate the previously documented hyperphagic response (Dulloo et al., 1997), increases in hunger sensations (Turicchi et al., 2020) and weight regain (Vink et al., 2016). However, more data is needed to fully

understand the difference between these dietary patterns since other studies reported no advantages from IER over CER regarding changes in body composition (Seimon et al., 2015, Coutinho et al., 2018a, Harris et al., 2018).

Regarding changes in RMR, Coutinho et al. observed greater decreases (-120 vs -40 kcal/day) after 12 weeks of IER in comparison to CER despite similar changes in body weight (-12.5%), but these disappeared after adjusting for losses of FFM (Coutinho et al., 2018a). On the other hand, Catenacci et al. observed a reduction in RMR in the CER group at week 8 after adjusting for FFM, but not in the IER group (Catenacci et al., 2016). Coutinho et al. and Klempel et al. did not observe any significant differences in physical activity behaviours after 12.5% and 5.8% WL through IER, respectively (Coutinho et al., 2018a, Klempel et al., 2010). Therefore, it remains unknown if IER leads to a smaller decrease in EE and physical activity behaviours as compared to CER.

Sustained increases in appetite after WL are proposed to be a factor responsible for weight regain (Sumithran et al., 2011, Polidori et al., 2016), although evidence is contradictory and few studies have examined changes in appetite after IER (Coutinho et al., 2018a, Heilbronn et al., 2005, Klempel et al., 2010, Varady et al., 2013). For instance, Coutinho et al. observed no significant differences in fasting and postprandial sensations of hunger or fullness despite a 12.5% WL via IER or CER (Coutinho et al., 2018a). Additionally, Varady et al. observed no changes in daily hunger but an increase in daily fullness during 'fast days' after a 6.5% WL (Varady et al., 2013), while Bhutani et al. observed a reduction in daily hunger and increase in daily fullness during 'fast days' after ~3.2% WL (Bhutani et al., 2013). Interestingly, Klempel et al. observed a short-term increase in daily hunger after 1 week of IER, but hunger decreased after 2 weeks and remained lower throughout the trial (Klempel et al., 2010). The effects of IER on eating behaviours traits have only been assessed in two interventions in which Bhutani et al. observed an increase in restraint and decrease in uncontrolled eating while Kroeger et al. observed no changes (Bhutani et al., 2013, Kroeger et al., 2018). Thus, the effect of these dietary patterns on eating behaviour traits remains unknown.

A number of important limitations also exist in the aforementioned studies. Firstly, some studies did not have a comparison group (Kroeger et al., 2018, Klempel et al., 2010, Heilbronn et al., 2005), and therefore it is not possible to know how IER

would differ from CER. Secondly, when compared to CER, some studies did not match the degree of WL which could lead to different compensatory responses. Thirdly, food is not usually provided in the CER group, with adherence being reported using self-reported daily food records. Lastly, appetite was usually measured in a fasting state or prior to sleep, but postprandial and daily sensations have rarely been measured. The data presented in Chapters 6-8 address these issues.

6.1.1 Objective & Hypotheses

The main aim of this study was to compare the effects of matched WL ($\geq 5\%$) through CER or IER on changes in body composition, EE, physical activity, appetite sensations and eating behaviour traits in healthy women with overweight and obesity. The following hypotheses were tested:

1. A greater retention of FFM (and a lower p-ratio) and a lower adaptive thermogenesis would be observed in IER.
2. Reductions in EE and physical activity, if they occurred, would be lower in IER.
3. Hunger sensations would not increase in IER.
4. Weight loss through CER and IER would lead to increases in cognitive restraint and decreases in disinhibition.

6.2 Methods

6.2.1 Participants

Women with overweight and obesity were recruited from the University of Leeds and the surrounding area via posters and email lists for a study examining 'The effects of a personalised WL meal plan on body composition and metabolism'. The study ran from February to December 2018. Volunteers were included if they were aged between 18-55 years and had a BMI between 25.0-34.9kg/m². Exclusion criteria are described in Chapter 3.2. Volunteers were remunerated £100 for participating. The study received approval from the School of Psychology Research Ethics Committee (ref: PSC-238, date: 10/01/2018). This trial was registered at clinicaltrials.gov as NCT03447600.

6.2.2 Screening

Following an online pre-screening questionnaire assessing general eligibility criteria, participants were invited to the laboratory for a full screening session where the protocol was thoroughly explained, eligibility was determined, consent forms were signed and preferences for study foods were analysed. Participants were instructed not to change their physical activity habits for the duration of the study. The specifics of the study meal plans were not explicitly explained during the screening session. Participants were told that once their baseline measurements were completed, they would be informed about their individualised meal plan.

6.2.3 Randomisation & Blinding

Participants were blinded to the 2 arms of the study as it was advertised as 'a personalised WL meal plan'. Upon consenting to participate, participants were randomised (randomization.com) to IER or CER on a 1:1 ratio in blocks of 6 stratified by age (18-36 / 37-55 years) and BMI (25.0-29.9 / 30.0-34.9 kg/m²). Participants and investigators were blinded to the allocated treatment until the baseline measurements were completed. At this point, the participants were given the details of their meal plan (i.e., IER or CER) by the research dietitian who retrieved each diet allocation on a case-by-case basis from an independent co-investigator. To minimise attrition bias, the diet allocation of those that withdrew from the study were re-allocated to new participants (8 pre-diet intervention, 6 during). Outcome assessors remained blinded to the diet allocations throughout the entire intervention. Participants were debriefed about the 2 arms of the trial at the end of the intervention.

6.2.4 Procedure

As shown in Figure 6.1, at baseline, week 2 and in the final week of the intervention, participants completed a free-living week of measurements where body weight was measured fasted and nude each morning (with the exception of week 2) with a scale provided (Salter scale model 9206, UK), and an online food diary (myfood24), alongside CoEQ to retrospectively assess free-living daily appetite sensations, was completed at the end of each day. Moreover, a physical activity monitor (SenseWear Armband) was worn continuously throughout the week (concurrently with myfood24 and CoEQ) to assess minutes of physical activity and estimate TDEE. Upon completion of the measures week, participants attended the laboratory for a measures day (for IER this was completed after a fast day in week 2 and in the final week in order to standardise that all participants were assessed in a negative energy balance condition).

All testing took place after a 10-12-h overnight fast. Fasting appetite, body composition, and RMR were assessed. This was followed by a fixed breakfast (25% of RMR measured with indirect calorimetry) and an *ad libitum* lunch 3 hours later. Both meals have been described in Chapter 3.3.1.1. Appetite sensations were assessed pre- and post-breakfast, every 30 minutes between meals and post-lunch. Participants were then provided with paper versions of an eating behaviour questionnaire (Three-Factor Eating Questionnaire) to complete at home that evening.

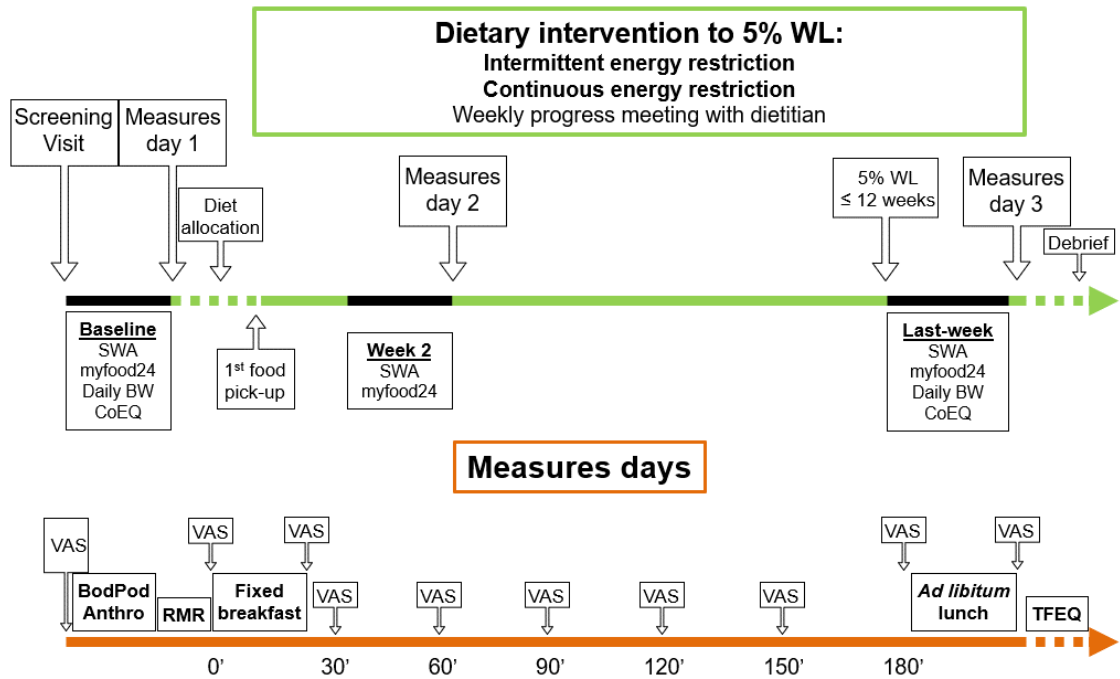


Figure 6.1 – Schematic of the trial. WL, Weight loss; SWA, SenseWear Armband; BW, body weight; CoEQ, control of eating questionnaire; VAS, visual analogue scales; Anthro, anthropometrics; RMR, resting metabolic rate; TFEQ, Three-Factor Eating Questionnaire.

6.2.5 Measurements

Anthropometrics, body composition, RMR, physical activity and TDEE, appetite sensations (fasting, AUC and free-living), EI (free-living and laboratory-measured) and eating behaviour traits (Three-Factor Eating Questionnaire) were assessed as previously described in Chapter 3.

6.2.5.1 Adaptive Thermogenesis

Adaptive thermogenesis in RMR was calculated using a method that has been previously used (Bosy-Westphal et al., 2009), which compares the difference between baseline predicted (using a created regression equation based on baseline data) and measured RMR, with the difference between post-WL predicted and measured RMR:

$$\text{Adaptive Thermogenesis} = (RMR_{m2} - RMR_{p2}) - (RMR_{m1} - RMR_{p1})$$

Where $m1$ and $p1$ represent the measured and predicted RMR values at baseline and $m2$ and $p2$ at post-WL. Using this method, a negative value represents the presence of adaptive thermogenesis (i.e., a greater than predicted decrease in RMR).

6.2.6 Diet Intervention

Following the first day of measurements, participants met with the research dietitian for their diet allocation meeting in which the specifics of their allocated meal plan were explained (IER or CER) and they were given a checklist of the specific foods they would be able to eat for the duration of the intervention. After agreeing and consenting to the terms of their meal plan, a food collection / weekly meeting appointment was scheduled. The research dietitian then calculated energy requirements based on measured RMR \times physical activity levels obtained from the SenseWear Armband. Meal plans were adapted for each participant based on energy requirements and food preferences, and modified on a weekly basis based on feedback from participants. Foods were all pre-portioned (except for the milk where a measuring cup was also provided) with minimal preparation required and accompanied by daily food check lists. Participants were permitted to consume coffee / tea with the milk provided by the researchers (otherwise only black coffee / tea and herbal teas were allowed) and other energy-free beverages, sugar-free gum, and were encouraged to drink plenty of water. Participants were instructed to note whether all foods were consumed, or specify how much was left, and the time eaten. Additionally, participants noted if any foods or drinks not on the meal plan were consumed (and if yes, to specify what and how much). Two 'days off' per month were allowed.

During IER, on fast days, volunteers consumed 25% of their daily energy requirements from total diet replacement products (LighterLife Ltd, UK) provided by the researchers, whereas on the alternate days, volunteers ate *ad libitum* using their own foods. The calorie content (~150 kcal) and macronutrient composition (~36% carbohydrate, ~27% fat and ~37% protein) was similar for each product, and ensured a daily protein intake of 49.2 ± 8.2 g, in line with the 50 g recommended by the European guidelines on total diet replacement products for weight management (Products, 2015). There were no time restrictions on when participants could consume the food packs (ranging from 3

to 5 full packs plus an additional bar portion to make up the difference if needed). Participants were also provided milk portions for coffee/tea, if requested (and deducted from the daily allocated calories), but were required not to consume any other energetic beverages. During the weekly meetings with the dietitian, if WL was not achieved with full compliance, food intake on feed days was discussed and general guidance was offered.

During CER, participants consumed 75% of their daily energy requirements each day from commercially available products provided by the researchers, estimated to induce a similar WL based on current clinical nutrition practices (Association, 2017). The macronutrient composition of the diet was 50-55% carbohydrate, 30-35% fat and 15-20% protein, in line with national guidelines (Foundation, 2017). Three main meals and snacks were provided. Similar to IER, no time restrictions or specific number of eating episodes were given for the consumption of the foods. During the weekly meetings with the dietitian, if WL was not achieved or plateaued with full adherence, prescribed food intake was adjusted.

Experience of the meal plan and WL was monitored each week, and EI adjusted if needed. Upon reaching ~5%WL at a weekly weigh in, participants repeated a final measures week while continuing the dietary intervention and emailed their fasted body weight (Salter scale model 9206, UK) each day to the research dietitian. Participants were included in this analysis ($\geq 5\%$ WL) if self-reported body weight was $\geq 5\%$ WL on at least 4/7 days leading to the last measures day and objectively confirmed during the final measures day. Participants who did not achieve the $\geq 5\%$ WL criterion were still tested at 12 weeks but not included in the per protocol analyses.

6.2.7 Adherence to the Intervention

Adherence to the meal plans was considered when actual EI did not exceed the prescribed EI by more than 75 kcal (Hoddy et al., 2014). If this occurred, that day was considered non-adherent. Adherence (%) was calculated on a weekly basis using a previously published method (Hoddy et al., 2014):

$$\text{Adherence (\%)} = \frac{\text{Adherent days}}{\text{Prescribed days}} \times 100$$

Additionally, EI was calculated by using the 'intake-balance method' which has been described in Chapter 3.3.1.3. This was calculated to examine the mean energy deficit throughout the intervention for each individual and dietary group.

6.2.8 Statistical Analyses

This chapter only presents data on the changes from baseline to post-WL in the participants that reached $\geq 5\%$ WL ($n = 30$). Previous research has shown that the compensatory responses in EE and appetite that occur during periods of negative energy balance may vary at different amounts of total WL (Nymo et al., 2017, Nymo et al., 2018). As the aim of this analysis was to examine whether CER and IER induce different compensatory responses (and not which intervention induced a greater WL), total WL would need to be matched between groups and participants. However, not all individuals reached the target WL and therefore, only the ones achieving $\geq 5\%$ WL were analysed so assessments could be conducted under similar weight change conditions. If all participants have been included, the large variability in WL between individuals could be a confounding factor. Consequently, reducing the number of participants examined could slightly lower the statistical power. However, as shown below, the number of participants analysed was within the power calculation and thus do not influence or undermine the conclusions of this chapter.

Independent samples t-tests were used to analyse baseline differences between groups. Baseline to post-WL changes were analysed with repeated measures ANOVA with group as the between-subject factor and time as the within-subject factor. Effect sizes are reported as partial eta-squared (η_p^2) for the ANOVAs.

Power calculations (G*Power v3.1) conducted revealed that 24 participants would be needed to detect a difference between groups regarding changes in daily appetite sensations (effect size $F = 0.49$) between 2 groups and 2 repeated measurements ($r = 0.25$) with $\alpha = 0.05$ and $1-\beta = 0.8$.

6.3 Results

6.3.1 Consort Diagram

As shown in Figure 6.2, 400 interested volunteers were screened online and 66 were assessed for eligibility in the laboratory. Of these, 54 were included into the study and 46 completed the baseline measurements (measures week and measures day). Twenty-four were allocated to IER and 22 to CER. In IER, 6 discontinued the intervention (25%) and a further 6 did not reach $\geq 5\%$ WL within 12 weeks (25%). In CER, 1 was lost to follow-up (no contact after first week on meal plan), 2 discontinued the intervention (14%), and 1 did not reach $\geq 5\%$ WL within 12 weeks (6%). There were significantly more completers achieving $\geq 5\%$ WL within 12 weeks in CER than in IER ($p = 0.03$).

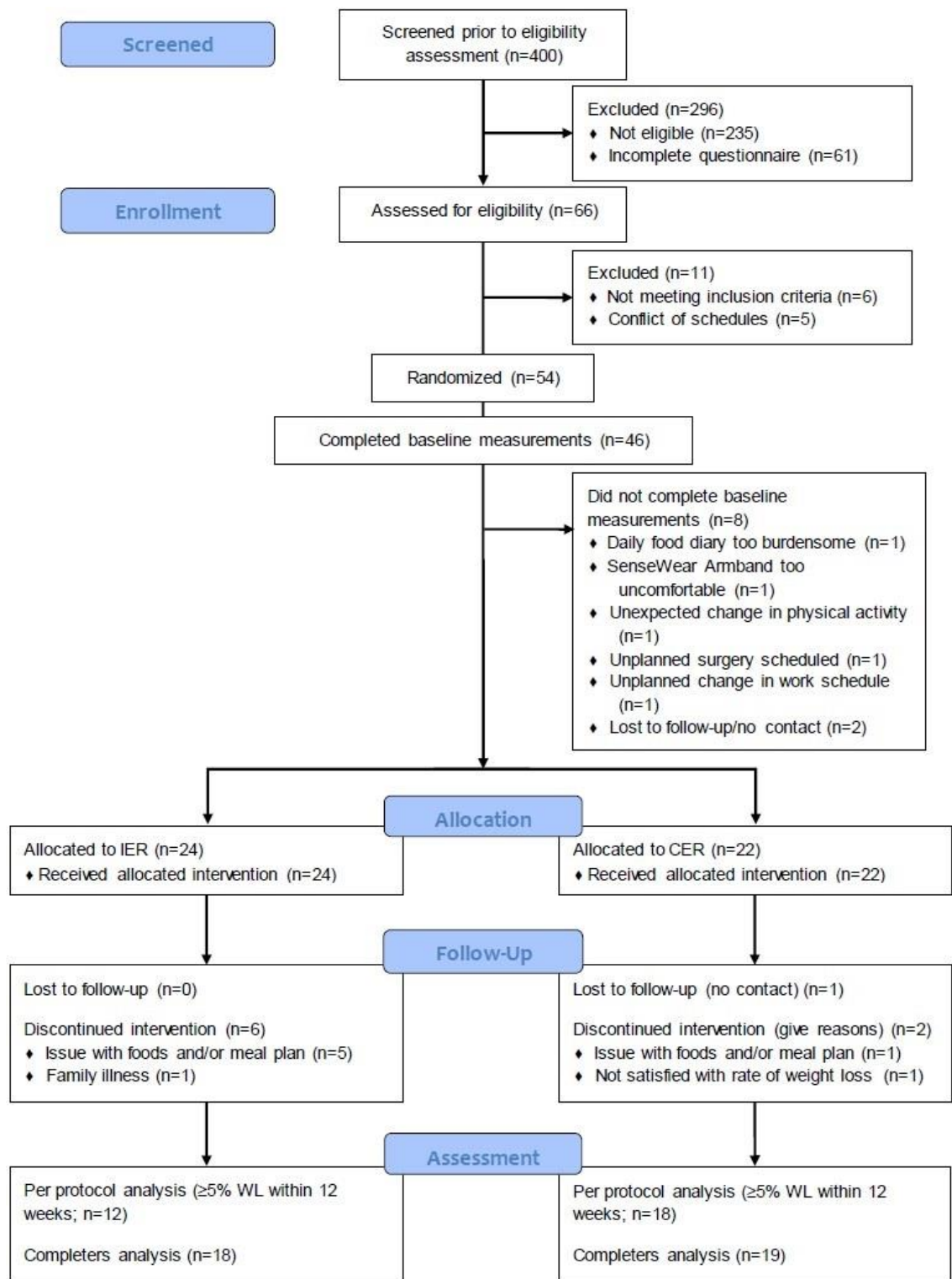


Figure 6.2 – Consort flow diagram. IER, intermittent energy restriction; CER, continuous energy restriction; WL, weight loss.

6.3.2 Baseline Characteristics

As the main aim of this study was to compare a matched WL to $\geq 5\%$ between IER and CER, the following analyses will be conducted in the participants that reached the minimum criteria (i.e., at least 5% WL). In the following chapters, data from the completers ($n = 37$) will be provided and analysed. As shown in Table 6.1, there were no significant baseline differences between groups in the participants that achieved the $\geq 5\%$ WL criteria.

Table 6.1 – Baseline group characteristics of participants who reached $\geq 5\%$ WL within 12 weeks through CER ($n = 18$) or IER ($n = 12$).

	CER	IER	p-value
Age (years)	35 \pm 9	34 \pm 10	0.80
Body weight (kg)	79.2 \pm 10.4	81.1 \pm 12.2	0.64
Height (cm)	164.7 \pm 7.8	166.5 \pm 9.2	0.58
BMI (kg/m ²)	29.1 \pm 2.4	29.1 \pm 2.5	0.95
Fat mass (kg)	32.5 \pm 8.3	34.0 \pm 7.2	0.60
Fat-free mass (kg)	46.7 \pm 5.5	47.1 \pm 6.6	0.85
Body fat (%)	40.6 \pm 6.2	41.7 \pm 4.1	0.60
RMR (kcal/day)	1435 \pm 220	1443 \pm 211	0.92
TDEE (kcal/day)	2335 \pm 287	2444 \pm 353 ¹	0.36
AEE (kcal/day)	645 \pm 161	759 \pm 265 ¹	0.15
Total PA (min/day)	233 \pm 93	252 \pm 92 ¹	0.59
Sedentary (min/day)	762 \pm 108	756 \pm 92 ¹	0.88
MVPA (min/day)	69 \pm 31	73 \pm 40 ¹	0.76

CER, continuous energy restriction; IER, continuous energy restriction; WL, weight loss; BMI, body mass index; RMR, resting metabolic rate; TDEE, total daily energy expenditure; AEE, activity energy expenditure; PA, physical activity, MVPA, moderate-to-vigorous physical activity. ¹ Data available from 11 participants.

