The Impact of Intermittent Fasting on Energy Balance and Associated Health Outcomes

Templeman, Iain

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THE IMPACT OF INTERMITTENT FASTING ON ENERGY BALANCE AND ASSOCIATED HEALTH OUTCOMES

Iain Templeman

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Abstract

Intermittent fasting may be an effective strategy for managing obesity and the associated dysfunction. It involves punctuating typical patterns of nutritional intake with scheduled periods of abstinence from all energy-providing nutrients. The few prior studies of this approach have yielded promising insights; yet there remains a dearth of knowledge regarding how intermittent fasting affects energy metabolism and health in humans, which this research sought to address. Initially, diurnal variations in subjective appetite ratings were established as a robust foundation upon which to design temporal nutritional interventions (Chapter 4). Specifically, appetite increased throughout the day to a peak in the evening, despite the apparent inversion of the accompanying rhythm in key regulatory peptides. This led to the development of a novel intermittent fasting intervention in which a complete fast was applied in alternating 24-hour periods for 20 days, with transitions from fasting to feeding occurring at 15:00 each day. In separate lean and overweight/obese cohorts, the impact of this diet, both eucaloric (i.e. complete refeeding in fed periods) and with a 25% calorie restriction (i.e. 50% refeeding during fed periods), was contrasted against a standard 25% calorie restriction. The experiments featured measures of postprandial metabolic responses and free-living physical activity (combined heart rate/accelerometry). In lean adults (Chapter 5), combining intermittent fasting with calorie restriction decreased physical activity thermogenesis relative to intermittent fasting or calorie restriction in isolation, largely due to reduced spontaneous activity during fasting. However, there were no improvements in metabolic health, whilst intermittent fasting also resulted in smaller declines in fat mass than daily calorie restriction. A similar pattern was seen for body composition in overweight/obese individuals (Chapter 6) but none of the interventions caused adaptive changes in energy expenditure. Instead, combining intermittent fasting with calorie restriction reduced postprandial insulinaemia and improved fasted and postprandial plasma lipid concentrations. Collectively, this suggests that the amount and timing of energy intake exert interactive effects on metabolism and health, with baseline adiposity being an important determinant of responses.

Tags: intermittent fasting, energy metabolism, physical activity, metabolic health, body composition, obesity, diurnal, circadian rhythms, insulin, feeding.

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List of Abbreviations

Abbreviation	Full Term
AgRP	Agouti-related Peptide
AHEAD	Action for Health in Diabetes
ANOVA	Analysis of Variance
ATGL	Adipose Triglyceride Lipase
ATP	Adenosine Triphosphate
AUC	Area Under Curve
BMAL1	Brain and Muscle ARNT-Like 1
BMI	Body Mass Index
CALERIE	Comprehensive Assessment of the Long-term Effects of Reducing Intake of Energy
CART	Cocaine- and Amphetamine-regulated Transcript
CD36	Cluster of Differentiation 36
CHO	Carbohydrate
CI	Confidence Interval
CK1	Casein Kinase 1
CLOCK	Circadian Locomotor Output Cycles Kaput
CO_2	Carbon Dioxide
Con	Control
COX	Carbohydrate Oxidation
CPT1	Carnitine Palmitoyltransferase 1
CRY	Cryptochrome
DEXA	Dual-energy X-ray Absorptiometry
DIT	Diet-Induced Thermogenesis
DLMO	Dim Light Melatonin Onset
EDE-Q	Eating Disorder Examination Questionnaire
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-linked Immunosorbent Assay
EtOH	Alcohol
FAT	Lipid
FMI	Fat Mass Index
GLUT	Glucose Transporter
H_2O	Water
HDL	High-Density Lipoprotein
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
HSL	Hormone Sensitive Lipase
iAUC	Incremental Area Under Curve