6.3.3 Adherence to the Interventions

There were no differences in mean weekly adherence reported in the weekly self-reported logbook between groups (CER: 89.0 \pm 9.7%, IER: 81.4 \pm 14.6%; $p = 0.13$), although the CER group presented higher levels. Additionally, there were

no significant differences in calculated EI (CER: 1593 ± 306 kcal/day, IER: 1678 ± 336 kcal/day; $p = 0.49$) or percentage of energy requirements (CER: $70.5 \pm 8.7\%$, IER: $71.6 \pm 10.3\%$; $p = 0.75$), indicating that the average EI throughout the intervention was similar between groups.

6.3.4 Changes in Body Weight and Body Composition

Changes in body weight and body composition can be observed in Table 6.2. The CER group achieved a final WL of $6.3 \pm 0.8\%$ in 57 ± 16 days, and IER a final WL of $6.6 \pm 1.1\%$ in 67 ± 13 days. Although there was a decrease in body weight over time ($p < 0.001$; $\eta_p^2 = 0.96$), there were no significant differences between groups or interaction effects. Additionally, there were no significant differences between groups in the number of days until final measures day ($p = 0.10$), although IER took 10 days longer to reach the final measures day ($\geq 5\%$ WL).

Fat mass ($\eta_p^2 = 0.91$), FFM ($\eta_p^2 = 0.70$) and body fat percentage ($\eta_p^2 = 0.78$) decreased over time (all $p < 0.001$) but there were no significant differences between groups or interaction effects. The p-ratio was not significantly different between conditions (CER: 0.26 ± 0.19 ; IER: 0.25 ± 0.20 ; $p = 0.94$), suggesting that the fraction of FFM lost was similar between groups. These demonstrate that approximately $\frac{1}{4}$ of the body weight lost was in form of FFM, although a large variability was observed between individuals.

Table 6.2 – Changes between baseline and post-WL for body weight, body fat percentage, fat mass and fat-free mass.

	CER Baseline	CER Post-WL	Change	IER Baseline	IER Post-WL	Change
Body weight (kg) *	79.1 ± 10.4	74.2 ± 10.0	-5.0 ± 0.8	81.1 ± 12.2	75.8 ± 11.3	-5.3 ± 1.4
Body fat (%) *	40.6 ± 6.2	38.4 ± 6.4	-2.2 ± 1.1	41.7 ± 4.1	39.3 ± 7.8	-2.4 ± 1.5
Fat mass (kg) *	32.5 ± 8.3	28.8 ± 7.7	-3.7 ± 1.0	34.0 ± 7.2	30.0 ± 6.8	-4.1 ± 1.6
Fat-free mass (kg) *	46.7 ± 5.5	45.4 ± 5.5	-1.3 ± 0.9	47.1 ± 6.6	45.8 ± 6.3	-1.3 ± 0.8

CER, continuous energy restriction; IER, continuous energy restriction; WL, weight loss. * Significant difference between baseline and post-WL ($p < 0.001$).

Section Summary:

- Body weight, FM, body fat percentage and FFM decreased in both IER and CER.
- On average, 25% of the body weight lost was comprised of FFM.
- No significant differences in body composition were observed between groups.

6.3.5 Changes in Energy Expenditure and Physical Activity

6.3.5.1 Resting Metabolic Rate

No significant changes were observed from pre to post-WL in RMR ($p = 0.65$; $\eta_p^2 = 0.01$), and no differences between groups were observed in RMR (CER vs IER; $p = 0.65$).

6.3.5.2 Adaptive Thermogenesis

Adaptive thermogenesis was not significantly different between groups (CER: -48 ± 123 kcal/day; IER: -89 ± 116 kcal/day; $p = 0.35$). However, a large variability between individuals was present (CER: -271 to 207 kcal/day; IER: -266 to 118 kcal/day) as can be seen in Figure 6.3.

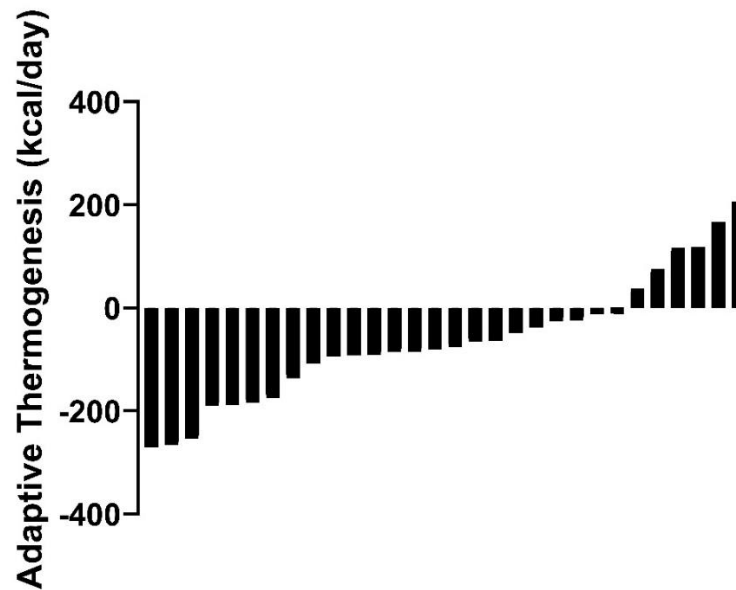


Figure 6.3 – Adaptive thermogenesis across the whole sample ($n = 30$). A negative value means that adaptive thermogenesis was present, i.e., a greater than predicted decrease in RMR occurred during WL.

6.3.5.3 Total and Activity Energy Expenditure

A decrease in TDEE ($p = 0.002$; $\eta_p^2 = 0.30$) and AEE ($p = 0.007$; $\eta_p^2 = 0.24$) was observed from baseline to the last week of intervention, but no differences between groups or interaction effects existed. However, after including changes in body weight from baseline to post-WL as a covariate (as AEE and TDEE in part reflect body size), the changes in TDEE and AEE disappeared (TDEE, $p = 0.68$, $\eta_p^2 = 0.007$; AEE, $p = 0.06$; $\eta_p^2 = 0.13$).

6.3.5.4 Physical Activity and Sedentary Behaviour

No significant changes were observed from baseline to the last week of the intervention for total physical activity ($p = 0.37$; $\eta_p^2 = 0.03$), minutes of light ($p = 0.48$; $\eta_p^2 = 0.02$), moderate-to-vigorous physical activity ($p = 0.34$; $\eta_p^2 = 0.03$) or sedentary time ($p = 0.08$; $\eta_p^2 = 0.11$) with no differences between groups or interaction effects. Although the overall changes in EE and physical activity behaviours were small, the variability between individuals was large as can be seen in Figure 6.4. The impact of this inter-individual variability on WL will be examined in Chapter 8.

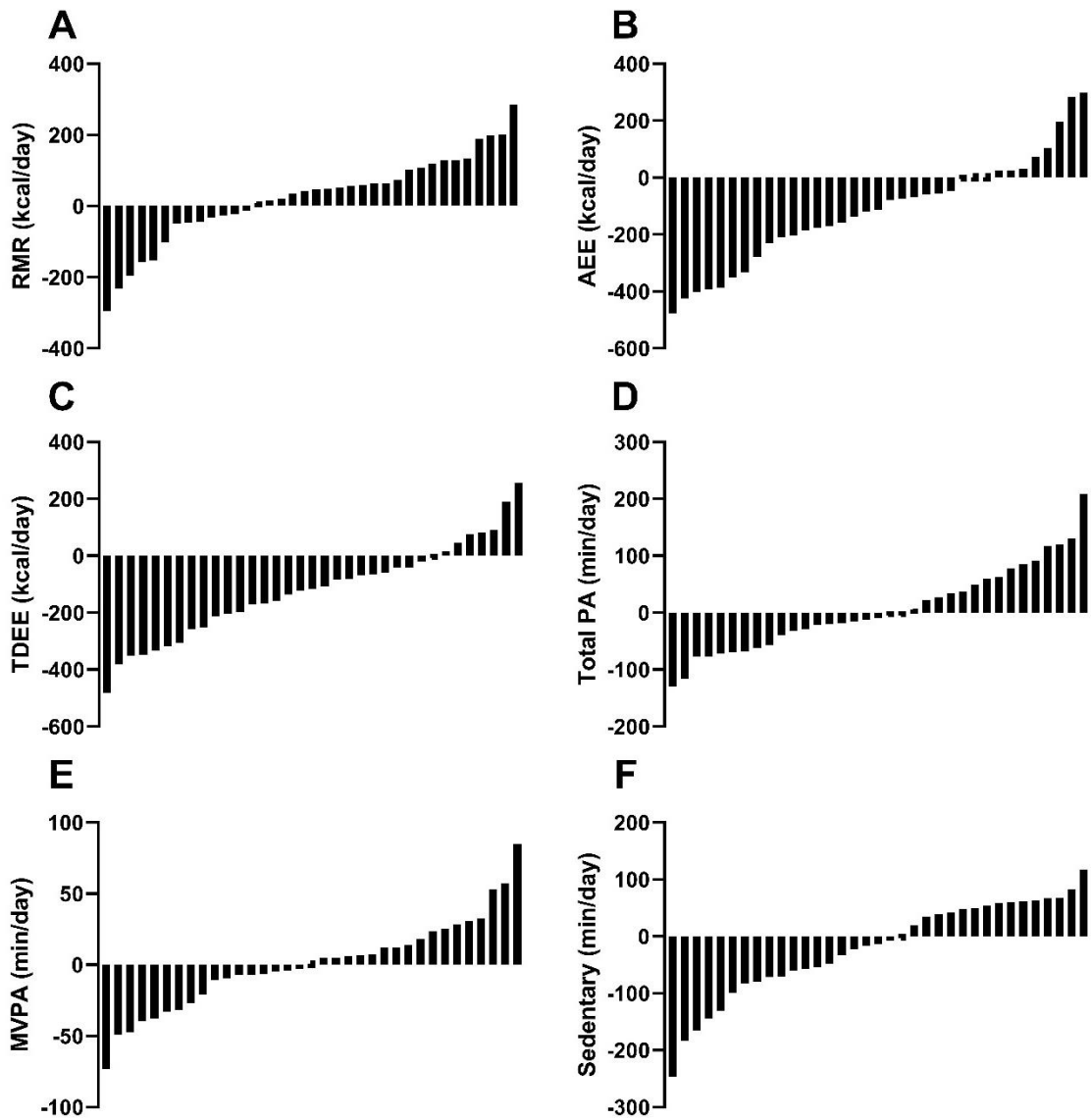


Figure 6.4 – Individual changes in energy expenditure and physical activity behaviours from baseline to the last week of intervention. A) Resting metabolic rate; B) Total daily energy expenditure; C) AEE, Activity energy expenditure; D) Total physical activity; E) Moderate-to-vigorous physical activity; F) Sedentary time.

Table 6.3 – Changes between baseline and last week of intervention for energy expenditure and physical activity behaviours in CER (n = 18) and IER (n = 12).

	CER Baseline	CER Post-WL	Change	IER Baseline	IER Post-WL	Change
RMR (kcal/day)	1435 ± 220	1426 ± 203	-9 ± 128	1443 ± 211	1473 ± 187	30 ± 118
TDEE (kcal/day) *	2335 ± 287	2262 ± 328	-73 ± 155	2477 ± 350	2338 ± 249	-139 ± 179
AEE (kcal/day) *	645 ± 161	604 ± 230	-41 ± 175	794 ± 247	639 ± 162	-154 ± 178
Total PA (min/day)	233 ± 93	260 ± 108	28 ± 74	260 ± 92	259 ± 79	-1 ± 79
Light PA (min/day)	164 ± 70	184 ± 75	20 ± 55	184 ± 68	179 ± 52	-5 ± 56
MVPA (min/day)	69 ± 31	77 ± 37	8 ± 32	77 ± 40	81 ± 38	4 ± 32
Sedentary (min/day)	762 ± 108	719 ± 126	-43 ± 82	760 ± 95	743 ± 103	-17 ± 94

CER, continuous energy restriction; IER, continuous energy restriction; WL, weight loss; RMR, resting metabolic rate; TDEE, total daily energy expenditure; AEE, activity energy expenditure; PA, physical activity; MVPA, moderate-to-vigorous physical activity. * Significant difference from baseline to the last week of intervention ($p < 0.05$). These differences disappeared after adjusting for changes in body weight.

Section Summary:

- Activity energy expenditure and TDEE decreased during the intervention with no differences between groups, but these changes disappeared after adjusting for changes in body weight.
- No significant changes in RMR and physical activity behaviours were observed during the intervention or between groups.
- Adaptive thermogenesis occurred but no differences between groups were observed.
- Large inter-individual variability was present regarding changes in EE and physical activity.

6.3.6 Changes in Appetite Sensations

6.3.6.1 Fasting Appetite Sensations

No significant changes were observed in fasting desire to eat (CER: 5 ± 25 mm; IER: 0 ± 30 mm; $p = 0.62$; $\eta_p^2 = 0.01$), fullness (CER: -4 ± 24 mm; IER: 3 ± 20 mm; $p = 0.88$; $\eta_p^2 = 0.001$), hunger (CER: 2 ± 31 mm; IER: 1 ± 36 mm; $p = 0.81$; $\eta_p^2 = 0.002$) and prospective food consumption (CER: 6 ± 19 mm; IER: 7 ± 15 mm; $p = 0.06$; $\eta_p^2 = 0.12$) or differences between groups. Changes in fasting desire to eat, fullness, hunger and prospective food consumption can be observed in Figure 6.5. When fasting appetite sensations were examined collectively (i.e., composite scores, explained in Chapter 3.7.1), no significant changes were observed from baseline to post-WL ($p = 0.61$; $\eta_p^2 = 0.01$) or between groups ($p = 0.87$; $\eta_p^2 = 0.001$).

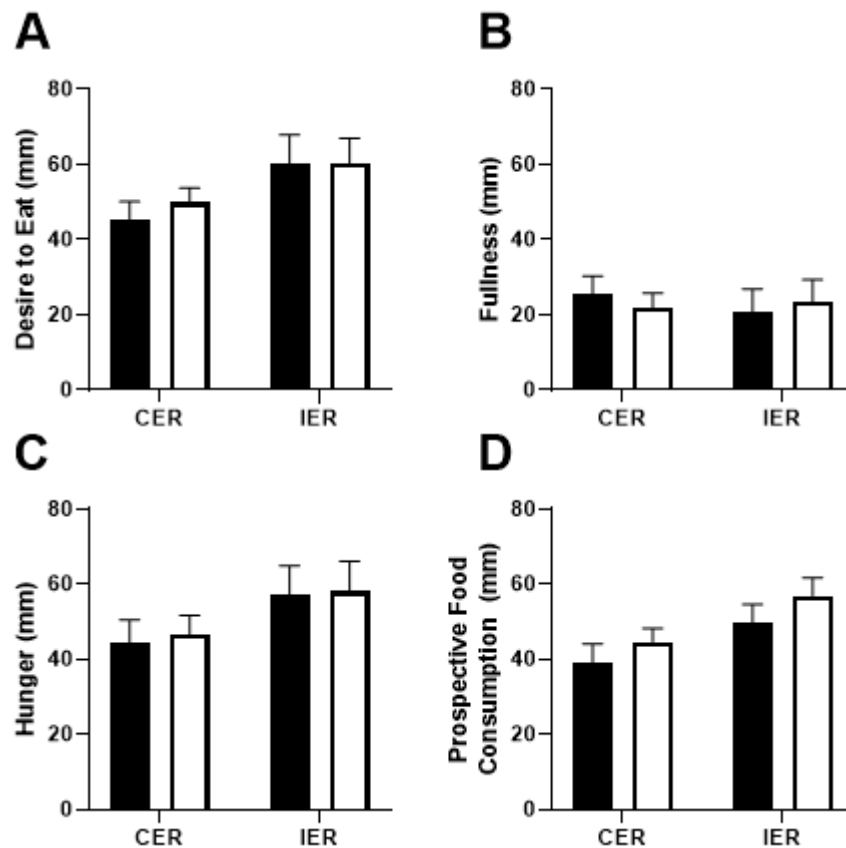


Figure 6.5 – Changes in fasting A) desire to eat, B) fullness, C) hunger and D) prospective food consumption from baseline to post-weight loss. Black bars: baseline; white bars: post-weight loss.

6.3.6.2 Area Under the Curve Appetite Responses

A decrease in AUC desire to eat [CER: -988 ± 2008 mm x min; IER: -1056 ± 2306 mm x min; $p = 0.02$, $\eta_p^2 = 0.19$] and hunger [CER: -931 ± 1742 mm x min; IER: -828 ± 2184 mm x min; $p = 0.02$, $\eta_p^2 = 0.18$] was observed from baseline to post-WL, with no significant differences between groups or interaction effects. No significant changes in fullness (CER: 118 ± 1889 mm x min; IER: 1242 ± 2800 mm x min; $p = 0.12$, $\eta_p^2 = 0.08$) or prospective food consumption (CER: -620 ± 2245 mm x min; IER: -461 ± 1773 mm x min; $p = 0.17$, $\eta_p^2 = 0.07$) were observed. Changes in AUC desire to eat, fullness, hunger and prospective food consumption can be observed in Figure 6.6. When AUC appetite sensations were examined collectively (i.e., composite scores), significant decreases were observed from baseline to post-WL ($p = 0.02$; $\eta_p^2 = 0.18$) but no differences between groups were found ($p = 0.86$; $\eta_p^2 = 0.001$).

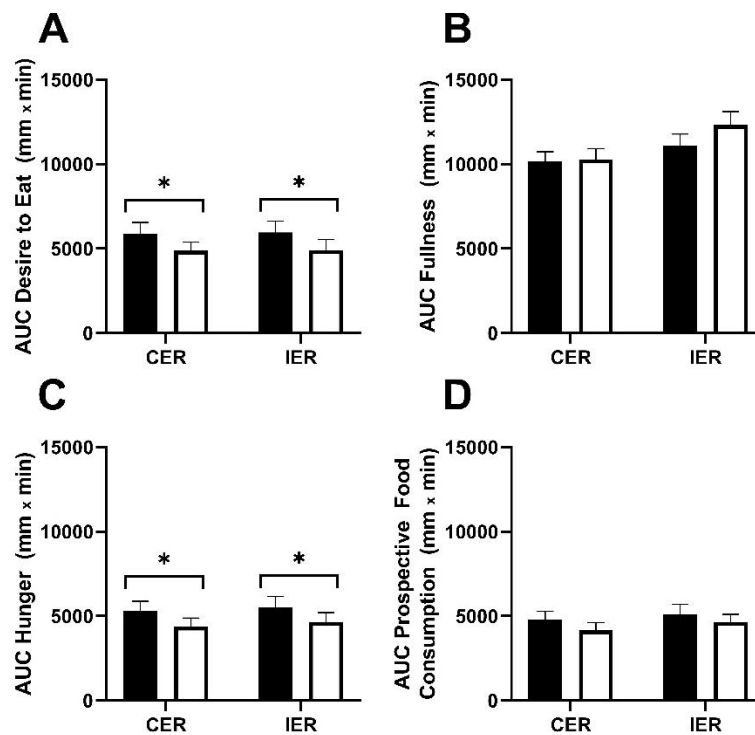


Figure 6.6 – Changes in AUC A) desire to eat, B) fullness, C) hunger and D) prospective food consumption from baseline to post-weight loss. Black bars: baseline; white bars: post-weight loss. * Significant difference from baseline to post-WL ($p < 0.05$).

6.3.6.3 Free-living Retrospective Daily Appetite Sensations

Composite scores of free-living daily appetite sensations assessed using CoEQ (end of day measure) did not significantly change from baseline to post-WL in the whole group ($p = 0.16$; $\eta_p^2 = 0.07$). However, an interaction between time and group emerged ($p = 0.004$; $\eta_p^2 = 0.27$). Post hoc analyses revealed that daily appetite sensations increased in IER (37 ± 7 mm to 47 ± 6 mm; $p = 0.003$, $\eta_p^2 = 0.57$) but not in CER (42 ± 9 mm to 38 ± 14 mm; $p = 0.26$, $\eta_p^2 = 0.08$).

6.3.7 Changes in *Ad Libitum* Energy Intake

Breakfast was standardised to 25% of measured RMR (baseline – CER: 364 ± 54 kcal, IER: 360 ± 50 kcal, $p = 0.89$; post-WL – CER: 358 ± 50 kcal, IER: 368 ± 45 kcal, $p = 0.57$), and therefore, no significant differences were observed over the intervention ($p = 0.95$; $\eta_p^2 < 0.001$) or between groups.

For *ad libitum* EI at lunch, there were no significant changes from baseline to post-WL ($p = 0.66$; $\eta_p^2 = 0.01$) or differences between groups ($p = 0.27$; $\eta_p^2 =$

0.04), but there was an interaction between time and group ($p = 0.02$; $\eta_p^2 = 0.19$). Post hoc analyses revealed that CER consumed more than IER at baseline ($p = 0.05$; $\eta_p^2 = 0.14$) and reduced intake after WL to a similar level as IER ($p = 0.05$; $\eta_p^2 = 0.20$). Changes in *ad libitum* EI at the test meal can be observed in Figure 6.7.

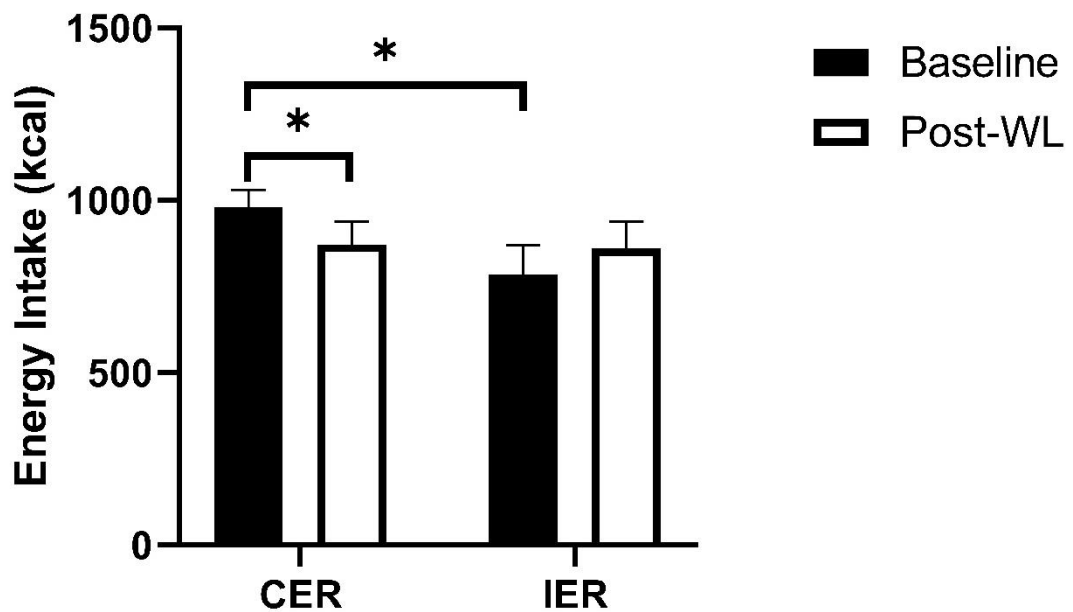


Figure 6.7 - Changes in energy intake during the *ad libitum* test meal (lunch). Black bars: baseline; white bars: post-weight loss. CER, continuous energy restriction; IER, intermittent energy restriction. * Statistically significant differences ($p < 0.05$).

6.3.8 Changes in Self-reported Energy Intake

At baseline, no differences were observed between groups in self-reported EI (CER: 1951 ± 374 kcal; IER: 1783 ± 438 kcal; $p = 0.27$). In the last week of the intervention, self-reported EI was higher in the CER (1400 ± 309 kcal vs IER: 1095 ± 209 kcal; $p = 0.006$). Self-reported EI significantly decreased from baseline to the final week of the intervention ($p < 0.001$; $\eta_p^2 = 0.78$) with no differences between groups being observed ($p = 0.28$; $\eta_p^2 = 0.04$).

6.3.9 Changes in Eating Behaviour Traits

Dietary restraint (CER: 4.6 ± 3.2 ; IER: 3.8 ± 4.6 ; $p < 0.001$; $\eta_p^2 = 0.55$) and both flexible (CER: 2.1 ± 1.4 ; IER: 1.0 ± 2.4 ; $p < 0.001$; $\eta_p^2 = 0.42$) and rigid restraint (CER: $0.9 \pm 1.6 \pm 2.0 \pm 2.1$; $p < 0.001$; $\eta_p^2 = 0.41$) increased from baseline to post-WL with no differences between groups or interaction effects. Disinhibition decreased from baseline to post-WL (CER: -2.8 ± 2.8 ; IER: -0.9 ± 2.6 ; $p = 0.001$; $\eta_p^2 = 0.32$) and a group effect was observed ($p = 0.03$; $\eta_p^2 = 0.15$). Post hoc analyses demonstrated that disinhibition only decreased in the CER group (CER: $p = 0.001$, $\eta_p^2 = 0.51$; IER: $p = 0.24$, $\eta_p^2 = 0.12$). Susceptibility to hunger (CER: -2.8 ± 3.4 ; IER: -2.5 ± 2.5 ; $p < 0.001$; $\eta_p^2 = 0.43$) decreased from baseline to post-WL with no differences between groups or interaction effects.

Section Summary:

- Fasting appetite sensations did not significantly change from baseline to post-WL.
- A significant decrease in AUC hunger, desire to eat and appetite composite scores from baseline to post-WL was observed in both groups.
- Daily retrospective assessments of appetite sensations increased from baseline to the final week of the intervention in the IER group, but not in CER.
- No significant changes in test meal EI were observed from baseline to post-WL. However, CER decreased EI due to having higher baseline values.
- Cognitive restraint increased while susceptibility to hunger decreased in both groups with no differences between groups. Levels of disinhibition decreased in CER, but not in IER.

6.4 Discussion

In this study, the differences in the physiological, psychological and behavioural compensatory responses following matched WL to $\geq 5\%$ through CER or IER were investigated. It was hypothesised that IER would mitigate some of the previously observed responses that occur during CER and oppose WL such as losses of FFM, decreases in RMR and increases in appetite sensations. However, in this study, IER did not lead to any superior physiological, psychological or behavioural outcomes in comparison to CER. Interestingly, postprandial hunger, desire to eat and appetite composite scores decreased after WL achieved by both CER and IER. However, free-living daily appetite sensations increased during WL in IER, but not in CER.

6.4.1 Changes in Body Composition

A recent systematic-review and meta-analyses concluded that alternate day fasting, an IER dietary pattern, was superior to CER in preserving FFM (Alhamdan et al., 2016). However, in this analysis, CER was represented by very low energy diets (<800 kcal/day). Therefore, this finding could be attributed to the higher severity of energy restriction since it has been observed that greater losses of FFM occur with greater energy deficits (Hall, 2007). In the current study, percentage of WL and average EI were similar between groups (percentage of energy requirements - CER: $\sim 71\%$ vs IER: $\sim 72\%$). Although a decrease in body weight, FM, FFM and body fat percentage was observed from baseline to post-WL, no differences were observed between groups. Additionally, the p-ratio did not differ between groups, meaning that IER did not lead to a better retention of FFM in comparison to CER. These findings, which are in agreement with previous studies (Coutinho et al., 2018a), suggest that a possible reason for the different observations of Alhamdan et al. might be the severity of the energy deficit in CER (that promotes greater and faster rates of WL) and not the inclusion of days with a higher EI in IER.

6.4.2 Changes in Energy Expenditure and Physical Activity

During periods of negative energy balance, a decrease in RMR is usually observed due to changes in body composition (Bosy-Westphal et al., 2009, Casanova et al., 2019). However, a greater than predicted decrease in RMR (i.e.,

adaptive thermogenesis) has also been observed in several studies (Leibel et al., 1995, Dulloo et al., 2012, Muller et al., 2016), even after adjusting for specific changes in tissue-organ specific components of FFM (Bosy-Westphal et al., 2009). In this study, no significant changes in RMR from baseline to post-WL or differences between groups were observed. However, considering that it was a modest WL intervention (i.e., only $\geq 5\%$ WL) and losses of FFM were minimal (~ 1.3 kg), it is plausible that RMR would not significantly change.

Adaptive thermogenesis was evident in this study although there were no significant differences between groups. The large variability observed between individuals should not be overlooked as previous research has suggested that adaptive thermogenesis could be associated with appetite sensations and EI (Tremblay et al., 2013, Hopkins et al., 2014), a topic that will be discussed in Chapter 7. It has been proposed that adaptive thermogenesis hinders WL efforts (Major et al., 2007) and this will be further discussed in Chapter 8. Nonetheless, the present results suggest that IER did not lead to a lower adaptive thermogenesis as compared to CER. In fact, the mean adaptive thermogenesis was almost double in IER (-48 vs -90 kcal/day for CER and IER, respectively).

Decreases in TDEE are usually observed during WL interventions, partially as a result of a reduction in body size (Melby et al., 2017). Leibel et al. observed a decrease of ~ 550 kcal/day after 10% of WL in individuals with obesity, 244 kcal/day lower than predicted, even after adjusting for changes in FFM (Leibel et al., 1995). Additionally, in the same group of individuals, a ~ 260 kcal/day decrease in non-resting EE was also observed, 165 kcal/day lower than predicted. Furthermore, in response to 24-hour fasting, a ~ 180 kcal decrease in TDEE was observed (Schlögl et al., 2015). In the current study, a decrease in both TDEE and AEE was observed from baseline to the last week of intervention, with no differences between groups. However, this effect disappeared after adjusting for changes in body weight, suggesting that the decrease in EE was mainly due to changes in body size and not in physical activity.

The main determinant of AEE, apart from body size and composition, are physical activity behaviours. Whether levels of physical activity change during dietary-induced WL remains unclear, although a recent systematic review observed that a decrease was present in 63% of the studies analysed (Silva et al., 2018). In the current study, no statistically significant differences were observed from baseline

to the last week of the intervention for physical activity behaviours. It could be that a modest WL intervention was not a strong enough stimulus to elicit a compensatory response in EE and physical activity behaviours. Nonetheless, Coutinho et al. observed no differences in physical activity behaviours from baseline to post 12.5% WL with no differences between IER or CER (Coutinho et al., 2018a) suggesting that a decrease in physical activity is not an inevitable consequence despite substantial WL.

Despite no changes at a group (mean) level, it is important to consider that large inter-individual variability was observed. While the mean change in total physical activity was 16 min/day, this ranged from -130 to +209 min/day. Focusing exclusively on the mean value could erroneously suggest that there were no changes in physical activity behaviours. Considering that AEE represents approximately 20-30% of someone's energy requirements, changes in physical activity at the individual level should not be overlooked. For instance, evidence suggests that even short-term (e.g., 24-hour) changes in TDEE in response to fasting can predict long-term magnitude and rate of WL (Reinhardt et al., 2015), and that physical activity levels are better predictors of WL maintenance success than differences in RMR (Ostendorf et al., 2019). The influence of physical activity on WL at the individual level will be further examined in Chapter 8.

6.4.3 Changes in Appetite Sensations, Energy Intake and Eating Behaviour Traits

One of the commonly proposed reasons underlying WL resistance and weight regain after successful WL interventions is an increase in hunger and decrease in satiety and satiation (Sumithran et al., 2011). In the present study, a 10% decrease in AUC sensations of hunger and desire to eat (and appetite composite scores) was observed, with no concomitant changes in fullness. It could be suggested that these changes occurred because the modest WL in the present study was not a strong enough stimulus to induce compensatory appetite responses. However, similar findings have been observed with higher degrees of WL. For instance, Andriessen et al. observed a 18.1% and 20.2% decrease in AUC hunger and desire to eat after $\geq 8\%$ WL through a low-calorie diet for 8 weeks (Andriessen et al., 2018). Additionally, Bhutani et al. observed a decrease in daily hunger and increased fasting fullness after 3 kg WL ($\sim 3.3\%$) while Coutinho et al.

and Varady et al. observed no changes in perceptions of appetite after 12.5% and 6.5% WL, respectively, although the latter observed an increase in daily fullness sensations (Varady et al., 2013, Bhutani et al., 2013, Coutinho et al., 2018a). Hoddy et al. observed no changes in AUC hunger, but increases in AUC fullness after 10 weeks through IER. Contrastingly, in the present study, free-living daily appetite sensations increased in IER (but not in CER). This finding is in agreement with a previous intervention that compared CER to a 5:2 dietary pattern, observing higher daily hunger scores in the IER group.

Another point to consider is that the post-WL measurements were conducted still in a negative energy balance. It has been postulated that the timing of the measurements could influence appetite sensations (e.g., energy deficit vs energy balance). For instance, although no changes in hunger sensations were observed after 8 weeks of a very low energy ketogenic diet, those levels increased after 4 weeks in a weight stabilisation phase despite no changes in body weight (Nymo et al., 2017). However, although these observations suggest that the timing of the measurement could influence the outcomes of interest, these should not be generalised as participants were following a ketogenic diet. It could be that leaving the state of ketosis (which has been suggested to suppress appetite) leads to a compensatory increase in appetite (Deemer et al., 2020).

Furthermore, previous research suggests that altered appetite-related peptide secretion kinetics can be observed in individuals with overweight and obesity (Korek et al., 2013, Romon et al., 2003, Lean and Malkova, 2016), as well insulin and leptin resistance (Lustig et al., 2004, de Luca and Olefsky, 2008, Areias and Prada, 2015). For instance, a smaller decrease in ghrelin concentration after a meal has been observed in individuals with obesity in comparison to lean, which could influence satiation and meal termination (Korek et al., 2013). Furthermore, after 7 weeks of energy restriction (~11.2kg WL), an improvement (i.e., decrease) in postprandial ghrelin secretion was observed in response to a carbohydrate meal, but not to a fat meal (Romon et al., 2006). As appetite-related peptides were not assessed in the current study, it is not possible to conclude that this decrease was due to physiological changes as other factors could also be involved. However, as appetite-related anorexigenic and orexigenic peptides mainly exert episodic (short-term) influences on appetite, it could be that these could contribute to an improved satiation and satiety response to meals.

Therefore, it could be postulated that WL would normalise the peptide-secretion kinetics and thus improve appetite control, which would in part explain the decrease observed in postprandial appetite sensations.

The nature of the intervention (i.e., individualised meal plan with food provision) could also have led to 'habituation', a process in which a response to a stimulus decreases over time (Epstein et al., 2009). For example, daily exposure to the same meal led to a spontaneous reduction in EI over time in women with or without obesity, although this could be due to other processes independent of hunger like cognitive learning and memory (Epstein et al., 2011). Therefore, it could be that habituation to smaller meals would lead to lower postprandial appetite sensations.

Lastly, it is important to consider that free-living daily appetite sensations increased in IER from baseline to post-WL, which goes in agreement with a previous IER study (Sundfør et al., 2018). This would also go in line with the commonly proposed idea that the motivation to eat increases during WL (Hintze et al., 2017). The reasons for the opposite findings between postprandial and free-living measurements of appetite could be due to the different processes they reflect. While postprandial appetite ratings may be more representative of an episodic appetite response that is specific to that meal and context, it is possible that free-living daily measurements would be more reflective of overall motivation to eat which could be more influenced by tonic processes. Nonetheless, although the variables in this study do not allow for the examination of potential mechanisms, it could be that the feeding pattern in IER (i.e., very-low energy day alternated with *ad libitum* feeding) would lead to a greater increase in overall motivation to eat. As previously suggested, future studies should look at both fasting, postprandial and daily measurements of appetite assessed in the laboratory and free-living conditions, in order to get a complete understanding of how appetite sensations change in response to negative energy balance.

Regarding eating behaviour traits, dietary restraint (both flexible and rigid) increased after WL with no differences between groups, while disinhibition decreased only in the CER group. Since disinhibition reflects a tendency to overeat in response to the presence of different stimuli, the fact that all the food was provided during the intervention for the CER but not IER group could at least partially explain this difference. Additionally, the '*ad libitum* and fast' pattern in the

IER group could lead to more episodes of uncontrolled eating. The absence of a decrease in disinhibition, alongside the increase in free-living daily appetite sensations, could in part explain why it took more time for IER participants to reach 5% WL. In fact, changes in disinhibition were positively associated with changes in free-living appetite ($r = 0.52$; $p = 0.004$) which together could contribute to a lower energy deficit.

Moreover, as higher levels of disinhibition are associated with increased adiposity (Williamson et al., 1995, Westenhoefer et al., 1999), the decrease observed during CER alongside increases in restraint could have important beneficial implications for WL and maintenance. Levels of cognitive restraint (both flexible and rigid) increased in both groups from baseline to post-WL. Although increased levels of rigid restraint could be associated with increased BMI (Westenhoefer, 1991, Westenhoefer et al., 1999, Provencher et al., 2003), a concomitant increase in flexible restraint and decrease in disinhibition, observed in this study, could lead to better outcomes. Therefore, the findings of this study suggest that eating behaviour traits can improve after WL through CER and IER, although CER could potentially be superior in reducing disinhibition.

6.4.4 Limitations

Some limitations in the current study need to be acknowledged. Firstly, although both groups received similar amounts of food packs, the lack of food provision during the 'feeding days' in IER could have influenced some of the outcome measures. However, as a similar mean EI was observed between groups, it could be postulated that the impact, if present, would be small. Nonetheless, it is possible that the lack of food provision could influence eating behaviours during IER in which a decrease in disinhibition was not observed. Secondly, the timing of the measurements was not adjusted according to the phase of the menstrual cycle. However, since the timing of the measures day was dependent on the week participants reached $\geq 5\%$ WL, it would be impossible to adjust for the phase of the menstrual cycle. Lastly, as the WL was modest and the intervention was conducted in a relatively small number of women, which could lead to an insufficient statistical power to detect significant changes in some variables, these results may not be generalisable to a larger number of participants or with more

intensive WL interventions although our findings are similar to previous studies (Coutinho et al., 2018a).

6.5 Conclusion

A matched WL to $\geq 5\%$ through CER or IER in healthy women with overweight and obesity induced similar changes in physiological, psychological and behavioural outcomes. Intermittent energy restriction did not lead to a better preservation of FFM or a lower compensatory reduction in RMR. Additionally, postprandial sensations of hunger and desire to eat decreased after both dietary interventions, but free-living daily appetite sensations increased in IER. Lastly, the results from this study demonstrate that eating behaviour traits can be improved with WL, although levels of disinhibition seemed to only decrease with CER. Importantly, there was a large inter-individual variability regarding the variables examined in this study, highlighting the importance of looking beyond just the mean changes. Lastly, as the number of participants discontinuing the intervention and not achieving the $\geq 5\%$ WL criteria was higher in IER, alongside an increase in daily appetite sensations and no change in disinhibition, it suggests that this dietary pattern may not be suitable for everyone. Overall, these findings support the idea that metabolic, psychological and behavioural compensatory responses, albeit variable, are not inevitable consequences in modest diet-induced WL in women with overweight and obesity.

Chapter Summary:

- No significant changes were observed in body composition, EE (including adaptive thermogenesis) and physical activity following a matched WL to $\geq 5\%$ through IER or CER.
- Although no significant changes were observed in fasting appetite sensations, a decrease in postprandial hunger, desire to eat and appetite composite scores was seen in both groups.
- Daily appetite sensations, reported retrospectively at the end of the day, increased in IER, but not in CER.
- While levels of restraint (both flexible and rigid) increased in both groups, disinhibition only decreased in CER.

Chapter 7 – Associations Between Changes in Body Composition, Energy Expenditure and Physical Activity during Weight Loss with Appetite and Energy Intake

Chapter Aim:

- Examine the associations between changes in body composition, energy expenditure and physical activity during diet-induced weight loss with changes in free-living and laboratory measurements of appetite sensations and energy intake at a test meal.

7.1 Introduction

The amount and composition of the weight lost during periods of negative energy balance induced by diet and/or exercise differs substantially between individuals (Gardner et al., 2018, King et al., 2008, Hopkins et al., 2014, Astrup et al., 1995, Sorbris et al., 1982). The fraction of FFM lost during WL is an emerging and novel area of interest in relation to its effects on appetite and weight management (Forbes, 1987, Hall, 2007). It has been previously suggested that losses of FFM might act as an orexigenic signal during WL, but data to support this hypothesis are limited and often related to studies of extreme WL. For example, it has been observed that greater losses of FFM during semi-starvation or military training were associated with a hyperphagic response that only ceased once FFM was restored to baseline levels (Dulloo et al., 1997, Nindl et al., 1997). More recent evidence has also examined the effect of FFM losses during more modest WL (~10% of initial body weight). Here, greater losses of FFM during WL have also been associated with subsequent weight regain (Vink et al., 2016, Turicchi et al., 2019) and increases in sensations of hunger and desire to eat in men but not in women (Turicchi et al., 2020). However, evidence remains limited and it has yet to be investigated if the postulated increase in appetite sensations arising from losses of FFM is observed in interventions with lower amounts of WL (e.g., 5%)

as the previously mentioned studies reported mean body weight losses of at least 10% (Dulloo et al., 1997, Vink et al., 2016, Turicchi et al., 2019, Turicchi et al., 2020).

Greater than predicted decreases in RMR (i.e., adaptive thermogenesis) and changes in physical activity behaviours (i.e., minutes per day) and associated EE (AEE) have been reported during periods of negative energy balance induced by diet and/or exercise (Bosy-Westphal et al., 2009, Hopkins et al., 2014, Tremblay and Chaput, 2009, Silva et al., 2018). Interestingly, adaptive thermogenesis has been observed to be associated with changes in 24-hour EI after a 12-week supervised exercise intervention (~1.5% WL) (Hopkins et al., 2014), and with changes in hunger after a diet and exercise intervention (~12.4% WL) in a group of 8 men with obesity (Tremblay et al., 2013). In these studies, a greater than predicted decline in RMR was associated with increases in EI and hunger, respectively. These data suggest that individuals who experience compensatory changes in EE may also display compensatory changes in EI. However, few have sought to examine the associations between adaptive thermogenesis and appetite / food intake in the context of WL.

Lastly, no study has ever investigated the associations between changes in free-living physical activity and changes in appetite sensations or EI during diet-induced WL. As it has been suggested in cross-sectional research that physical activity may influence appetite through AEE (Hopkins et al., 2019) and changes in the sensitivity of appetite control (Mayer et al., 1956, Beaulieu et al., 2016), it is important to examine how longitudinal free-living changes during dietary energy restriction influence appetite. For instance, it could be that an increase in physical activity in inactive individuals (as the ones recruited for this study) could lead to a better coupling between EE and EI, with concomitant reductions in EI and appetite sensations.

7.1.1 Objective & Hypotheses

The aim of this chapter was to investigate whether changes in body composition, EE and physical activity during diet-induced WL were associated with changes in appetite sensations (free-living or laboratory composite scores) and EI at a test meal, by testing the following hypotheses:

1. Greater (absolute and relative) losses of FFM would be associated with increases in appetite composite scores (free-living and laboratory measures) and EI at a test meal.
2. Adaptive thermogenesis and reductions in physical activity, AEE and TDEE would be associated with increases in appetite composite scores and EI at a test meal.