Abbreviation	Full Term
JNK	c-Jun N-terminal Kinase
LDL	Low-density Lipoprotein
LOX	Lipid Oxidation
METs	Metabolic Equivalents
mTOR	mammalian Target of Rapamycin
NEFA	Non-esterified Fatty Acids
NHANES	National Health and Nutrition Examination Survey
NHS	National Health Service
NPY	Neuropeptide Y
O_2	Oxygen
PAL	Physical Activity Level
PAT	Physical Activity Thermogenesis
PER	Period
POMC	Preopiomelanocortin
POX	Protein Oxidation
PRO	Protein
RER	Respiratory Exchange Ratio
RIA	Radioimmunoassay
RMR	Resting Metabolic Rate
SCN	Suprachiasmatic Nucleus
SD	Standard Deviation
SEM	Standard Error of the Mean
SIRT1	Sirtuin 1
tAUC	Total Area Under Curve
TNF	Tumour Necrosis Factor
UK	United Kingdom
V.Vigorous	Very Vigorous
VAS	Visual Analogue Scales
VCO_2	Carbon Dioxide Production
VLDL	Very Low-density Lipoprotein
VO_2	Oxygen Uptake
$\dot{V}O_2$ max	Maximal Oxygen Uptake
75:75	Daily Calorie Restiction Intervention
0:150	Intermittent Fasting with Calorie Restriction Intervention
0:200	Intermittent Fasting without Calorie Restriction Intervention

Chapter 1: Lay Summary

Obesity is a prevalent health concern due to its association with conditions such as type 2 diabetes and cardiovascular disease. Despite efforts to address this in recent decades, the incidence of obesity has proved resilient to a host of evidence-based solutions. The scale of this issue is well-characterised by Ng *et al.* (2014), who highlight that at a population level, there have been no successful reductions in obesity prevalence in 33 years. Part of this may lie in the shortcomings of conventional strategies to treat obesity and the associated dysfunction, which target reductions in energy intake via restrictions on the type and/or amount of foods consumed (i.e. typical dieting), increases in energy expenditure (i.e. being more physically active), or a combination of the two. Although effective initially, these strategies struggle to deliver sustainable weight losses and health improvement in the long-term. This is often ascribed to the difficulty of sustaining such strategies, whilst there are also compensatory changes in both physiology and behaviour which seemingly counter weight loss efforts.

In recent years, there has been growing public interest in time-related approaches to dieting. These methods restrict calories within defined time periods, as opposed to applying a continuous restriction every day. Collectively, such strategies are commonly referred to as intermittent fasting, with the popular 5:2 diet being but one example. A number of arguments have been put forward to justify such an approach, including reduced motivational demands, ease relative to alternative methods, no requirements for any knowledge of nutrition or foods, and possibly offering better alignment with the dietary conditions which shaped human physiology over several millennia. However, the rise in the popularity of these approaches to dieting has vastly outpaced the research to support or refute their application in an obesity management context. Although the results emerging from the few scientific studies that have been undertaken are promising, several potential attributes of intermittent fasting have been largely overlooked, which could be vital in ascertaining the utility of these strategies.

Of particular interest is the extent to which intermittent fasting may elicit compensatory changes in energy use, either at rest or through physical activity. Even at an intuitive level, it seems reasonable to speculate that short but intense periods of

energy restriction would be less likely to induce a persistent reduction in energy use than a continuous restriction. If this is indeed the case, it would be a key strength of these intermittent approaches relative to the continuous dietary alternatives. Furthermore, the reduced number of eating occasions may also offer health benefits that are independent of weight loss. In most Western cultures, consuming at least three meals per day is considered a societal norm. This typically takes the form of breakfast, lunch and dinner, which are ordinarily separated by a matter of hours. Each of these eating occasions results in an influx of energy from varying amounts of carbohydrate, fat and protein, which the body handles by utilising what is required and storing the excess. This process takes a number of hours to resolve, meaning that an ensuing meal is usually consumed shortly after this initial influx of nutrients has been buffered, if not before. Consequently, there are but a few hours each day where nutrient and hormone levels are at basal values, which has been implicated in some of the dysfunction that accompanies obesity. Therefore, in providing these extended fasting periods, intermittent approaches may hold another advantage over continuous methods, wherein the frequency of meals is usually preserved.

The studies described in this thesis explored these potential facets to provide a more thorough appraisal of the utility of intermittent fasting as a strategy for managing obesity and the associated dysfunction. In an initial step, Chapter 4 measured rhythms in appetite and related regulatory hormones over 24-hours, to help establish how an intermittent fasting diet might best be designed to maximise therapeutic potential whilst minimising motivational demands. The resulting intervention used a complete alternate-day approach to intermittent fasting, where 24-hour periods of feeding were alternated with 24-hour periods of fasting, in which energy intake was not permitted whatsoever. Although extreme, this duration of uninterrupted fasting had previously been associated with improvements in health even in the absence of weight loss, making it the best choice considering the aims of the research. This particular design was also novel in so far as the transition from feeding to fasting and *vice versa* occurred at 15:00 each day. This was better aligned with the observed rhythms in hunger in Chapter 4 than previous formats, whilst enabling some form of feeding behaviour within each sleep-wake cycle to facilitate adherence to the diet.