7.2 Methods

The analyses presented in this chapter were conducted using the data collected from the study described in Chapter 6. Analyses were done in the participants that completed the intervention ($n = 37$), and data for CER and IER groups were combined as there were no differences between groups regarding changes in body composition, physical activity and EE (see Chapter 6 for a full description of the results and comparison between interventions). A detailed description of the methods used and of the dietary intervention can be found in Chapter 3 and Chapter 6, respectively. In this chapter, the following variables measured at baseline and post-intervention (or last week of the intervention) were utilised to test the aforementioned hypotheses:

- Body composition (FM, FFM and p-ratio)
- RMR (absolute values and adaptive thermogenesis)
- TDEE and AEE
- Minutes of daily physical activity and sedentary behaviour
- Objectively measured *ad libitum* test meal intake
- Laboratory appetite sensations (fasting and AUC composite scores)
- Free-living retrospective daily appetite sensations (composite score)

7.2.1 Statistical Analyses

A detailed description of the statistical approach used in this thesis can be found in Chapter 3.8. In this chapter, changes from baseline to post-intervention (or last week of the intervention for TDEE, AEE, physical activity components and free-living appetite composite scores) were examined using repeated measures ANOVA. Pearson correlations were conducted to examine the associations between changes in body composition, physical activity components and EE with changes in appetite sensations (free-living and laboratory composite scores) and EI at a test meal. Partial correlations with EE variables were conducted after bivariate correlations were detected to examine whether these were independent of changes in body weight.

7.3 Results

7.3.1 Descriptive Characteristics

Changes from baseline to post-intervention for body composition, EE, physical activity components, appetite sensations and test meal EI can be observed in Table 7.1, but are summarised briefly here: body weight ($\eta_p^2 = 0.92$), FM ($\eta_p^2 = 0.85$) and FFM ($\eta_p^2 = 0.70$) decreased over time (all $p < 0.001$), and the mean p-ratio was 0.28 ± 0.31 . Mean RMR and minutes of physical activity did not significantly change over time (all $p \geq 0.16$), while statistically significant decreases in TDEE ($p < 0.001$; $\eta_p^2 = 0.35$) and AEE ($p = 0.003$; $\eta_p^2 = 0.25$) were observed. However, decreases in TDEE ($p = 0.27$; $\eta_p^2 = 0.04$) and AEE ($p = 0.65$; $\eta_p^2 = 0.006$) became non-significant after adjusting for changes in body weight. Lastly, except for a decrease in laboratory AUC appetite composite scores ($p = 0.03$; $\eta_p^2 = 0.13$), no other significant changes were observed for appetite sensations (fasting or free-living) or EI at a test meal (all $p \geq 0.18$) in the whole sample. However, free-living appetite composite scores increased in IER ($p = 0.003$; $\eta_p^2 = 0.57$), but not in CER ($p = 0.26$; $\eta_p^2 = 0.08$).

Table 7.1 – Changes in body composition, energy expenditure, physical activity components, appetite sensations and test meal energy intake between baseline and post-intervention (or last week of the intervention).

	Change ± SD
Body Composition	
Body Weight (kg)	-4.7 ± 1.5 *
Body Weight (%)	-5.9 ± 1.6
Fat Mass (kg)	-3.4 ± 1.7 *
Fat-free Mass (kg)	-1.3 ± 0.9 *
Energy Expenditure	
Resting Metabolic Rate (kcal/day)	11 ± 142
Adaptive Thermogenesis (kcal/day)	-66 ± 138
Total Daily Energy Expenditure (kcal/day)	-124 ± 167 *
Activity Energy Expenditure (kcal/day)	-117 ± 193 *
Physical Activity Components	
Total Physical Activity (min/day)	5 ± 75
Sedentary Time (min/day)	-21 ± 86
MVPA (min/day)	0 ± 32
Appetite Composite Scores and Energy Intake	
Laboratory Fasting Appetite (mm)	3 ± 20
Laboratory AUC Appetite (mm x min)	-719 ± 1936 *
Free-living Retrospective Appetite (mm)	3 ± 13
<i>Ad libitum</i> Test Meal Energy Intake (kcal)	-44 ± 213

MVPA, moderate-to-vigorous physical activity; AUC, area under the curve. N = 37 except for total daily and activity energy expenditure, physical activity components and free-living retrospective appetite composite scores (n = 35). * Statistically significant changes from baseline to post-intervention, p < 0.05.

7.3.2 Associations Between Changes in Body Composition with Appetite Sensations and Energy Intake at a Test Meal

Bivariate correlations between changes in body weight and body composition with changes in appetite sensations (free-living and laboratory composites scores) and EI at a test meal were conducted (Table 7.2). No associations were observed between changes in body weight or body composition (FM, FFM or p-ratio) and changes in appetite sensations (free-living or laboratory composite scores) or EI at the test meal (all $p \geq 0.12$).

Table 7.2 – Associations between changes in body weight and body composition with test meal energy intake and free-living / laboratory measurements of appetite sensations.

		Δ Test Meal EI	Δ Lab Fasting Appetite	Δ Lab AUC Appetite	Δ Free-living Appetite
Δ BW	r	0.08	-0.02	-0.08	0.03
	p	0.66	0.92	0.66	0.88
Δ FM	r	0.02	-0.06	-0.05	-0.14
	p	0.93	0.73	0.78	0.44
Δ FFM	r	0.10	0.08	-0.04	0.27
	p	0.57	0.64	0.83	0.12
P-ratio	r	-0.19	-0.15	-0.10	-0.20
	p	0.25	0.38	0.56	0.24

BM, body weight; FM, fat mass; FFM, fat-free mass; EI, energy intake; Lab, laboratory.

7.3.3 Associations Between Changes in Energy Expenditure and Physical Activity with Appetite Sensations and Energy Intake at the Test Meal

Bivariate correlations between changes in EE (TDEE, AEE and adaptive thermogenesis) and physical activity components with changes in appetite sensations (free-living and laboratory composites scores) and EI at a test meal were conducted (Table 7.3).

Table 7.3 – Associations between changes in energy expenditure and physical activity components with test meal energy intake and free-living / laboratory measurements of appetite sensations.

		Δ Test Meal EI	Δ Lab Fasting Appetite	Δ Lab AUC Appetite	Δ Free-living Appetite
Δ TDEE	r	0.06	0.11	0.17	-0.14
	p	0.74	0.53	0.34	0.46
Δ AEE	r	-0.13	0.08	0.21	-0.34
	p	0.46	0.66	0.23	0.05
AT	r	-0.05	-0.04	0.10	-0.41
	p	0.78	0.83	0.54	0.02
Δ Total PA	r	-0.05	0.12	0.13	-0.13
	p	0.78	0.48	0.45	0.46
Δ Sed	r	-0.12	<0.001	-0.14	0.17
	p	0.49	1.00	0.42	0.35
Δ MVPA	r	0.03	-0.04	0.07	-0.21
	p	0.85	0.81	0.70	0.24

TDEE, total daily energy expenditure; AEE, activity energy expenditure; AT, adaptive thermogenesis; Total PA, total physical activity; Sed, sedentary time; MVPA, moderate-to-vigorous physical activity; Lab, laboratory.

No associations were observed between changes in TDEE or any of the physical activity components with changes in free-living and laboratory appetite composite scores or EI at the test meal ($p \geq 0.24$). However, while adaptive thermogenesis was negatively associated with changes in free-living appetite composite scores ($r = -0.41$, $p = 0.02$) after adjusting for changes in body weight (Figure 7.1), the same was not observed for changes in AEE ($r = -0.34$; $p = 0.06$). Therefore, greater adaptive thermogenesis was associated with increases in free-living appetite sensations. The lack of associations between AEE and free-living appetite sensations was corroborated by the absence of associations between physical activity components (min/day) and appetite sensations (free-living or laboratory composite scores).

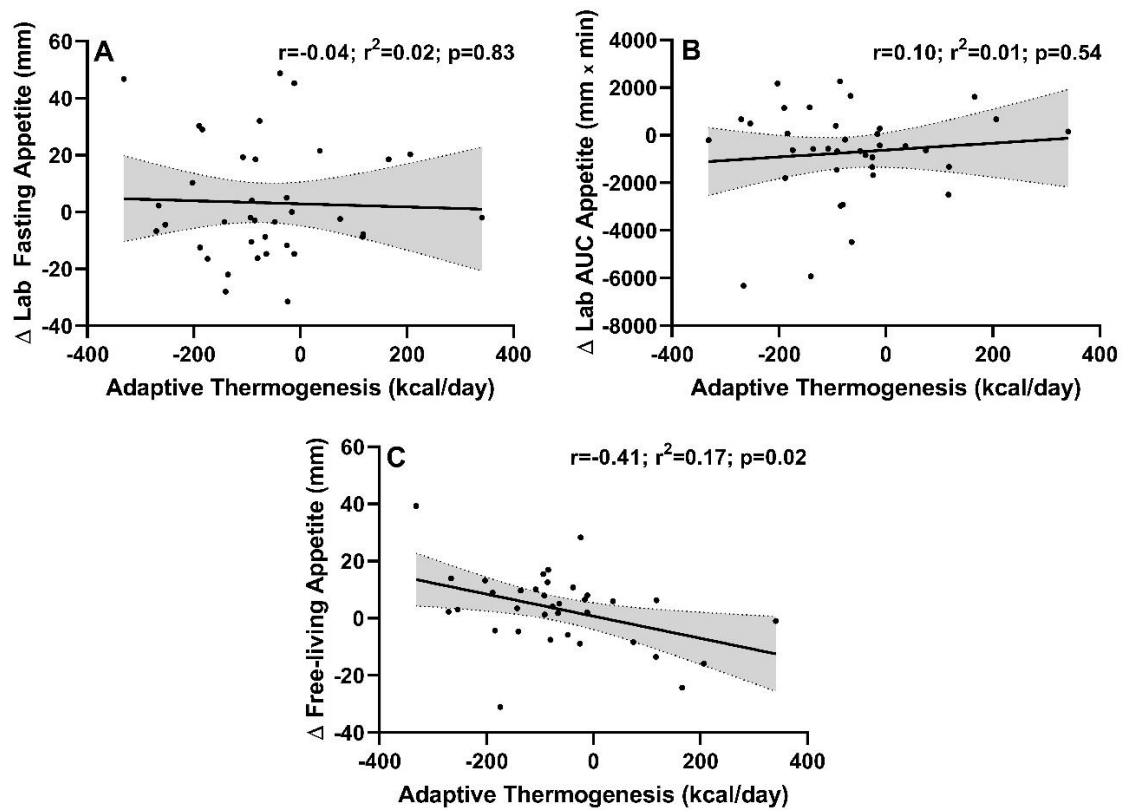


Figure 7.1 – Associations between adaptive thermogenesis and changes in A) laboratory fasting appetite composite scores; B) laboratory AUC appetite composite scores; and C) free-living appetite composite scores. Grey bands represent the 95% confidence intervals.

7.4 Discussion

The aim of this chapter was to investigate whether changes in body composition, EE and physical activity components during WL were associated with changes in appetite sensations (free-living and laboratory composite scores) or EI during a test meal. It was hypothesised that greater losses of FFM (and a higher p-ratio), reductions in physical activity and a greater than predicted decrease in RMR (i.e., adaptive thermogenesis) would be associated with increases in free-living and laboratory appetite composite scores, and EI at a test meal. Results indicated that changes in body composition and physical activity (and AEE) were not associated with changes in appetite sensations (free-living or laboratory composite scores), or EI at the test meal. However, a greater adaptive thermogenesis was associated with increases in free-living appetite sensations, independently of changes in body weight.

7.4.1 Associations Between Changes in Body Composition with Appetite Sensations and Test Meal Energy Intake

Previous studies have demonstrated that greater losses of FFM during WL were associated with a hyperphagic response that only ceased once these tissues were restored to baseline levels (Dulloo et al., 1997, Nindl et al., 1997). However, these findings were reported in interventions comprising of severe energy deficits (> 1000 kcal/day) with body weight losses of ~10kg (Nindl et al., 1997) and ~17kg (Dulloo et al., 1997) in lean individuals. Furthermore, associations have been observed between losses of FFM during WL with subsequent weight regain (Vink et al., 2016, Turicchi et al., 2019) and with increases in sensations of hunger and desire to eat in men but not in women (Turicchi et al., 2020). In the current study, no associations were observed between changes in body composition (FM, FFM or p-ratio) with changes in appetite sensations (free-living or laboratory composite scores) or EI at the test meal.

A possible explanation for these contrasting findings could be the different magnitude of WL / FFM loss and initial body fat percentage between studies. Mean FFM loss in the Minnesota semi-starvation experiment was close to 10kg while the initial mean percentage of body fat was 12.6% (Dulloo et al., 1997). In the study conducted by Nindl et al. (1997), initial body fat percentage was 12% while the mean loss of FFM was 5kg. Turicchi et al. (2020) reported a mean loss of FFM of 4.6kg in men with an initial 32.8% body fat percentage. Interestingly, in the analyses conducted by Turicchi et al. (2020), associations between losses of FFM and changes in appetite sensations were not observed in women, possibly due to the higher initial body fat percentage (44.6% vs 32.8%, respectively) and smaller loss of FFM (27.5% vs 35.3% or 2.7kg vs 4.6kg, respectively). In the current study, mean initial body fat percentage was 41.1% while mean losses of FFM were only 1.3kg. Additionally, mean WL was 4.7kg (5.9%) while other studies reported body weight losses of 17kg (25%) (Dulloo et al., 1997), 8-9kg (9-10%) (Vink et al., 2016), 10kg (11.3%) (Nindl et al., 1997) and 13kg (12%) (Turicchi et al., 2020).

Considering the available data, it is plausible to postulate that a larger amount of WL and greater losses of FFM may be necessary to observe compensatory increases in appetite sensations and a hyperphagic response. Additionally, it may be that leaner individuals are more prone to these compensatory increases in

hunger, possibly due to the higher p-ratio during WL (Hall, 2007). However, more studies are required to investigate the existence of a dose-response effect in which compensatory increases in appetite occur with changes in FFM, and whether specific components of body composition [e.g., specific changes in components of adipose tissue (visceral vs subcutaneous) or FFM (skeletal muscle vs organ mass)] are necessary to invoke a hyperphagic response.

7.4.2 Associations Between Changes in Energy Expenditure and Physical Activity with Appetite Sensations and Test Meal Energy Intake

A greater than predicted decrease in RMR (i.e., adaptive thermogenesis) has been shown to be associated with increases in hunger sensations after a diet and exercise intervention (Tremblay et al., 2013) and 24-hour EI after a 12-week supervised exercise intervention (Hopkins et al., 2014). In the current study, a diet-only intervention, a greater adaptive thermogenesis was associated with increases in free-living appetite sensations (composite scores). These results are in agreement with the findings reported by Hopkins et al. (2014) and Tremblay et al. (2013) by demonstrating that a greater adaptive thermogenesis was associated with increases in free-living appetite sensations (i.e., overall motivation to eat). The combined effects of a greater than predicted decline in RMR and an increase in appetite could potentially hinder WL and weight maintenance efforts. In contrast, there were no associations between adaptive thermogenesis and laboratory-measured appetite sensations. However, it is important to note that they may not reflect the same appetite sensations. While free-living appetite sensations may reflect the overall daily motivation to eat, laboratory (fasting and AUC) measurements of appetite may be more indicative of acute / episodic responses to the test meal which could be influenced by factors such as peptide secretion and gut mechanoreceptors.

Although this is an important finding, the methodology used in this study does not allow for the examination of potential mechanisms. However, Hopkins et al. (2014) suggested that this could be influenced by leptin, as a greater adaptive thermogenesis was associated with greater reductions in leptin ($r = 0.46$; $p = 0.048$). Whilst the influence of changes in leptin on adaptive thermogenesis has

been questioned (Muller et al., 2016), exogenous leptin administration has been shown to attenuate the decline in satiation in weight reduced individuals (Kissileff et al., 2012). Therefore, considering that leptin has been considered an important tonic mediator of food intake (Heini et al., 1998, Blundell et al., 2020), it could be that greater decreases in leptin could underly these increases in appetite.

Interestingly, in this study, adaptive thermogenesis was not associated with total WL or rate of WL (both $p > 0.41$; see Chapter 8 for more details). Therefore, although adaptive thermogenesis was associated with increases in appetite sensations, its direct implications in energy balance regulation and weight management remains unclear. For instance, although a study reported that those that regained weight after WL had a 3-fold higher adaptive thermogenesis (~33 vs ~93kcal/day) after a 13 ± 3 week diet-induced WL intervention to ~10-13% (Bosy-Westphal et al., 2013), another study demonstrated that the associations between adaptive thermogenesis and weight change (WL and weight regain) were weak or non-existent (Martins et al., 2020a) after a 12 ± 3 kg WL (15%) and a weight regain of $52 \pm 36\%$ (1-year) and $83 \pm 52\%$ (2 years).

In the current study, changes in physical activity components and AEE were not associated with changes in free-living or laboratory appetite sensations, or EI at the test meal. This is an interesting finding as previous exercise-only interventions observed changes, albeit highly variable between individuals, in appetite sensations and EI (King et al., 2008, Whybrow et al., 2008, Flack et al., 2018). However, these studies only manipulated physical activity behaviours (by implementing exercise) without providing specific diet instructions. In these studies, by solely influencing EE through exercise, it was possible to examine whether changes in EI and/or appetite sensations would spontaneously occur. In the current study, diet was manipulated while physical activity spontaneously changed (participants were asked to keep their physical activity habits stable throughout the intervention). Although it is possible that changes in physical activity influenced appetite sensations, the concomitant variability in EI between individuals and different degrees of energy deficit (observed by the variability in total WL and mean rate of WL; Chapter 8) may make it difficult to disentangle the independent effects of each energy balance component. Furthermore, studies comparing diet-only with combined diet and exercise / physical activity interventions could provide a better insight into the role of physical activity in

energy balance regulation (i.e., influence in both markers of appetite and EE) in a context of negative energy balance.

7.4.3 Limitations

Some limitations in the current study need to be acknowledged. Previous studies suggesting an influence of losses of FFM on appetite sensations reported mean WL of at least 10%, as well lower initial body fat percentages, which could lead to different compensatory changes from the ones observed in this study. Therefore, while this is not a methodological limitation per se, the findings from this study should not be generalised, although they suggest that compensatory responses in appetite may not be observed in groups of individuals with higher initial levels of body fatness and with lower amounts of WL and FFM loss.

Furthermore, the absence of associations between body composition, EE and physical activity components with test meal EI could be due to the usage of a different methodology from Hopkins et al. (2014). While in this study only one *ad libitum* test meal was provided (3 hours after a fixed breakfast adjusted to 25% of RMR), in the study by Hopkins et al. (2014) individuals consumed 4 *ad libitum* meals throughout the day (24-hour EI). It could be that providing a higher number of meals would have provided a better indication of tonic appetite changes, which would explain the absence of findings using this variable (i.e., no associations between body composition or EE with test meal EI), similar to what was observed in Chapter 4.

7.5 Conclusion

Contrary to previous research and to what was hypothesised, greater losses of FFM and a higher p-ratio were not associated with increases in free-living or laboratory appetite sensations, or EI at the test meal after WL. However, a greater adaptive thermogenesis was associated with increases in free-living appetite sensations. Taken together, the findings from this study do not support the notion that a greater p-ratio leads to compensatory increases in appetite sensations, but highlight the need to further investigate the influence of adaptive thermogenesis on appetite control and energy balance regulation.

Chapter Summary:

- Changes in body composition, physical activity and AEE during WL were not associated with changes in appetite sensations or EI at the test meal.
- A greater adaptive thermogenesis was associated with increases in free-living appetite sensations.

Chapter 8 – Factors Associated with the Mean Rate of Weight Loss and Changes in Body Composition During Diet-Induced Energy Restriction in Women with Overweight and Obesity

Chapter Aims:

- Examine the metabolic, behavioural and psychological factors associated with the mean rate of weight loss during diet-induced energy restriction.
- Investigate the metabolic, behavioural and psychological factors associated with the composition of the weight lost during diet-induced energy restriction.

8.1 Introduction

A faster rate of WL throughout the intervention has been shown to be associated with greater longer-term WL and maintenance (Yank et al., 2014). Furthermore, greater WL (Leung et al., 2017) and BMI reductions (Yackobovitch-Gavan et al., 2015) after 6 and 2 weeks, respectively, have been shown to be associated with higher adherence, a robust predictor of longer-term WL (Alhassan et al., 2008, Del Corral et al., 2011, Lemstra et al., 2016, Acharya et al., 2009). However, although identifying predictors of rate of WL and composition of the weight lost – including appetite sensations, eating behaviour traits and physical activity – could be crucial for longer-term weight management, research on this topic is limited.

Baseline characteristics have generally been shown to be poor predictors of longer-term outcomes (Carraca et al., 2018, Teixeira et al., 2005), although some factors have been previously highlighted. For instance, a higher initial body weight and BMI have been found to be associated with a faster rate of WL (Finkler et al., 2012) and greater WL (Braet, 2006), respectively. Furthermore, higher

baseline ratings of fasting hunger, prospective food consumption and desire to eat have been shown to be associated with a lower WL (Drapeau et al., 2007). Baseline eating behaviour traits (e.g., cognitive restraint) (Teixeira et al., 2005, Filiatrault et al., 2014) and physical activity levels (Yank et al., 2014) have also been shown to be modest predictors of longer-term WL and rate of WL, respectively, but not always (Jakicic et al., 2015, Akter et al., 2019).

An early identification of how someone will respond to an intervention could allow for proactive adjustments to maintain faster rates of WL and higher levels of adherence. Therefore, highlighting predictors of rate of WL in the first weeks of an intervention could be critical to improve longer-term weight management. Higher WL in the first 4-6 weeks has been found to be associated with a greater total WL (Stotland and Larocque, 2005, Miller et al., 2015, Unick et al., 2017, Barnes et al., 2018, James et al., 2018). Notably, a study observed that very early responders ($\geq 0.5\%$ WL) after 2 weeks were 5 times more likely to achieve the 6-month WL goal (Garvin et al., 2016). This suggests that the rate of WL in the first 2 weeks may indicate how an individual is going to respond in the longer-term. Furthermore, changes in eating behaviour traits (e.g., increases in cognitive restraint) after 4 weeks have been observed to be associated with greater total WL (James et al., 2018). While no research regarding the influence of early changes in free-living physical activity on the rate of WL currently exists, a smaller decrease in TDEE and a smaller increase in sedentary time in response to a 24-hour fast was associated with greater WL after 6 weeks (Reinhardt et al., 2015). These studies suggest that shorter-term changes may be important indicators of longer-term WL. However, whether changes in metabolic, behavioural or psychological factors after 2 weeks are associated with longer-term WL outcomes has yet to be examined.

In addition to baseline and short-term predictors of WL, changes across an entire intervention have been shown to be associated with total WL. For instance, increases in fasting desire to eat and prospective food consumption from baseline to post-intervention have been found to be weakly associated with greater WL (Drapeau et al., 2007). Furthermore, increases in cognitive restraint and decreases in disinhibition and susceptibility to hunger have also been observed to be associated with greater WL (Batra et al., 2013). Although data on physical activity is limited, a study observed that the group that achieved a higher WL had

greater increases in moderate-to-vigorous physical activity (Fazzino et al., 2017). It has also been suggested that adaptive thermogenesis could hinder WL efforts (Tremblay and Chaput, 2009), but studies supporting this hypothesis are limited. Lastly, as well as the amount of WL, the composition of the weight lost may vary between individuals during diet-induced energy restriction (Turicchi et al., 2020). Research suggests that the fraction of FFM lost during diet-induced WL (i.e., p-ratio = Δ FFM / Δ body weight) is mainly influenced by initial body fatness, total WL and rate of WL (Forbes, 1987, Hall, 2007). However, it remains unknown if other factors are associated with the composition of the weight lost such as shorter-term p-ratio or changes in free-living physical activity. For instance, a study reported an almost 3-fold greater reduction in body fat percentage (with 65% greater WL and no loss of FFM) after 6-months of a hypocaloric diet combined with moderate physical activity (10.000 steps per day at a moderate-vigorous rate) in comparison to diet only (sedentary condition; only general physical activity recommendations) in previously inactive individuals with overweight and obesity (Hernández-Reyes et al., 2019). Therefore, it could be that changes in physical activity could lead to a better preservation of FFM (i.e., lower p-ratio). As greater losses of FFM have been found to be associated with greater increases in hunger and desire to eat in men (Turicchi et al., 2020), hyperphagia (Dulloo et al., 1997) and weight regain (Vink et al., 2016, Turicchi et al., 2019), identifying factors that lead to these unfavourable changes in body composition may be critical to improve longer-term weight management.

8.1.1 Objective & Hypotheses

The aim of this chapter was to investigate the metabolic, behavioural and psychological factors associated with the mean rate of WL and the composition of the weight lost during diet-induced energy restriction by testing the following hypotheses:

1. Higher baseline body weight, body fat percentage, cognitive restraint and physical activity, and lower appetite sensations and disinhibition would be associated with a faster mean rate of WL.

2. A faster mean rate of WL and a greater decrease in sedentary time after 2 weeks would be associated with a faster mean rate of WL throughout the intervention.
3. Greater increases in appetite sensations, cognitive restraint and physical activity, and decreases in disinhibition and susceptibility to hunger throughout the intervention would be associated with a faster mean rate of WL.
4. Higher initial body fat percentage, greater total WL, a faster mean rate of WL and a lower amount of physical activity (baseline levels and greater decreases from baseline to post-intervention) would be associated with a higher p-ratio.

8.2 Methods

The analyses presented in this chapter were conducted using the data collected from the study described in Chapter 6. Analyses were done in the participants that completed the intervention ($n = 37$). Although data were combined for analyses, these were adjusted by condition as the mean rate of WL was different between groups (CER: $0.8 \pm 0.3\%/week$; IER: $0.6 \pm 0.3\%/week$; $p = 0.01$). In the current chapter, data collected during week 2 (both measures week and measures day – conducted after 16 ± 3 days) will be included to examine the associations between shorter-term changes and longer-term outcomes. The following variables will be utilised to test the previously mentioned hypotheses:

- Body weight (absolute value and rate of WL)
- Body composition (FM, FFM and p-ratio)
- RMR (absolute value and adaptive thermogenesis)
- TDEE and AEE
- Minutes of physical activity and sedentary behaviour
- Laboratory appetite sensations (fasting and AUC composite scores)
- Free-living retrospective daily appetite sensations (composite score)
- Eating behaviour traits (cognitive restraint, disinhibition and susceptibility to hunger)

8.2.1 Mean Rate of Weight Loss

In the present study, total WL and the time to complete the intervention ranged from -0.7 to 8.3% and 35 to 99 days, respectively. Therefore, to control for the variability in intervention duration and total WL between participants, mean rate of WL throughout the intervention was calculated. In the scientific literature (Finkler et al., 2012, Nackers et al., 2010, Vink et al., 2016, Coutinho et al., 2018b, Turicchi et al., 2019), rate of WL has been calculated using the following equation:

$$\text{Rate of Weight Loss (\% or kg per week)} = \frac{\text{Total Weight Loss (\% or kg)}}{\text{Time (weeks)}}$$

8.2.2 Statistical Analyses

A detailed description of the statistical approach used in this thesis can be found in Chapter 3.8. In this chapter, repeated measures ANOVA were conducted to examine changes between baseline and week 2. Effect sizes are reported as partial eta-squared (η_p^2) for the ANOVAs. Furthermore, partial correlations (adjusting for group, i.e., CER or IER) were conducted to examine the associations between body weight, body composition, appetite sensations (free-living and laboratory composite scores), test meal EI, eating behaviour traits, EE and physical activity components at baseline, changes from baseline to week 2 and changes from baseline to post-intervention with mean rate of WL. Partial correlations with EE variables were further adjusted for total WL to examine whether these were independent of changes in body weight. As changes in body composition were similar between groups (see Chapter 6), analyses regarding the factors associated with the p-ratio were conducted without adjusting for group. Analyses were conducted in the 37 participants that completed the intervention (i.e., completers that reached $\geq 5\%$ WL or within 12 weeks). Analyses were also performed after removing one participant that gained weight (0.6kg; 0.7%) as it could influence the results. Differences that emerged from these analyses were highlighted in each section.

8.3 Results

8.3.1 Descriptive Characteristics

Baseline values and changes from baseline to post-intervention (or last week of the intervention) can be found in Chapters 6 and 7. Changes from baseline to week 2 for body weight, body composition, EE, physical activity components, appetite sensations (free-living and laboratory composite scores), test meal EI and eating behaviour traits can be found in Table 8.1. As can be observed, statistically significant decreases in body weight ($p < 0.001$; $\eta_p^2 = 0.84$), FM ($p < 0.001$; $\eta_p^2 = 0.57$), FFM ($p = 0.001$; $\eta_p^2 = 0.31$), body fat percentage ($p = 0.009$; $\eta_p^2 = 0.17$), TDEE ($p = 0.002$; $\eta_p^2 = 0.23$), AEE ($p = 0.02$; $\eta_p^2 = 0.14$) and increases in dietary restraint ($p = 0.006$; $\eta_p^2 = 0.20$), flexible restraint ($p = 0.02$; $\eta_p^2 = 0.15$), rigid restraint ($p = 0.01$; $\eta_p^2 = 0.17$), and free-living appetite sensations ($p = 0.001$; $\eta_p^2 = 0.27$) occurred during the first 2 weeks of the intervention. As in Chapter 6, changes in TDEE ($p = 0.16$; $\eta_p^2 = 0.06$) and AEE ($p = 0.66$; $\eta_p^2 = 0.006$) became non-significant after adjusting for changes in body weight. Apart from flexible restraint (difference between groups - $p = 0.04$; $\eta_p^2 = 0.11$) that increased in CER but not in IER, no differences were observed between conditions (all $p \geq 0.27$; $\eta_p^2 \leq 0.04$). At week 2, mean rate of WL was $0.2 \pm 0.1\%$ /week with no differences between groups ($p = 0.80$).

Table 8.1 – Changes in body weight, body composition, energy expenditure, physical activity components, composite scores for appetite sensations, test meal energy intake and eating behaviour traits between baseline and week 2.

	Change ± SD (Baseline to week 2)
Body Composition	
Body Weight (kg)	-1.9 ± 0.9 *
Body Weight (%)	-2.4 ± 1.0
Fat Mass (kg)	-1.2 ± 1.1 *
Body Fat Percentage (%)	-0.5 ± 1.2 *
Fat-free Mass (kg)	-0.7 ± 1.0 *
P-ratio	0.38 ± 0.60
Energy Expenditure	
Resting Metabolic Rate (kcal/day)	0 ± 112
Total Daily Energy Expenditure (kcal/day)	-124 ± 167 *
Activity Energy Expenditure (kcal/day)	-117 ± 193 *
Physical Activity Components	
Total Physical Activity (min/day)	-4 ± 46
Sedentary Time (min/day)	-12 ± 54
MVPA (min/day)	-4 ± 25
Appetite Composite Scores and Energy Intake	
Laboratory Fasting Appetite (mm)	6 ± 23
Laboratory AUC Appetite (mm x min)	-142 ± 1556
Free-living Retrospective Appetite (mm)	7 ± 11 *
<i>Ad libitum</i> Test Meal Energy Intake (kcal)	-36 ± 183
Eating Behaviour Traits	
Dietary Restraint	1.5 ± 3.1 *
Rigid Restraint	0.6 ± 1.3 *
Flexible Restraint	0.6 ± 1.6 *
Disinhibition	-0.4 ± 2.2
Susceptibility to Hunger	-0.03 ± 2.3

MVPA, moderate-to-vigorous physical activity; AUC, area under the curve. * Significant change between baseline and week 2 ($p < 0.05$). N = 37 except for TDEE, AEE and physical activity components (n = 36).

The mean rate of WL throughout the intervention for each participant can be seen in Figure 8.1. While the mean value of the group was $0.7 \pm 0.3\%$ /week, it ranged from -0.1% to 1.2% /week. The next sections will be focused on examining the metabolic, behavioural and psychological factors associated with the inter-individual variability in the mean rate of WL throughout the intervention.

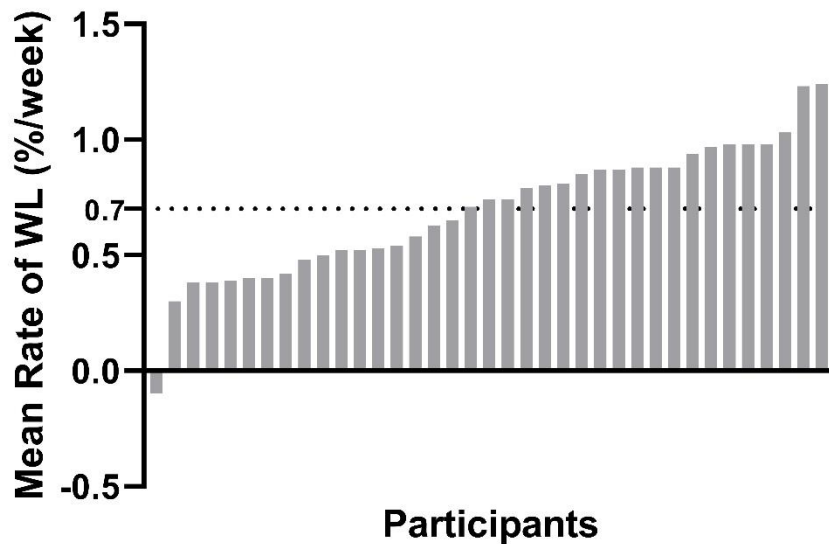


Figure 8.1 – Mean rate of weight loss of the 37 participants that completed the intervention. The dotted horizontal line represents the mean value of the group.

8.3.2 Associations Between Baseline Characteristics and Mean Rate of Weight Loss

Correlations between baseline body weight, body fat percentage, appetite sensations (free-living and laboratory composite scores), test meal EI, eating behaviour traits, EE and physical activity components with mean rate of WL were conducted. No associations were observed between any baseline characteristics with mean rate of WL throughout the intervention (all $p \geq 0.06$). Similar findings were obtained when the participant who gained weight was removed.

8.3.3 Associations Between Changes from Baseline to Week 2 and Mean Rate of Weight Loss

Mean rate of WL at week 2 was positively associated with mean rate of WL throughout the intervention ($r = 0.43$; $p = 0.01$; Figure 8.2-A), indicating that the participants that had a faster initial mean rate of WL presented a faster mean rate of WL throughout the intervention. No associations were observed between changes in appetite sensations (free-living or laboratory composite scores), test meal EI or eating behaviour traits from baseline to week 2 and mean rate of WL throughout the intervention (all $p \geq 0.21$). Similar findings were obtained when the participant who gained weight was removed.

Changes in TDEE, AEE and physical activity components after 2 weeks were not associated with mean rate of WL (all $p \geq 0.12$). However, after removing the participant that gained weight, associations between changes in sedentary time ($r = -0.38$; $p = 0.03$) and mean rate of rate WL throughout the intervention emerged ($r = -0.38$, $p = 0.03$; Figure 8.2-B). The participants that had the greatest decreases (or smaller increases) in sedentary time after 2 weeks of intervention had a faster mean rate of WL throughout the intervention.

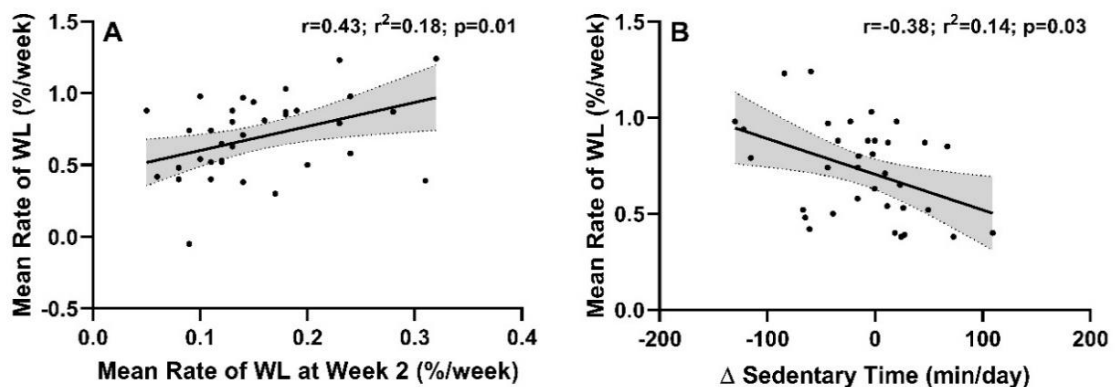


Figure 8.2 – Associations between mean rate of weight loss with A) mean rate of weight loss at week 2; and B) changes between baseline and week 2 in sedentary time. Grey bands represent the 95% confidence intervals. $N = 37$ for A and $n = 36$ for B (participant that gained weight was removed).

8.3.4 Associations Between Changes from Baseline to Post-intervention and Mean Rate of Weight Loss

No associations were observed between changes in appetite sensations (free-living or laboratory composite scores) or test meal EI across the intervention and mean rate of WL (all $p \geq 0.53$). Furthermore, no associations were observed between changes in eating behaviour traits and mean rate of WL (all $p \geq 0.16$). Similar findings were obtained when the participant who gained weight was removed ($p \geq 0.06$).

Regarding EE, changes in TDEE ($r = 0.46$; $p = 0.006$) and AEE ($r = 0.51$; $p = 0.002$), but not in RMR ($r = -0.06$; $p = 0.72$) or adaptive thermogenesis ($r = 0.01$; $p = 0.98$), were positively associated with mean rate of WL (Figure 8.3). These were unaffected after adjusting for changes in body weight (TDEE, $r = 0.46$ $p = 0.007$; AEE, $r = 0.42$, $p = 0.02$) or when the participant who gained weight was removed.

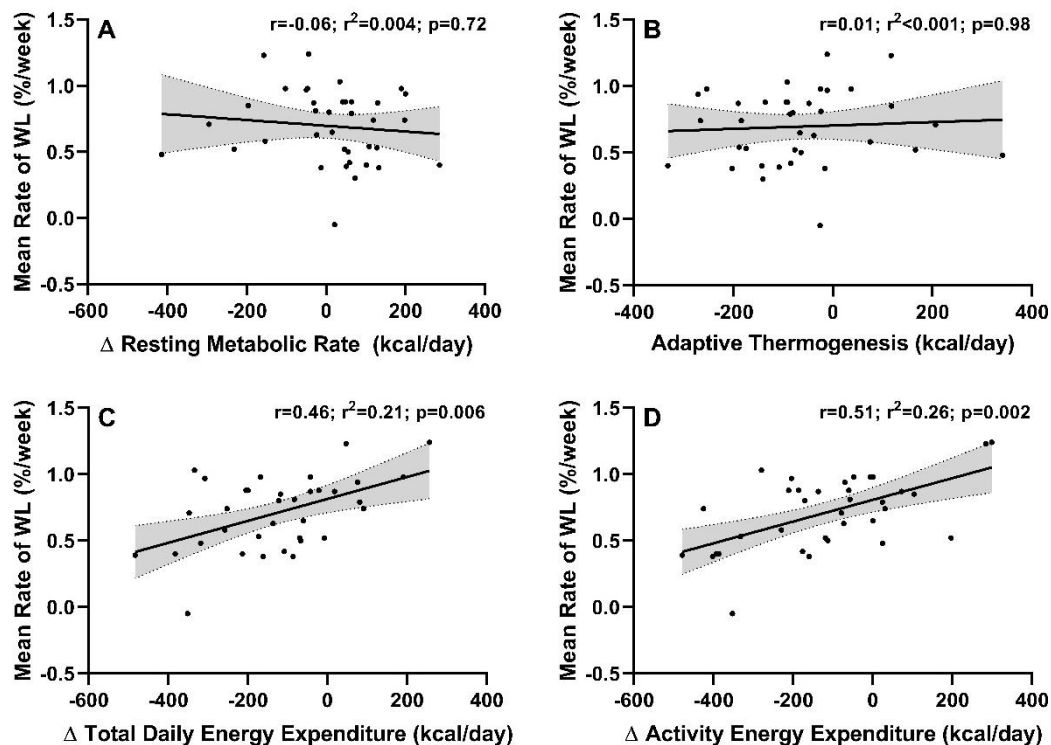


Figure 8.3 – Associations between mean rate of weight loss and A) changes in resting metabolic rate; B) adaptive thermogenesis; C) changes in total daily energy expenditure and D) changes in activity energy expenditure. Grey bands represent the 95% confidence intervals. $N = 37$ (A & B) and $n = 36$ (C & D).

Associations between changes in physical activity components and the mean rate of WL can be observed in Figure 8.4. Positive associations were observed between mean rate of WL and changes in total physical activity ($r = 0.54$; $p = 0.001$) and moderate-to-vigorous physical activity ($r = 0.55$; $p = 0.001$) while a negative association was observed with changes in sedentary time ($r = -0.52$; $p = 0.002$). These demonstrate that participants that increased EE and time in physical activity behaviours throughout the intervention had a faster mean rate of WL.

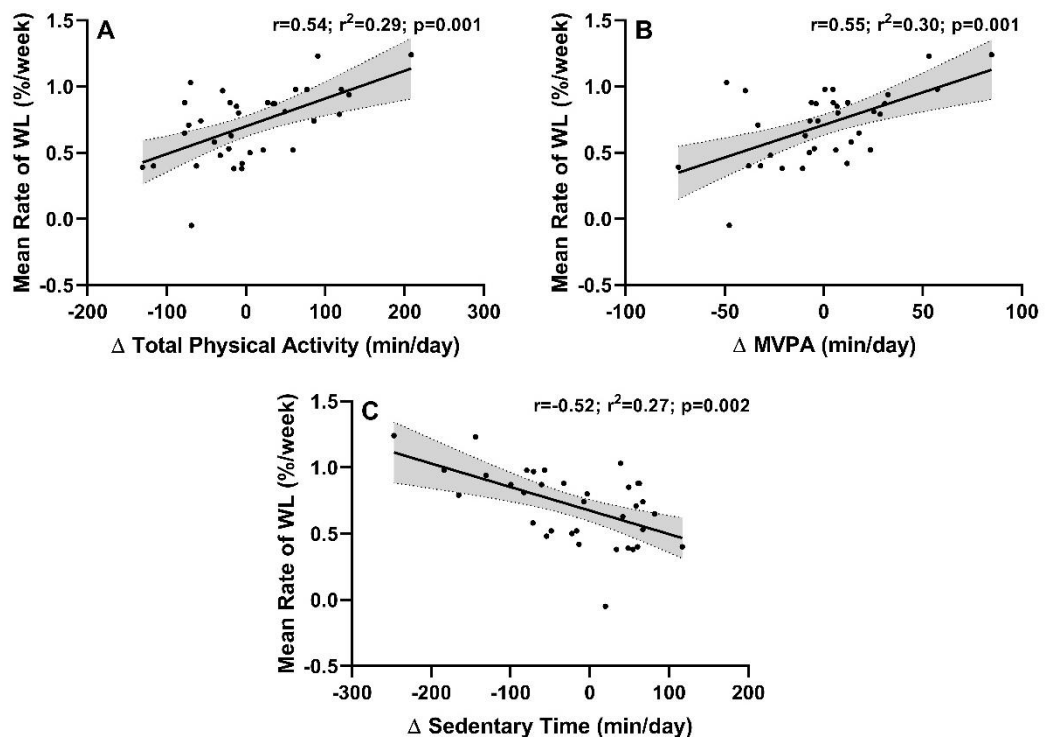


Figure 8.4 – Associations between mean rate of weight loss and changes in A) total physical activity; B) moderate-to-vigorous physical activity (MVPA) and C) sedentary time. Grey bands represent the 95% confidence intervals. $N = 36$.

8.3.5 Factors Associated with the Composition of the Weight Lost

In addition to assessing the factors associated with the mean rate of WL during the intervention, it was also of interest to examine the factors associated with changes in body composition (i.e., p-ratio). Initial body fat percentage, mean rate of WL and changes in body weight were not associated with the p-ratio throughout the intervention (all $p \geq 0.15$). Baseline levels and changes in physical activity components did not influence the p-ratio (all $p \geq 0.50$). The only variable that was associated with p-ratio throughout the intervention was the p-ratio at

week 2 ($r = 0.40$; $p = 0.002$) and this got stronger when the participant that gained weight was excluded ($r = 0.57$; $p < 0.001$; Figure 8.5).