In Chapter 5, this novel intermittent fasting intervention was combined with a net 25% reduction in energy intake and applied to a randomly allocated group of lean adults for 20 days. At the same time, two further groups of lean adults were randomly prescribed either a daily energy restriction of 25% or the same intermittent fasting strategy without any energy restriction. All three interventions were accompanied by comprehensive assessments of energy balance and metabolic health. Consequently, in comparing these three groups, it was possible to answer two key questions. Firstly, is intermittent fasting a better approach to weight loss and improving metabolic health than restricting calories every day? Secondly, are the effects of intermittent fasting a product of weight loss or the routine extension of fasting periods? In Chapter 6, the same method was then applied to a group of overweight/obese adults to address the same questions within the context of a pre-existing energy surplus.

Collectively, this thesis yields fascinating and original insights as to how intermittent fasting impacts upon energy expenditure and metabolic health, impacts which were seemingly dependent on body fat levels. In Chapter 7, the potential underpinnings of these observed effects are discussed, spanning the broad areas of body composition, physical activity and metabolic health. The conclusions drawn in the collective light of these studies not only carry implications for the utility of intermittent fasting and future research on the topic, but perhaps our understanding of the causes of obesity as well.

Chapter 2: Literature Review

2.0 – Obesity

2.0.1 – Definition

Obesity broadly describes a metabolic state characterised by excessive fat accumulation to an extent that can present a risk to health (World Health Organization, 2000; Abdelaal, le Roux and Docherty, 2017). In clinical terms, this is commonly established through a calculation of body mass index (BMI); body mass (kg) divided by height (m) squared (Keys *et al.*, 1972). Values of 30 $kg \cdot m^{-2}$ and above are considered indicative of obesity in most humans and do typically associate with deteriorations in health, which is in accordance with the aforementioned definition (Flegal *et al.*, 2013). Although this relies upon the assumption that elevated body mass is a consequence of increases in adiposity alone, which is incorrect (Romero-Corral *et al.*, 2008), these associations with adverse health outcomes persist across a range of direct and indirect adiposity measures (Janssen, Katzmarzyk and Ross, 2004; Flegal *et al.*, 2009; Ashwell, Gunn and Gibson, 2012; Rohan *et al.*, 2013; Qi *et al.*, 2015; Kim and Park, 2018). Therefore, courtesy of its relative ease of application (Adab, Pallan and Whincup, 2018), BMI remains at the centre of most statistics on obesity prevalence (Ranasinghe *et al.*, 2013).

2.0.2 – Prevalence

The scale of the issue that obesity presents is perhaps best illustrated by the systematic review of Ng *et al.* (2014), which summarised the prevalence statistics from 183 countries between 1980 and 2013. This provides a clear appraisal of the progression of obesity as a global epidemic, the cost of which is suggested to account for 2.8% of global spending (McKinsey Global Institute, 2014). The findings of this review suggest that 2.1 billion people are currently considered overweight or obese, with the prevalence continuing to rise in both developed and developing countries. In-part, this can be attributed to the ageing population in several developed countries, such as the UK, given the typical accumulation of adipose tissue over adulthood in Westernised

countries (Wang *et al.*, 2011). However, these increases in prevalence were reflected across all age groups, including both children and adolescents, with the most rapid rises seen between 20 and 40 years of age (Ng *et al.*, 2014). Although it is worth noting that the rate of increase was greatest from 1992 to 2002, and that in the last 10 years there has been a slowing of the rise in developed countries, globally there have been no reductions in obesity prevalence in 33 years (Ng *et al.*, 2014). This highlights the need for improved strategies for treating obesity.