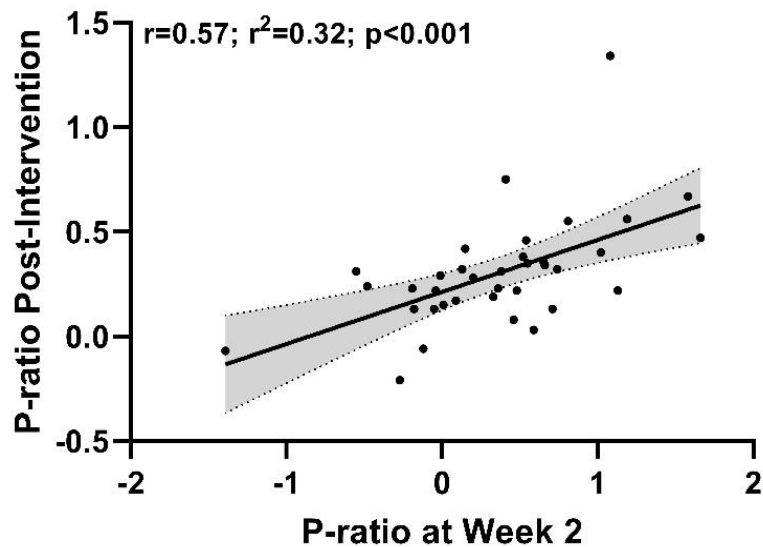


Figure 8.5 – Associations between the p-ratio at week 2 and p-ratio after the intervention. Grey bands represent the 95% confidence intervals. $N = 36$.

8.4 Discussion

The aim of this chapter was to investigate the metabolic, behavioural and psychological factors associated with the mean rate of WL and the composition of the weight lost during diet-induced energy restriction. Contrary to what was hypothesised, baseline characteristics (e.g., body weight, body fatness, eating behaviour traits, appetite sensations and levels of physical activity) were not associated with mean rate of WL throughout the intervention. Furthermore, changes in appetite sensations (free-living or fasting / AUC laboratory composite scores), test meal EI and eating behaviour traits at week 2 or across the intervention were not associated with mean rate of WL. However, mean rate of WL and changes in sedentary time at week 2 were associated with mean rate of WL throughout the intervention. Favourable changes in EE (i.e., increases in TDEE and AEE) and physical activity components (i.e., increases in daily minutes of physical activity and decreases in sedentary time), but not in RMR or adaptive thermogenesis, across the entire intervention were associated with a faster mean rate of WL. Lastly, p-ratio at week 2 (but not initial body fat percentage, total WL, mean rate of WL or physical activity) was strongly associated with p-ratio post-intervention.

8.4.1 Associations Between Baseline Characteristics and Mean Rate of Weight Loss

A previous review highlighted initial body weight as a predictor of rate of WL expressed as kg/week (Finkler et al., 2012). Although a trend for a weak negative association ($r = -0.30$; $p = 0.08$) between initial body weight and mean rate of WL (expressed as kg/week) emerged in the current study, the same was not observed when mean rate of WL was expressed as % WL/week ($p = 0.76$). Therefore, the observation by Frinkler et al. could be due to the fact that the same relative energy restriction (i.e., percentage of energy restriction) may lead to a similar percentage of WL (e.g., 5% WL), but a greater absolute WL (in kg). For example, a 10% WL for a 100kg individual would correspond to 10kg, but only 6kg for a 60kg individual, although the relative WL (i.e., as a percentage) would be the same. Thus, a higher baseline body weight could be associated with a greater absolute WL, as observed by Finkler et al., but not relative (as a percentage) WL. As no baseline characteristics were associated with mean rate of WL, the data from the current study corroborates previous evidence suggesting that these may not be good predictors of longer-term outcomes (Teixeira et al., 2005, Carraca et al., 2018).

8.4.2 Associations Between Changes from Baseline to Week 2 with Mean Rate of Weight Loss

Research assessing shorter-term changes as predictors of longer-term outcomes is limited. However, several publications have now reported that WL in the first weeks of an intervention (usually ~4-6 weeks) is a significant predictor of total WL (Barnes et al., 2018, James et al., 2018, Tronieri et al., 2019). Furthermore, changes in eating behaviour traits after 4 weeks have also been observed to be associated with total WL (James et al., 2018). In the current study, a faster rate of WL in the first 2 weeks was positively associated with mean rate of WL throughout the intervention. This corroborates earlier findings suggesting that WL after 2 weeks may indicate how an individual will respond in the longer-term (Garvin et al., 2016).

No associations were observed between changes in eating behaviour traits (or appetite sensations and test meal EI) after 2 weeks and mean rate of WL

throughout the intervention. Although significant changes in eating behaviour traits were observed after 2 weeks, it could be that these were not large or variable enough to detect an association with the mean rate of WL. These findings contrast with the ones by James et al., possibly due to 2 reasons: 1) in this study, changes were assessed after 2 weeks instead of 4 in which larger changes could possibly be detected; and 2) the smaller sample size in the present study could have limited the statistical power to detect significant results. For instance, in the study by James et al., not only data was assessed in 186 women (vs 37 in the current study), the association between changes in eating behaviour traits and WL was only detected in some dimensions (e.g., cognitive restraint but not disinhibition) and not in all models. This suggests that if present, the associations between shorter-term changes in eating behaviours and WL outcomes may not be robust and therefore hard to detect.

Lastly, decreases in sedentary time after 2 weeks were associated with a faster mean rate of WL throughout the intervention. Although the study design differs substantially, these findings are in agreement with the results reported by Reinhardt et al. in which smaller increases in sedentary time in response to a 24-hour fast were associated with a greater total WL (and rate of WL). Altogether, these findings are of importance as they demonstrate that changes in body weight and physical activity after 2 weeks of diet-induced energy restriction may permit an identification of how well someone will respond in the longer-term. Importantly, these changes occurred despite participants being asked not to change their physical activity habits during the intervention and therefore these occurred spontaneously. This information could be of critical importance to improve weight management success as practitioners would be able to be proactive and adjust the intervention early based on shorter-term responses, possibly avoiding a decline in adherence that tends to occur over time (Unick et al., 2017). For instance, if an individual demonstrates a spontaneous reduction in physical activity during diet-induced energy restriction, a focus on increasing physical activity behaviours could be important to sustain a higher EE and maintain a faster rate of WL.

8.4.3 Associations Between Changes from Baseline to Post-intervention with Mean Rate of Weight Loss

Contrary to previous research (Drapeau et al., 2007) and to what was hypothesised, changes in appetite sensations from baseline to post-intervention were not associated with the mean rate of WL. Regarding eating behaviour traits, these were also not associated with mean rate of WL throughout the intervention. These findings contrast with previous evidence suggesting that changes in eating behaviour traits (e.g., increases in cognitive restraint and decreases in disinhibition) are associated with changes in body weight (Ahern et al., 2012, Koopman et al., 2018). As mentioned in the previous section, a possible explanation for this finding could be the sample size. For instance, Koopman et al. and Ahern et al. investigated 120 and 772 individuals, respectively. In the 37 individuals that took part in the current study, trends for associations between changes in cognitive restraint ($r = 0.27$; $p = 0.12$) and susceptibility to hunger ($r = -0.33$; $p = 0.06$) with mean rate of WL emerged. These associations follow the same direction as previous evidence suggesting that increases in cognitive restraint and decreases in susceptibility to hunger are associated with greater WL. Cognitive restraint reflects a trait that represents a pressure to limit food intake (usually for weight control) while susceptibility to hunger represents a higher propensity to eat in response to hunger sensations. Therefore, these associations would suggest that a higher ability to restraint food intake, even in the presence of higher hunger sensations, could facilitate the maintenance of a greater energy deficit and therefore a faster rate of WL.

Changes in RMR and adaptive thermogenesis have been highlighted as factors that could potentially resist WL and complicate weight management (Tremblay and Chaput, 2009, Johannsen et al., 2012). In the current study, although a large inter-individual variability in adaptive thermogenesis was present (see Chapter 6 for a detailed description), no associations were observed between changes in RMR or adaptive thermogenesis and mean rate of WL. These findings are in contrast with the idea that higher adaptive thermogenesis resists WL. Moreover, although it has been suggested that a higher adaptive thermogenesis could predispose individuals for weight regain (Bosy-Westphal et al., 2013), a recent study demonstrated that if it has an effect, it is limited (Martins et al., 2020a). Therefore, although adaptive thermogenesis is a common and reproducible

compensatory response (Muller et al., 2016), its influence on weight management warrants further investigation.

An important finding from the current study was that both TDEE and AEE were strongly associated with the mean rate of WL, suggesting that individuals that increased EE (or had a lower decrease over time) presented faster mean rates of WL. These findings were further confirmed after assessing physical activity components, with strong associations emerging and suggesting that participants that had greater increases in physical activity and decreases in sedentary time had faster mean rates of WL. These findings are in agreement with a previous study in which 142 women with overweight and obesity went through a 6-month WL with a structured meal plan and instructions to increase physical activity levels (Fazzino et al., 2017). Researchers observed that the group of individuals who had greater increases in the amount of moderate-to-vigorous physical activity lost more weight. As in the current study changes in physical activity components were strongly correlated with changes in EE ($r = 0.71 - 0.89$), the main mechanism to explain these associations may be the higher energy deficit induced by the greater EE through increases in physical activity. An important observation was that participants were asked to keep their physical activity habits constant throughout the intervention and the prescribed EI was based on baseline energy requirements/physical activity levels. Therefore, changes in physical activity and EE would have the potential to modulate the energy deficit and thus influence the mean rate of WL. However, although changes in physical activity behaviours during periods of negative energy balance have previously been observed (Silva et al., 2018), it is not possible with the current data to identify whether these were voluntary (e.g., increased motivation to lose weight due to being part of an intervention) or involuntary changes.

Nonetheless, these findings and the ones from studies reporting greater WL with combined diet-exercise interventions vs diet alone (Clark, 2015) highlight the importance of physical activity for weight management and energy balance regulation. Not only it may help in increasing or maintaining a higher EE, but it may also contribute to a better control of appetite (Beaulieu et al., 2016, Beaulieu et al., 2018, Mayer et al., 1956, Blundell et al., 2015b). As previous research typically emphasizes the benefits of physical activity for weight maintenance (Ostendorf et al., 2019, Varkevisser et al., 2019), this study demonstrates and

supports the idea that it could also be a critical coadjutant during WL and highlights the need to monitor both sides of the energy balance equation in weight management interventions. Likewise, this may be crucial in empowering people as these data suggest that behaviour seems to have a stronger impact on weight management and energy balance regulation than metabolic adaptations (e.g., adaptive thermogenesis), that are mainly out of the individual's ability to control. Lastly, although genetics may influence the predisposition to be physically active, genes do not seem to be too tight to prevent individuals from exercising and voluntarily being more active (Zhang and Speakman, 2019), and are beyond the scope of this thesis.

8.4.4 Factors Associated with the Composition of the Weight Lost

Although investigating predictors of total WL and rate of WL may be crucial for longer-term weight management, it is also important to understand the factors associated with changes in the composition of the weight lost. As greater losses of FFM have been associated with increases in hunger and desire to eat in men (Turicchi et al., 2020), hyperphagic responses (Dulloo et al., 1997) and greater weight regain (Vink et al., 2016, Turicchi et al., 2019), it is critical to investigate strategies that may allow for a better preservation of FFM and potentially improve longer-term weight management. Previous studies highlighted initial body fat percentage, total WL and rate of WL as the strongest predictors of p-ratio during diet-induced energy restriction (Forbes, 1987, Hall, 2007). However, in the current study, these associations were not observed, possibly due to 3 reasons: 1) the range of body fat percentage in this study was restricted to individuals with overweight and obesity; losses of FFM may be greater in leaner individuals; 2) the curves introduced by Forbes (1987) and Hall (2007) comprised of greater amounts of WL (> 10kg) and therefore, the range of WL in the current study (+0.7 to -8.3%; +0.6 to 8.9kg) may not have been enough to observe an association between these variables (i.e., association between p-ratio and initial body fat percentage, total WL and mean rate of WL); and 3) the studies used to create the models generally utilised fasting, very-low energy diets or surgery, which could substantially influence not only total WL, but the composition of the weight lost. Interestingly, in the current study, p-ratio at week 2 was strongly associated with p-ratio post-intervention. This suggests that during diet-induced energy

restriction, the p-ratio may be relatively stable and determined by the individual's genetics, but this could not be tested in the current study and therefore remains to be confirmed.

Interestingly, a recent analysis demonstrated a strong association ($r = 0.60$) between the changes in body composition in 8 individuals during 2 identical 1-week diet-induced WL interventions (50% energy restriction) separated by 1 year (Müller and Bosy-Westphal, 2019). These suggest that changes in body composition (i.e., loss of FFM during WL) may be replicable and an individual trait. If this is the case, it may be that in individuals predisposed to having a higher p-ratio in response to energy restriction, it would be important to utilise strategies known to better preserve FFM during periods of negative energy balance. This could include performing exercise (especially resistance training) (Stiegler and Cunliffe, 2006, Clark, 2015) and higher protein intakes (Helms et al., 2014).

8.4.5 Limitations

Some limitations in the current study need to be acknowledged. Firstly, the equation used to calculate the rate of WL in this (and previous studies) assumes that this is linear over time. However, this may be inaccurate due to factors such as changes in both EI and EE / physical activity that may occur during periods of negative energy balance (Melby et al., 2017, Casanova et al., 2019). However, the main aim of this study was to identify the factors associated with WL variability (i.e., why on average some individuals lost weight at a faster rate) and not with the intra-individual variability in weekly changes in body weight. Therefore, calculating a 'mean rate of WL' as it was conducted in this study allowed for an examination of the factors that explain why some individuals lose weight faster (on average).

Assessing changes in EE and physical activity at baseline, week 2 and post-intervention assumes that the changes between these time points are linear and does not guarantee that the participants' responses in the shorter-term and longer-term follow the same pattern / direction. However, although it is recognised that changes in EE and physical activity are unlikely to be linear, changes at week 2 and post-intervention were strongly correlated (all $r = 0.60 - 0.70$; $p < 0.001$). This suggests that participants that had greater decreases in EE and physical

activity after 2 weeks were the ones that also had the greatest decrease between baseline and post-intervention.

8.5 Conclusion

The results from this study corroborate previous findings demonstrating that baseline characteristics may not be good indicators of longer-term WL. Furthermore, a faster mean rate of WL and decreases in sedentary time after 2 weeks were both associated with a faster mean rate of WL throughout the intervention. In this study, changes (at week 2 and across the intervention) in appetite sensations, EI or eating behaviour traits were not associated with mean rate of WL. However, increases in TDEE, AEE and physical activity behaviours throughout the intervention were associated with a faster mean rate of WL. Lastly, p-ratio at week 2 (but not initial body fat percentage, total WL and mean rate of WL) was associated with the p-ratio post-intervention. These findings highlight the importance of physical activity during WL interventions and suggest that shorter-term changes in physical activity behaviours and body weight may allow for an early identification of how well an individual will respond in the longer-term. This is of critical importance as it may allow for proactive adjustments to be made in order to maintain an efficient rate of WL and avoid a decline in adherence.

Chapter Summary:

- Baseline metabolic, behavioural and psychological factors were not associated with the mean rate of WL.
- Greater decreases in sedentary time and a faster mean rate of WL after 2 weeks were associated with a faster mean rate of WL throughout the intervention.
- Increases in TDEE, AEE and physical activity, and reductions sedentary time across the intervention were associated with a faster mean rate of WL.
- P-ratio after 2 weeks was strongly associated with the p-ratio post-intervention.

Chapter 9 – General Discussion

9.1 Thesis Overview

This thesis examined the associations between body composition and EE with appetite and EI under conditions of energy balance and across a spectrum of body fatness. Furthermore, the changes in body composition, EE and physical activity occurring during dietary-induced WL and their impact on compensatory changes in appetite, EI and body weight were also investigated.

These PhD aims were explored using a series of studies (one controlled WL randomised controlled trial and three cross-sectional) comprising of metabolic, psychological and behavioural measurements to assess several dimensions of appetite control and energy balance. The findings from this thesis contribute to a better understanding of the influence of body composition and EE on the drive to eat by including participants with a wide range of body fatness (Chapter 4), during energy balance (Chapters 4 and 5) and energy deficit (Chapter 7), and conducting body composition analysis at the tissue-organ level using MRI (Chapter 5). Furthermore, the findings from this thesis expanded the knowledge regarding the compensatory responses occurring during dietary-induced WL via CER or IER (Chapter 6), as well the factors associated with inter-individual differences in the rate and composition of the weight lost (Chapter 8). The main findings from this thesis were:

- The associations between FFM, RMR and TDEE with free-living 24-hour EI were moderated by body fatness (Chapter 4).
- The association between FM and test meal EI appeared to be non-linear, in which the strength (weakening) and direction (from negative to positive) changed at higher levels of body fatness (Chapter 4).
- High-metabolic-rate organs appear to better explain the variability in fasting hunger and free-living 24-hour EI between individuals than FFM as a uniform component (Chapter 5).

- IER was not superior to CER in attenuating the losses of FFM and compensatory responses occurring during WL (Chapter 6).
- Adaptive thermogenesis during WL, but not changes in body composition or physical activity, was associated with increases in free-living daily appetite sensations (Chapter 7).
- Changes in physical activity after 2 weeks and across the WL intervention were associated with the mean rate of WL (Chapter 8).

The importance and implications of these findings for future research and weight management interventions is described below.

9.2 The Associations Between Body Composition and Energy Expenditure with Appetite and Energy Intake are Influenced by Body Fatness

The main finding emerging from Chapter 4 was that the associations between body composition and EE with appetite and EI were influenced by the levels of body fatness in women. When participants were separated by BMI categories (lean vs overweight/obesity), stronger associations between FFM, RMR and TDEE with appetite sensations and free-living 24-hour EI were observed in the group of leaner participants. Furthermore, while a negative association was observed between FM and test meal EI in the participants that were lean, no associations were observed in the ones with overweight and obesity. Interestingly, although the sample size was limited, a positive association emerged when this association was assessed exclusively in the ones with obesity.

Importantly, these findings were confirmed in the whole sample where a moderation effect of body fat percentage (and FM, BMI and FMI) on the associations between FFM, RMR and TDEE with free-living 24-hour EI was observed. These data are relevant because they expand previous work and demonstrate statistically for the first time an influence of body fatness on the associations between body composition, EE and EI. It has been previously postulated that the tonic inhibitory influence of FM on appetite would be present

in lean individuals but not in those with overweight and obesity, but the present findings provide direct evidence to support this hypothesis (Blundell et al., 2001). The findings from this chapter also demonstrated for the first time the presence of a non-linear (quadratic) association between FM (and FMI, BMI and body fat percentage) and test meal EI. These data indicate that higher levels of body fatness are associated with a weaker coupling between EE and EI, as well a potential blunted inhibitory effect of FM on appetite.

These results expand on previous models of appetite by adding body fatness as a potential moderator of the tonic processes influencing EI. Weight gain leads to increases in both FM and FFM and an elevation in energy requirements. The work contained in this thesis and that of others suggest that these increases would increase the tonic drive to eat. Concomitantly, an increase in the levels of body fatness may lead to a weakening of the coupling between EE and EI, as well the proposed inhibitory influence of FM on appetite, promoting overconsumption of food (in excess of energy requirements). This could in part explain the apparent ease of gaining weight and difficulty in losing and maintaining a reduced body size.

These findings are relevant for clinical management of obesity because although leaner individuals seem to have appetite systems which are more in tune with their energy requirements, the same may not be observed in those with overweight and obesity. Consequently, this could increase the susceptibility of these individuals to passively overconsume in an obesogenic environment, complicating weight management (Blundell, 2018). Importantly, it has been shown that WL improves metabolic health and may normalise appetite-related peptide kinetics (Romon et al., 2006, Lean and Malkova, 2016, Barazzoni et al., 2020), while higher levels of physical activity may be associated with improved appetite sensitivity (Beaulieu et al., 2016). Therefore, it could be that a more restrained dietary approach alongside sustainable increases in physical activity leads to an improved sensitivity of the mechanisms influencing appetite, facilitating longer-term weight management. Of note, it should be recognised that higher levels of physical activity are present in individuals that successfully lose and maintain a reduced body size (Fazzino et al., 2017, Ostendorf et al., 2019).

9.3 The Variance in Appetite and Energy Intake Between Individuals is Better Explained When Body Composition is Assessed at the Tissue-Organ Level

The two studies described in Chapter 5 are the first to examine the associations between body composition with appetite sensations and EI at the tissue-organ level using MRI. In study 1 of this chapter, a moderate-to-strong positive association was observed between fasting hunger and the combined mass of high-metabolic rate organs and the liver, but not with FFM as a uniform component. These data are similar to studies demonstrating that body composition analysis at the tissue-organ level better explains the variability in RMR between individuals (Gallagher et al., 1998), and suggest that examining body composition at the tissue-organ level may better explain the variance in appetite and EI between individuals. This could be due to several reasons: 1) high-metabolic rate organs account for ~60% of RMR and could therefore exert a stronger influence over appetite and EI; and 2) FFM is a heterogeneous component in which small differences in its composition may substantially influence its energy density (e.g., ratio between skeletal muscle and high-metabolic rate organs). Therefore, body composition analysis at the tissue-organ level would better inform over the individuals' FFM composition and thus its specific energy requirements. Interestingly, from these organs, the liver emerged as the tissue with the strongest association with fasting hunger sensations. While it remains unclear whether the liver exerts a specific influence on appetite, it has been suggested that its glycogen availability, energy status (Friedman, 1995, Friedman, 2007), and endocrine signalling (Gonzalez et al., 2019) could play critical roles.

Study 2 of Chapter 5 aimed to extend these findings by including a more complete assessment of appetite (i.e., appetite sensations assessed fasted and in response to several meals; EI assessed through several *ad libitum* meals and during 7 days in free-living conditions). However, due to COVID-19, participant number was limited to 6, but importantly, the PhD candidate has been awarded a Wellcome Trust Early Career fellowship in which he will be able to expand this investigation with a larger sample size. As described in 'methodological considerations' below, this study helped to establish the feasibility of conducting body composition analysis at the tissue-organ level at the University of Leeds.

The findings from these studies highlight the importance of looking beyond 2-compartment models of body composition when assessing the determinants of appetite and EI. Although the previous research looking at the associations between FM and FFM with appetite and EI were important precursors in understanding the factors that modulate eating behaviour, it is now critical to take a step further and assess the influence of specific components of body composition on appetite and how these are altered in health and disease. As detailed body composition analysis at the tissue-organ level has been shown to better explain the variance between individuals in RMR (Gallagher et al., 1998), it is possible that the same will occur when examining the determinants of appetite and EI. Importantly, if particular components are indeed highlighted (e.g., the liver), more specific molecular and metabolic analyses should be conducted to examine the mechanisms by which these tissues modulate appetite.

9.3.1 Implications for Our Understanding of the Drive to Eat

An updated formulation of the determinants of EI including the potential influence of body fatness and specific components of body composition at the tissue-organ level is proposed below (Figure 9.1).

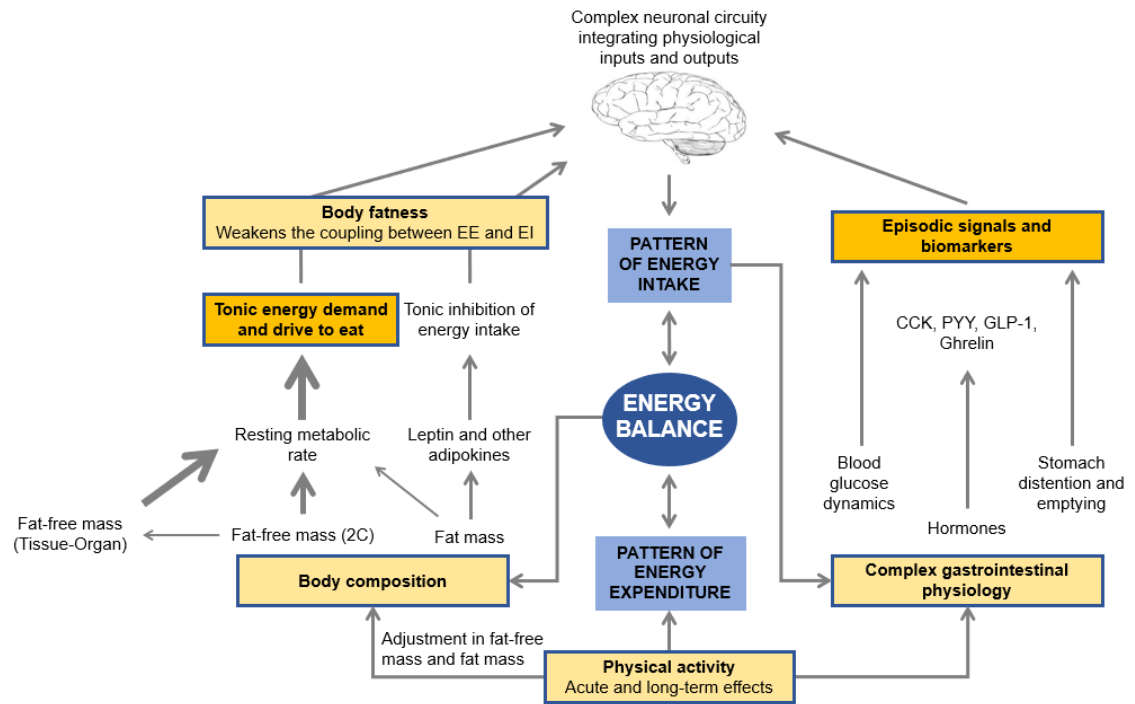


Figure 9.1 – Update of the figure by Blundell et al. (2020) regarding the interplay between tonic and episodic processes influencing appetite together with drive and inhibitory pathways. BC, body composition; EE, energy expenditure; EI, energy intake; 2C, 2-compartment model of body composition analysis.

It is known that appetite is influenced by several biological, psychological, behavioural and environmental factors. Furthermore, the interplay between episodic and tonic signalling strongly determines eating behaviours and eating patterns. An area of research that has started to emerge recently, and that was a main focus throughout this thesis, is the understanding of the influence of body composition and EE as tonic sources of feedback in the control of appetite and EI.

It has been postulated that FM, an endocrine organ, exerts a tonic inhibitory effect on EI to prevent an excessive accumulation of body fatness ('lipostatic model'). However, studies examining the associations between FM and EI often failed to demonstrate this which seemed to be contradictory to the theorised influence of FM on appetite (via leptin's anorexigenic action). On the other hand, FFM has consistently be found to be positively associated with EI. Additionally, it has been demonstrated that this association could be mediated by the energetic demands of FFM (Hopkins et al., 2018, Hopkins et al., 2016).

Interestingly, it has previously been hypothesised that the relationships between body composition and EE with appetite and EI could differ depending on the participants' level of body fatness. However, although it was a plausible hypothesis, it remained to be experimentally tested. In this thesis, not only this hypothesis was tested, but it was quantitatively demonstrated for the first time. The associations between FFM and EE with EI were found to be moderated by several markers of body fatness, in which these relationships were found to be weaker at higher levels of body fat. Furthermore, a non-linear relationship between FM and EI was also found in which a negative association was found in lean, but not in participants with overweight and obesity. These suggest that the higher levels of body fatness may lead to an uncoupling between EE and EI.

These findings have important implications for the understanding of the aetiology of obesity. Firstly, an obesogenic environment may facilitate passive overconsumption and physical inactivity. Alongside this environmental barrier, the concomitant increase in the drive to eat due to a higher body size, and a blunted inhibitory influence of FM on EI, could create a scenario that facilitates over-consumption and weight gain. As the compensatory forces resisting WL seem to be stronger than the ones opposing weight gain, it is understandable that weight management success rates are often disappointing. Although the data from this thesis do not allow to determine molecular mechanisms, these findings (which have been published in a peer-review journal) will serve an important precursor for future research. Future studies should look into replicating these results and examining the signalling pathways in which body fatness may dysregulate energy balance. This could open doors for the development of novel strategies for obesity management, including pharmacological products that specifically block the excitatory mechanisms that drive EI (rather than strengthen the inhibitory mechanisms that current pharmacology act on).

Another important finding from this thesis was the analyses of the associations between body composition and appetite at the tissue-organ level. This was a critical step in appetite-related research as FFM comprises of a heterogenous group of tissues with different metabolic rates. In the last 2 decades, obesity research started to incorporate MRI measurements to assess how body composition influences EE, and how metabolism changes during WL. However, it has never been purposefully used to conduct a complete examination of how

different components of FFM influence EI and appetite. This was a logic and important step as analysis at the tissue-organ level had allowed to better understand and explain the variability in RMR between individuals. As RMR is a strong determinant of appetite, assessing the influence of FFM on EI at the tissue-organ level could allow for a better explanation of the variability between individuals. Indeed, the findings from this thesis corroborate this hypothesis and suggest that a deeper body composition analysis may allow for a better understanding of the mechanisms driving EI. Future research, including the one the PhD candidate will conduct during his Wellcome Trust ISSF fellowship, should look into confirming these findings in greater sample sizes, and include potential moderators and mediators like cytokines and appetite-related peptides.

These findings were used to update a previously published figure by Blundell et al. (in which the PhD candidate is a co-author) (Blundell et al., 2020). The associations between FFM and EI have been consistently reported using 2-compartment models and this has been found to be mediated by EE. Body composition analyses at the tissue-organ level was added with a thicker arrow to represent two important ideas: 1) FFM better explains the variability in RMR between individuals than FFM as a uniform component (2-compartment model), and 2) inclusion of analysis at the tissue-organ level better explained the variability between subjects in appetite and EI in the data reported in this thesis. Furthermore, the influence of body fatness was also added by connecting the tonic drive and inhibitory effects of EE and FM on appetite and EI, respectively, to the brain. A critical finding from this thesis was that higher body fatness may weaken the associations between body composition and EE with EI. Not only the coupling between EE and EI seemed to be weaker in individuals with higher body fatness, the postulated inhibitory influence of FM on EI also seemed to be absent. These findings are important as they provided novel information regarding our understanding of factors influencing appetite and EI and how body fatness may contribute to energy balance dysregulation.

9.4 Is Intermittent Energy Restriction a Superior Dietary Strategy in Preserving Fat-Free Mass and Attenuating the Compensatory Responses Occurring During Weight Loss?

The study described in Chapter 6 aimed to match WL to $\geq 5\%$ in order to assess the differences between CER and IER in the compensatory responses occurring at a similar degree of WL (Beaulieu et al., 2019). Results from this study demonstrated that for a similar WL ($\sim 6\%$), changes in body composition, EE (including adaptive thermogenesis) and physical activity were similar between groups (albeit substantial inter-individual variability was observed). These findings are relevant as they demonstrate that IER (in this case alternating 75% energy deficit days with *ad libitum* feeding days) is not superior to CER in attenuating the losses of FFM or the compensatory responses in EE and physical activity behaviours when total WL is similar. However, as has been shown that periods of weight stabilisation after WL may reverse almost completely the greater than predicted decreases in RMR (Nymo et al., 2018), it would be interesting to examine whether other IER patterns exert different effects. For example, one study observed that alternating between 11 days in a 45% energy deficit with 3 days of a self-selective diet resulted in similar changes in body weight and composition (although EI was higher throughout the intervention) than CER (daily 55% energy deficit), but a smaller decrease in RMR (Davoodi et al., 2014). However, this could simply reflect a shorter-term rise in response to the acute increase in EI, and thus whether this would facilitate WL remains unknown.

Also in this study, changes in appetite sensations during WL measured in the laboratory (fasted and AUC) were similar between groups. Interestingly, AUC appetite sensations assessed in the laboratory decreased similarly between baseline and post-WL in both groups ($\sim 10\%$). However, when looking at free-living appetite sensations (average of 7 daily measurements using CoEQ), although they did not significantly change in CER, they increased in IER. While the former finding may suggest an improved satiety response to meals (i.e., episodic signalling), the latter may be reflective of an increase in the overall motivation to eat. Decreases in AUC appetite sensations have been previously found in WL interventions (Nymo et al., 2017, Andriessen et al., 2018) and this could be due to several factors (described in detail in Chapter 6) such as habituation to lower food portions and an improvement in appetite-related peptide

secretion kinetics. However, an increase in the systemic drive to eat observed in IER could in part explain the difference in the time to achieve the same amount of WL (more ~10 days). It could be that this eating pattern led to a lower (albeit not statistically different as estimated using the calculated 'intake-balance method') energy deficit in comparison to CER, slowing the rate of WL. Of note, the mean changes in physical activity behaviours and RMR were not statistically different from CER, and dropout numbers were also higher in IER (CER – 2 vs IER – 6). Importantly, while only 1 participant in CER terminated her participation due to the meal plan, this was the case for 5 participants in IER.

Overall, the findings from this study suggest that for a similar WL, IER does not seem to be superior in attenuating losses of FFM and compensatory responses in EE, physical activity or appetite. In fact, although some of the observations were not statistically significant (possibly due to a small sample size), it could be argued that IER was associated with stronger compensatory responses in free-living sensations of appetite, which could partially explain the lower (but not statistically significant) percentage of adherence and the extra days needed to reach a similar WL. Alongside a greater difficulty in adhering to the meal plan which could be observed by the higher number of dropouts, it indicates that this IER strategy may not be adequate for everyone. However, it may be used if it facilitates adhering to an energy deficit, as that has been repeatedly shown to be a key determinant of WL and maintenance success (Alhassan et al., 2008, Del Corral et al., 2011). Lastly, the findings from this study demonstrate two points that deserve to be highlighted: 1) compensatory responses to WL in EE, physical activity and appetite are not inevitable and do not occur in all individuals, and therefore generalised ideas (e.g., hunger increases during WL) should be avoided; and 2) although the average group values suggest that no changes occurred (e.g., amount of physical activity), the large inter-individual variability in responses during WL highlight the importance of looking beyond the mean and start examining responses at the individual level. This variability between individuals and its implication for weight management will be examined in more detail in the following two sections.

9.5 The Influence of Changes in Body Composition, Energy Expenditure and Physical Activity During Dietary-Induced Weight Loss on Appetite and Energy Intake

In the analyses presented in Chapter 7, no associations were observed between changes in body composition (including the fraction of FFM lost i.e., the p-ratio) and changes in markers of appetite. However, this could be due to the fact that participants only lost ~6% of their body weight (of which only ~1.3kg was FFM) and had an initial body fat percentage of ~41%. This is similar to the women included in the analyses conducted by Turicchi et al. (2020), which also did not find any associations between losses of FFM and changes in hunger sensations. Therefore, it is plausible that although compensatory appetite responses may occur as a consequence of greater losses of FFM, this may only occur under conditions in which total FFM loss is greater and initial body fat percentage is lower.

The findings from this thesis suggest that compensatory increases in appetite arising from losses of FFM are not observed in women with overweight and obesity undergoing moderate WL. However, it should be recognised that these compensatory responses have emerged in previous research in leaner individuals with greater total body weight and FFM loss (Dulloo et al., 1997, Vink et al., 2016, Turicchi et al., 2020). Of note, in these studies the influence of FFM on appetite and weight regain was only been examined using 2-compartment models of body composition. Therefore, if these findings are confirmed in future studies, it is critical to investigate which component of FFM (if specificity at the organ-level can be determined, e.g., the liver – see Chapter 5) is responsible for triggering these compensatory increases in hunger. The studies presented in Chapter 5 serve as an important precursor by highlighting the potential importance of analysing body composition at the tissue-organ level. By understanding the specific components of body composition that may trigger these compensatory responses, it may be possible to define adequate strategies to attenuate them. For instance, if skeletal muscle is observed to be the main trigger, this would reinforce the idea of promoting exercise, especially resistance training (Stiegler and Cunliffe, 2006), as well higher protein intakes (Jäger et al., 2017) during WL interventions to preserve these metabolically active tissues.

In the analyses presented in Chapter 7, a moderate negative association between adaptive thermogenesis in RMR during WL and free-living appetite composite scores (but not fasting or AUC measured in the laboratory) was observed. This finding corroborates the results from previous studies by demonstrating an association between a greater adaptive thermogenesis (i.e., greater than predicted decrease in RMR during WL) and increases in appetite sensations, which could undermine WL efforts and promote weight regain. For instance, an exercise-only (Hopkins et al., 2014) and a combined (diet + exercise) WL intervention (Tremblay et al., 2013) reported associations between adaptive thermogenesis in RMR with increases in 24-hour EI and hunger sensations, respectively. Although these findings need to be replicated, these suggest that compensatory responses in EE and appetite may occur in a co-ordinated fashion in an attempt to reduce the prescribed energy deficit. It would also be plausible to postulate that a 'WL resistant' phenotype exists, in which some individuals experience greater compensatory responses during periods of negative energy balance. For instance, it has been reported that individuals with a 'thrifty-' (Piaggi, 2019) or with a 'low-satiety-' (Buckland et al., 2019) phenotype demonstrate a greater resistance to WL. This has important implications for clinical practice as individuals that demonstrate a more WL resistant phenotype may require greater attention into attenuating the compensatory responses that attempt to reduce the planned energy deficit (i.e., strategies to maintain higher levels of EE and satiety through manipulations in physical activity and diet).

9.6 Changes in Physical Activity After 2 Weeks and Across the Intervention are Associated with the Rate of Weight Loss

The influence of physical activity during WL was examined in the study reported in Chapter 8. In this study, it was demonstrated that changes in the amount (i.e., minutes) of physical activity, sedentary behaviours and associated EE (i.e., AEE) were strongly associated with the rate of WL. Participants that presented greater decreases in physical activity and AEE, and increases in sedentary time, took longer to achieve a similar WL. As these changes in physical activity were not associated with changes in markers of appetite (as described in Chapter 7) or adherence to the meal plan, it is plausible that this influence on the rate of WL was mediated by changes in EE, decreasing/increasing the daily energy deficit.

These findings corroborate the ones from Fazzino et al. (2020) in which participants that achieved a greater WL ($\geq 10\%$) also had higher amounts of moderate-to-vigorous physical activity. Physical activity is usually highlighted in contexts of weight maintenance, possibly due to misinterpretation of the 'disappointing' results seen in exercise-induced WL interventions (i.e., lower than expected WL based on the increase in EE through exercise). However, it has been shown that when the energy deficit is controlled, exercise or diet only interventions lead to similar WL (Ross et al., 2000). Furthermore, combined (diet + physical activity) interventions usually lead to greater total WL than diet alone, possibly through the accumulation of a greater energy deficit (Clark, 2015). As there was a large inter-individual variability regarding changes in physical activity, these findings suggest that a greater WL may be achieved in interventions in which physical activity behaviours are monitored, especially in those susceptible to reductions in response to energy deficit.

A challenge in weight management research has been the ability to identify predictors of longer-term outcomes (Rosenbaum et al., 2018). This information would be critical in weight management as it would allow a practitioner to be proactive and prescribe an intervention based on baseline characteristics and make adjustments early on based on short-term responses. Notably, a relevant finding from this study was that the associations between changes in physical activity and rate of WL were detected just after 2 weeks of intervention (and changes between week 2 and across intervention were highly correlated). Furthermore, the mean rate of WL in the first 2 weeks was associated with the mean rate of loss across the intervention. These findings are of importance as it is known that a greater initial WL is associated with better adherence throughout and intervention (Leung et al., 2017). As higher levels of adherence are associated with better longer-term weight management (Alhassan et al., 2008, Del Corral et al., 2011, Lemstra et al., 2016), it reinforces the idea that physical activity should be promoted and included in WL interventions to: 1) achieve a larger energy deficit due to increases in EE; and 2) possibly enhance the sensitivity of the mechanisms influencing appetite. Furthermore, these novel findings demonstrate that changes in physical activity and body weight after 2 weeks of intervention can be indicative of how an individual will respond across the intervention. Therefore, based on these early responses, it may be possible to adjust the intervention (e.g., by prescribing increases in physical activity) to

maintain efficient rates of WL, potentially improving longer-term weight management success rates.

Until the analysis conducted in Chapter 8, it has been suggested that the main determinants of the fraction of FFM lost are initial body fat percentage, total WL and rate of WL (Forbes, 1987, Hall, 2007). In the analysis conducted in this thesis, the fraction of FFM lost was not associated with these variables, possibly due to a smaller amount of total WL in comparison to the examinations conducted by Forbes and Hall. However, the fraction of FFM lost in the first 2 weeks and across the intervention were strongly associated. While it makes sense that a greater fraction of FFM lost after 2 weeks and across the intervention are associated (as rate of WL at week 2 and across the intervention were), the absence of associations between composition of the weight lost (at any time point) with initial body fat percentage, rate of WL and levels of physical activity suggest that this could be under the influence of other factors such as allelic variation in genes. If indeed it is the case that some individuals have a greater susceptibility to lose FFM, this would reinforce the importance of including exercise, especially resistance training, in WL interventions (Clark, 2015).

9.6.1 Implications for Weight Management

The findings reported in this thesis corroborate previous studies and suggest that when the energy deficit and total WL is matched, the dietary pattern (e.g., CER or IER) used will have minimal impact on weight management and the compensatory responses occurring during WL. Therefore, as most (but not all) participants were able to achieve a $\geq 5\%$ WL through CER or IER, the individual's dietary preferences should be taken into consideration in order to ensure high levels of adherence and thus facilitate longer-term weight control.

Importantly, future studies should shift the focus from the paradigm of trying to determine the best dietary strategy for a group of individuals, but to examine which factors explain the variability in WL outcomes between individuals, as conducted in Chapter 8. This is critical as identifying the factors that resist or facilitate WL for each individual will allow to design personalised strategies (behavioural or pharmacological) according to specific phenotypes. In this chapter, physical activity, but not adaptive thermogenesis, was demonstrated to be an important determinant of the rate of WL and this could be observed after 2 weeks of dietary-induced energy restriction. This means that particularly in

individuals with a 'WL resistant' phenotype (i.e., greater decreases in EE and increases in hunger), promoting conscious increases in physical activity may be critical for longer-term weight management. However, it needs to be acknowledged that not only physical activity may be partially under genetic control (Zhang and Speakman, 2019), some data suggests that higher levels do not necessarily translate to increases in TDEE, as postulated by the constrained model (Pontzer et al., 2016). Nonetheless, as physical inactivity is highly prevalent (Church et al., 2011), it is unlikely that a strong constrained effect occurs when baseline levels of physical activity are low. Therefore, encouraging individuals to increase physical activity levels alongside dietary changes may be critical not only for improved weight management, but for overall health and longevity.

9.7 Methodological Considerations

By comprehensively assessing components of energy balance, this thesis allowed for the investigation of novel aspects regarding the determinants of appetite and factors influencing WL. The studies presented in this thesis included measurements of body composition (2-compartment model and at the tissue-organ level), EE, physical activity, appetite ratings, EI and eating behaviour traits.

Appetite is a complex biopsychological phenomenon and therefore, a multi-component assessment is essential for a proper and detailed understanding. A limitation present in the majority of studies is the insufficient methodology to assess appetite. In this thesis, the following measurements were conducted to assess appetite: appetite sensations (fasted; in response to meals – AUC; and free-living – in the end of the day retrospective self-rating of appetite); and EI (objectively measured in the laboratory via one to several test meals; and self-reported free-living 24-hour EI).

Incorporating a multi-component assessment of appetite allowed for a more comprehensive investigation of its dimensions and how they differed between individuals and in response to WL. For instance, while test meals and appetite ratings in response to meals may represent a more episodic (i.e., shorter-term) process of appetite, longer-term measurements of EI (as assessed using an online diet record tool during 7 days), as well daily measurements of appetite (as

assessed retrospectively during 7 days) may be more representative of tonic (i.e., longer-term) processes. This is important as episodic and tonic signalling may be influenced by different mechanisms. For instance, episodic signals are mainly (but not exclusively) inhibitory and related to satiation and satiety, being influenced by gut mechanoreceptors and appetite-related peptides, but also by environmental and contextual factors, modulating a constant tonic drive to eat stemming from cellular metabolism (energy needs arising from both FM, FFM and its specific components). An illustration of the importance of these multi-component assessments are the findings in Chapter 6. While AUC appetite sensations (ratings from pre-breakfast to post-lunch, ~195 minutes) decreased in both groups by ~10%, free-living daily assessments of appetite exhibited an increase in appetite in the IER group. This is a critical finding that could be overlooked in case only laboratory assessments had been conducted.

A similar rationale can be applied to EI. While objectively measured EI in a laboratory may allow for a more precise assessment than self-reported free-living conditions, it may not be ecologically valid due to the fact that participants are in an unrealistic environment and where food choices are limited. Although part of the inclusion/exclusion criteria were food preferences regarding the test meals, differences in individual's preferences may have influenced food intake. Furthermore, providing one meal may be more reflective of episodic signalling than tonic. This would explain why FM, but not FFM or RMR, was associated with test meal EI in Chapter 4. As it was recognised from the beginning that test meals would not be enough to capture all aspects of appetite, free-living assessments of EI were also included. While self-reported EI is usually criticised due to the presence of misreporting (Lichtman et al., 1992), several methods were used to account for this throughout the thesis. Firstly, logbooks were provided to aid participants with reporting accuracy. Furthermore, Goldberg cut-offs were used and compared between BMI categories. These indicated that although misreporting was likely present, it was not the reason for the findings presented in this thesis as no differences were noted between groups. Alongside Goldberg cut-offs, the 'intake-balance method' was used to compare measured and estimated EI (Hall and Chow, 2011). Although there were differences between measured and calculated EI, they were not statistically significant, which alongside the findings from the Goldberg cut-offs reinforce the confidence in the results collected using a free-living self-reported assessment of EI. A valuable

point from using free-living methods is that participants are able to consume what they typically eat (type and amount of food) over a longer period of time and therefore, it may be possible to have a better representation of the tonic processes influencing appetite. This probably explains why FFM and EE were associated with free-living 24-hour EI rather than intake at a single or several *ad libitum* meals (studies in Chapters 4 and 5). Measuring energy balance behaviours in free-living conditions is still one of researchers' greatest frustrations (Dhurandhar et al., 2015). Although wearable devices (an alternative to doubly labelled water, the gold standard method to assess TDEE via the ingestion and evaluation of the losses of the stable isotopes deuterium and oxygen-18) are getting more accurate at estimating EE, and these could be used alongside changes in body composition to estimate mean EI over a period of time, it still remains impractical to do these measurements and several assumptions may influence the accuracy of the results, such as considering that the metabolic rate per unit of FFM is constant, which does not account for the heterogeneity present in this compartment.

Another valuable energy balance assessment tool used throughout this thesis was the SenseWear Armband, a validated physical activity monitor. Although it is known that physical activity monitors usually misestimate EE in comparison to calorimetry or doubly labelled water, these were reported to be the most accurate devices to estimate assess EE behaviours, especially at lower intensities (O'Driscoll et al., 2020). This is a critical consideration as since one of the specific aims of this thesis was to examine the influence of body fatness on the associations between body composition and EE with appetite and EI. Importantly, participants were asked to use these devices during 7 days for at least 23 hours per day, only removing during activities that required contact with water (e.g., showering and swimming) and data was considered valid if participants used it for at least for 22 hours in 5 days (including at least 1 weekend day). This permitted to get a more accurate representation of habitual levels of physical activity and EE. Furthermore, measuring physical activity behaviours at 3 time points allowed for the examination of its relevance in WL efficiency. Of note, at the screening session participants were asked not to change their exercise and physical activity habits during the intervention, but compliance was not strictly monitored in order to allow for some degree of subconscious or compensatory changes to occur. It was therefore possible to demonstrate that changes in

physical activity during negative energy balance were strongly associated with the rate of WL, and thus highlight its importance for weight management.

Regarding the WL intervention described in Chapter 6, three methodological considerations deserve to be highlighted. Firstly, providing the food to participants (except for the *ad libitum* 'feeding days' in IER) allowed for a greater control and adherence. On the other hand, providing foods alongside weekly meetings with a dietitian could have also influenced the results (e.g., eating behaviour traits). Secondly, an important strength of this methodology was the attempt to match WL between participants and groups. This was a critical decision as previous research reported different compensatory responses at different amounts of WL (Nymo et al., 2017, Nymo et al., 2018). By matching the amount of WL, it would be possible to examine: 1) whether IER and CER would be superior in attenuating the compensatory responses occurring during WL; and 2) the inter-individual variability in compensatory responses to negative energy balance. Therefore, this study was an important intervention as it allowed to investigate whether one dietary strategy could be superior regarding compensatory metabolic, psychological and behavioural responses under similar conditions (i.e., matched WL). Lastly, the measurements 'post-WL' were collected while participants were still in a negative energy balance (i.e., ongoing WL). Although this should not be seen as limitation, it has been shown that periods of weight stabilisation may lead to a (at least partial) reverse of the compensatory responses occurring in EE (Nymo et al., 2018), and change in appetite sensations in comparison to during WL (Nymo et al., 2017). Therefore, the findings from the present analysis should be interpreted according to the timing of the measurements (during and not post-WL).

This thesis included body composition assessments at the tissue-organ level using MRI for the first time for examination of the associations between specific components of FFM with appetite and EI. Although 2-compartment models are important and provide more insight than only examining body weight, it was critical to take a step further and look into the influence of body composition at the tissue-organ level. Hopefully, these studies will be a precursor for future cross-sectional and longitudinal studies to include these assessments in order to create a deeper understanding of the mechanisms that influence appetite and energy balance regulation. Conducting study 2 in Chapter 5 also confirmed the feasibility

of conducting body composition analyses at the tissue-organ level in the University of Leeds. However, while conducting study 2, three major barriers emerged: 1) COVID-19; 2) conducting a standardised MRI protocol that could be easily replicated and that would provide high-quality images; and 3) manually segmentation of the images collected from the MRI scans.

In collaboration with the School of Medicine, a consistent and replicable protocol was defined after several pilot studies by mimicking the ones contemplated in previous research. The next steps regarding this will be to include heart mass and liver fat in order to get a complete body composition analysis. This will permit to include measurements in individuals with overweight and obesity in which levels of fat infiltrated in internal organs are higher. To accomplish this, pilot testing is going to be conducted to make the current protocol last less time so measurements of liver fat can be included.

Regarding the manual segmentation of images collected from the MRI scans, an intensive training process was conducted in the University of Kiel, Germany, with the aid of the researchers responsible for detailed body composition analyses in their laboratory. The PhD candidate had training regarding the usage of the software (SliceOmatic), as well as detailed anatomical slice-by-slice recognition, in which dozens of images were analysed and confirmed by the researcher leading MRI analyses in the University of Kiel. This training was of great importance as it allowed the PhD candidate to gather the required skills to confidently and autonomously analyse MRI images in future studies conducted in the University of Leeds.

9.8 Further Research

Producing this thesis has led to 3 first-author peer-reviewed journal articles, 2 second-author articles, and 6 conference abstracts (including 2 that were oral presentations; see pages ii-iv). Collaborations have led to a further 2 publications in related areas:

- Blundell, J., Gibbons, C., Beaulieu, K., **Casanova, N.**, Duarte, C., Hopkins, M., Stubbs, R.J., Finlayson, G. (2020). The drive to eat in *homo sapiens*: Energy expenditure drives energy intake. *Physiology & Behavior*. doi:10.1016/j.physbeh.2020.112846

- Turicchi, J., O'Driscoll, R., Finlayson, G., Duarte, C., Hopkins, M., **Casanova, N.**, Michalowska, J., Larsen, T.M., van Baak, M.A., Astrup, A., Stubbs, R.J. (2020). Associations between the proportion of fat-free mass loss during weight loss, changes in appetite, and subsequent weight change: results from a randomized 2-stage dietary intervention trial. *American Journal of Clinical Nutrition*. doi: 10.1093/ajcn/nqz331.

In addition to these, it is expected that the following publications will be submitted for publication:

- The Association Between Fat-Free Mass and Fasting Hunger Sensations is Stronger when Body Composition is Assessed at the Tissue-Organ Level (chapter 5 - Study 1) Target journal: *International Journal of Obesity*.
- Increases in Physical Activity are Associated with a Faster Rate of Weight Loss in Women with Overweight and Obesity (chapter 8) Target journal: *Physiology & Behavior*.

Although several important findings have emerged from this thesis, critical questions that should be addressed in the future also arose. In Chapter 4, an important finding was that levels of body fatness moderated the associations between RMR and TDEE with 24-hour EI. Of note, physical activity did not influence these relationships which is surprising considering the evidence suggesting an improved coupling between EE and EI, and a stronger inhibitory influence of FM on EI, at higher levels of physical activity (Mayer et al., 1956, Beaulieu et al., 2016, Beaulieu et al., 2018). Future studies should examine the independent effects of body fatness and physical activity by recruiting a range of participants with different levels (e.g., block stratification with 4 groups depending on body fatness and physical activity levels). This way, it would be possible to examine whether physical activity would have an independent influence on appetite and energy balance regulation, and if it could exert a protective effect (i.e., reduce the expression of the negative consequences observed at higher levels of body fatness) in individuals with overweight and obesity. Furthermore, a next step would be to examine the mechanisms by which a higher level of body fatness weakens the coupling between EE and EI (e.g., insulin and leptin sensitivity). This would be critical for weight management (research and clinical practice) since knowing the mechanisms linked to energy balance dysregulation

could allow for the conceptualisation of lifestyle and pharmacological strategies to attenuate these consequences arising from a higher body fatness.

Until the analyses conducted in Chapter 5, previous research has only assessed the associations between body composition and appetite using 2-compartment models. Future studies should look not only at the associations between components of FFM with appetite and EI, but also the influence of the type of fat (e.g., visceral vs subcutaneous) and its distribution on these relationships. This is important to consider as these have distinct structures and functions (Ibrahim, 2010), and seem to contribute differently to metabolic disease (Park and Lee, 2005, Patel and Abate, 2013). Assessing these relationships in larger sample sizes would also permit for a multiple regression analyses including all components of body composition at the tissue-organ level and thus examine their independent influences on appetite and EI. Furthermore, as evidence suggests that losses of FFM in leaner individuals achieving a high total WL may trigger compensatory responses in appetite that promote weight regain, it is critical that body composition analysis at the tissue-organ level is conducted in future WL studies. By identifying which specific components of FFM may be triggering these compensatory increases in hunger, it could be possible to define strategies to attenuate the losses of these metabolically active tissues that contribute to energy overconsumption and undermine WL efforts.

Lastly, future studies looking into changes in appetite during WL interventions should consider the following: 1) conduct a complete assessment of appetite including measurements in laboratory and free-living conditions; 2) measure these at different amounts of WL, including during weight stabilisation. A standardised way to examine changes in appetite and being able to perform these at different time points would allow for a deeper understanding of appetite and how it changes during WL, potentially allowing for the conception of strategies to attenuate or prevent this undesirable compensatory response that resists WL and promotes weight regain.

9.9 Conclusions

The findings from this thesis provide novel insight into the factors driving appetite by having investigated the associations between body composition and EE with appetite and EI at the tissue-organ level and how they differ across a spectrum of body fatness. It was demonstrated that the coupling between EE and EI, as well the proposed inhibitory influence of FM on EI, weaken at higher levels of body fatness. It was also observed that IER is not a superior nutritional strategy when it comes to preserving FFM and attenuating the metabolic, psychological and behavioural compensatory responses that occur during WL. Moreover, data from this study suggests that adaptive thermogenesis in RMR, but not losses of FFM, seem to be associated with increases in appetite sensations in women with overweight and obesity undergoing a moderate WL. Importantly, a crucial finding from this investigation was that a strong determinant of the rate of WL was changes in physical activity, a behavioural component that is at least partially under voluntary control. This finding is of great importance as it may be used to empower individuals and promote sustainable changes in physical activity to achieve longer-term weight management success. Ultimately, understanding individual variability in responses to WL interventions will help to create precision recommendations and better personalise weight management.

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Appendix

Supplementary Material for Chapter 4

Table S1 – Descriptive characteristics of participants separated by body mass index category.

	Lean (n = 45)		Overweight (n = 32)		Obesity (n = 16)	
	Mean	SD	Mean	SD	Mean	SD
Age (y)	35	10	33	10	39	9
Height (cm)	164.6	6.3	164.4	7.2	166.7	9.7
Body weight (kg)	59.0	5.9	74.6	8.3	89.1	11.8
BMI (kg/m ²)	21.8	1.5	27.5	1.3	31.9	1.3
Fat mass (kg)	16.3	3.6	29.0	5.2	40.9	8.0
FMI (kg/m ²)	6.0	1.3	10.7	1.6	14.6	1.8
Fat-free mass (kg)	42.8	4.0	45.7	5.7	48.2	5.9
FFMI (kg/m ²)	15.8	1.0	16.8	1.1	17.3	1.2
Body fat (%)	27.3	4.6	38.7	4.7	45.6	4.2

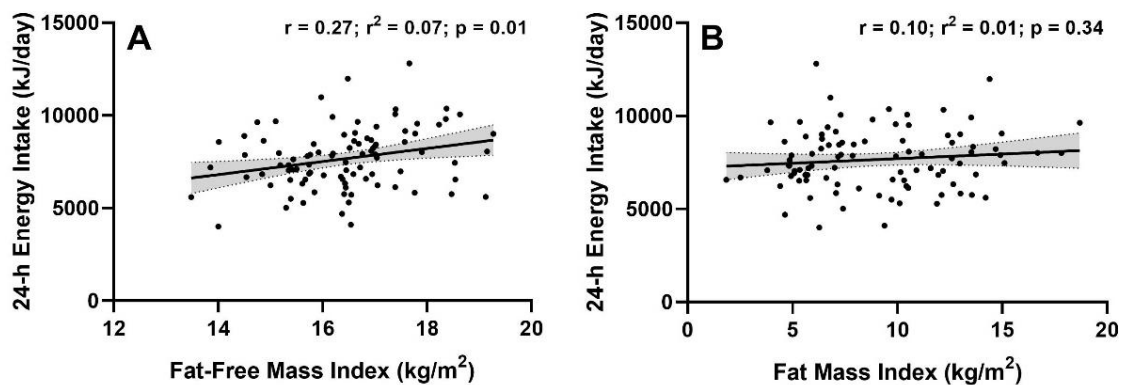


Figure S1 – Scatter plots illustrating the associations between A) fat-free mass index; and B) fat mass index with mean 24-hour energy intake (n = 93).

Table S2 - Multiple linear regression to examine the independent effects of fat mass index, fat-free mass index, resting metabolic rate and total daily energy expenditure on mean 24-hour energy intake.

	β	t	p-value
Model 1 - [F(2, 90)=3.5, r ² =0.05, p=0.04]			
Intercept		0.86	0.40
FMI	0.01	0.11	0.91
FFMI	0.26	2.44	0.02
Model 2 - [F(3, 89)=6.6, r ² =0.15, p<0.001]			
Intercept		0.15	0.88
FMI	-0.01	-0.10	0.93
FFMI	0.14	1.28	0.21
RMR	0.36	3.45	0.001
Model 3 - [F(3, 88)=6.4, r ² =0.15, p=0.001]			
Intercept		0.23	0.82
FMI	-0.12	-1.06	0.29
FFMI	0.17	1.54	0.13
TDEE	0.38	3.34	0.001

FMI, fat mass index; FFMI, fat-free mass index; RMR, resting metabolic rate; TDEE, total daily energy expenditure.

Table S3 – Correlations between fat mass index and fat-free mass index with 24-hour energy intake and test meal energy intake in the group of participants that are lean and in the ones that have overweight and obesity.

		24-hour EI	Test Meal EI
Lean (n = 45)			
Fat Mass Index	r	0.12	-0.42
	p	0.42	0.005
Fat-free Mass Index	r	0.36	0.17
	p	0.02	0.28
Overweight and Obesity (n = 48)			
Fat Mass Index	r	0.10	0.26
	p	0.52	0.07
Fat-free Mass Index	r	0.22	0.20
	p	0.14	0.17

EI, energy intake.

Table S4 – Multiple linear regression to examine the independent effects of fat mass index, fat-free mass index and resting metabolic rate on mean 24-hour energy intake in the participants with overweight and obesity and in those that are lean.

Overweight and Obesity (n = 48)			
	β	t	p-value
Model 1 - [F(2, 45)=1.7, r ² =0.03, p=0.20]			
Intercept		0.01	0.99
FMI	0.16	1.06	0.29
FFMI	0.26	1.72	0.09
Model 2 - [F(3, 44)=1.9, r ² =0.06, p=0.14]			
Intercept		0.22	0.83
FMI	0.08	0.53	0.60
FFMI	0.13	0.79	0.43
RMR	0.25	1.53	0.13
Lean (n = 45)			
	β	t	p-value
Model 1 - [F(2, 42)=3.8, r ² =0.11, p=0.03]			
Intercept		-0.79	0.44
FMI	0.15	1.10	0.29
FFMI	0.37	2.63	0.01
Model 2 - [F(3, 41)=7.0, r ² =0.29, p=0.001]			
Intercept		-1.48	0.15
FMI	0.07	0.55	0.59
FFMI	0.22	1.66	0.11
RMR	0.46	3.40	0.002

FMI, fat mass index; FFMI, fat-free mass index; RMR, resting metabolic rate.

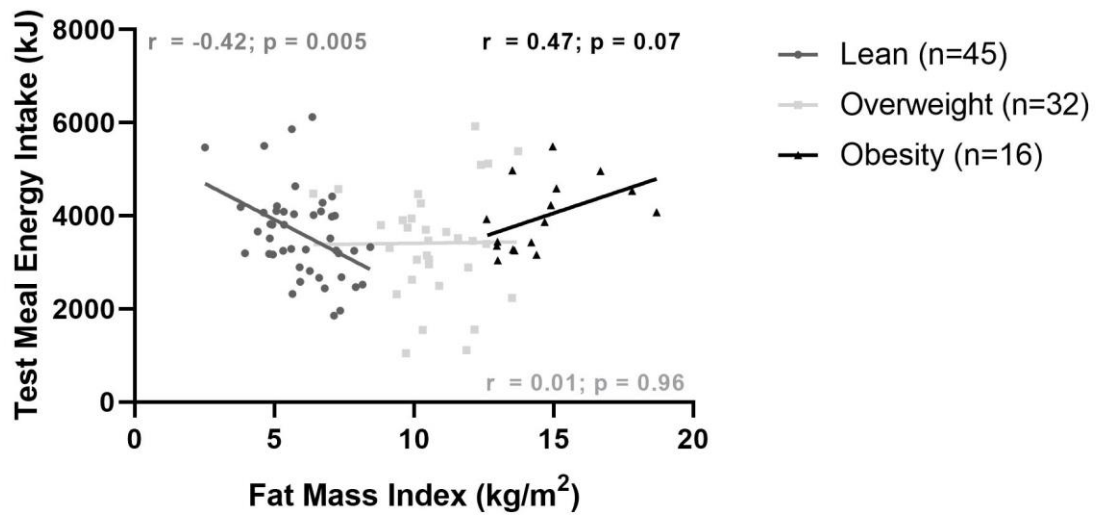


Figure S2 – Scatter plots illustrating the association between fat mass index and test meal energy intake by body mass index category.

Table S5 – Multiple regression to assess the non-linear associations between body fat percentage and fat mass index with test meal energy intake.

	β	t	p-value
Body Fat Percentage - [F(3, 88)=4.2, r ² =0.10, p=0.008]			
Intercept		4.72	< 0.001
BFP	-2.45	-3.46	0.001
FFM	0.08	0.77	0.45
BFP²	2.40	3.39	0.001
Fat Mass Index - [F(3, 88)=4.4, r ² =0.13, p=0.006]			
Intercept		4.84	<0.001
FMI	-1.81	-3.46	0.001
FFM	0.08	0.75	0.46
FMI²	1.85	3.55	0.001

BFP, body fat percentage; FFM, fat-free mass; FMI, fat mass index.

Table S6 – Moderation analyses using body mass index, fat mass index and total fat mass as moderators of the associations between resting metabolic rate and total daily energy expenditure with mean 24-hour energy intake.

Body Mass Index as the Moderator

	β	t	p-value
RMR - [F(3, 89)=9.2, $r^2=0.24$, $p<0.0001$]			
Intercept		-2.44	0.02
BMI	1.96	2.86	0.005
RMR	1.99	3.44	0.001
BMI x RMR	-2.80	-2.80	0.006
TDEE - [F(3, 88)=6.8, $r^2=0.19$, $p<0.0001$]			
Intercept		-1.46	0.15
BMI	1.33	1.94	0.06
TDEE	1.64	2.61	0.01
BMI x TDEE	-2.25	-2.00	0.04

Fat Mass Index as the Moderator

	β	t	p-value
RMR - [F(3, 89)=8.9, $r^2=0.23$, $p<0.0001$]			
Intercept		-1.73	0.09
FMI	-2.45	2.75	0.007
RMR	0.08	4.09	< 0.001
FMI x RMR	2.40	-2.73	0.008
TDEE - [F(3, 88)=7.3, $r^2=0.20$, $p<0.0001$]			
Intercept		-0.88	0.38
FMI	-1.81	2.05	0.04
TDEE	0.08	3.61	0.001
FMI x TDEE	1.85	-2.20	0.03

Total Fat Mass as the Moderator

	β	t	p-value
RMR - [F(3, 89)=9.4, r ² =0.24, p<0.0001]			
Intercept		-1.75	0.08
FM	1.86	2.96	0.004
RMR	1.00	4.38	< 0.001
FM x RMR	-2.12	-2.93	0.004
TDEE - [F(3, 88)=7.3, r ² =0.20, p<0.0001]			
Intercept		-0.76	0.45
FM	1.31	2.05	0.04
TDEE	0.89	3.72	< 0.001
FM x TDEE	-1.70	-2.21	0.03