2.0.3 – Obesity-Associated Chronic Diseases

Obesity has been acknowledged as a globally epidemic condition for almost two decades, which is due in no small part to its association with a number of chronic health conditions (World Health Organization, 2000). For instance, increasing BMI increments have been associated with a higher risk of type 2 diabetes (Abdullah *et al.*, 2010), cardiovascular disease (Liu *et al.*, 2014) and several cancers (Dobbins, Decorby and Choi, 2013). This notion is also reinforced by a recent meta-analysis from The Global BMI Mortality Collaboration (Di Angelantonio *et al.*, 2016). In a pooled cohort of over 10 million participants, the risk of all-cause mortality was found to be 45% greater in those with class I obesity (30.0-34.9 $kg \cdot m^{-2}$) relative to those with a normal BMI, increasing to 176% greater with class III obesity (40.0-59.9 $kg \cdot m^{-2}$).

Naturally, despite evidence using Mendelian randomisation that BMI may be causally related to disease (Nordestgaard *et al.*, 2012), the above observational studies can only identify such associations, and do not establish obesity *per se* as the causal factor. However, the proposed mechanistic underpinnings of such associations, as discussed later in Section 2.1.5, do lend credence to obesity having a direct role in numerous health conditions (Van Gaal, Mertens and De Block, 2006; Frayn *et al.*, 2012; Gilbert and Slingerland, 2013; Louie, Roberts and Nomura, 2013; Al-Goblan, Al-Alfi and Khan, 2014; Saltiel and Olefsky, 2017). Furthermore, reductions in the prevalence of a range of these associated conditions have been consistently reported following bariatric surgery (Pontiroli and Morabito, 2011; Cardoso *et al.*, 2017). These surgical treatments target larger and more sustainable weight losses compared to non-surgical alternatives and are often accompanied by remission of type 2 diabetes and resolution of hyperlipidaemia (Courcoulas *et al.*, 2014; Welbourn *et al.*, 2016). Unfortunately,

the upfront cost of these treatment strategies and the associated risk means that they are reserved for the most extreme cases, limiting their utility in resolving the burden of obesity at the population level (Welbourn *et al.*, 2016).

All the above collectively illustrate that obesity is a prevalent health concern, as evidenced by the inclusion of halting the rise in obesity as one of the nine targets in the 'Global Action Plan for the Prevention and Control of Non-Communicable Diseases: 2013-2020' from the World Health Organization (2013). Projections suggest that should current trends in obesity continue there will be a further 11 million obese adults in the UK by 2030, accompanied by an additional 600,000 cases of diabetes, 400,000 cases of coronary heart disease and stroke, and 110,000 cases of cancer (Wang *et al.*, 2011). Coupling this with a £2 billion increase in the cost to the National Health Service (NHS) (Wang *et al.*, 2011) and further losses of disability-adjusted life years (World Health Organization, 2009), finding more effective solutions to address the rising prevalence of obesity and the accompanying dysfunction remains paramount.

2.1 – Energy Balance

Fundamentally speaking, the aetiology of obesity distils down to conformity with the first law of thermodynamics; if energy stores within the body remain constant then the amount of energy entering the system must equal the amount of energy exiting the system (Hall *et al.*, 2012; Hill, Wyatt and Peters, 2012). Extending this premise, the chronic storage of energy which leads to obesity must reflect a sustained increase in intake, a sustained decrease in expenditure, or both (Wells and Siervo, 2011). However, whilst this reductionist view explains the central principle under examination, it ignores the complex interplay between these two facets of energy balance and the plethora of regulatory pathways which determine them (Spiegelman and Flier, 2001; Pan and Myers, 2018). Furthermore, when balance is not achieved, the change in stored energy in response to a surplus or a deficit is not as predictable as thermodynamic principles might suggest (Rosen and Spiegelman, 2006), as emphasised by the consistent observation that the regulatory system involved is seemingly biased toward weight gain (Schwartz *et al.*, 2003; Hill, Wyatt and Peters, 2012). Naturally, such disparities do not call the laws of thermodynamics in to question, but rather illustrate the complexity of the system when discussed in the context of human physiology.

The extent to which increases in fat mass are a result of increased intake or reduced expenditure remains a matter of debate, with the arguments to support both perspectives summarised by Hall *et al.* (2012). Of particular relevance here is where the locus of control lies for a given parameter. There can be no doubt that energy intake is a critical factor in terms of human physiology (Spiegelman and Flier, 2001), and whilst a physiological drive to procure food does exist in most organisms, the availability of energy to an organism is largely contingent upon the environment (Breslin, 2013). As such, beyond extreme or natural changes in either the physical capacity to ingest food (e.g. gastric bypass) or digestive/absorptive efficiency (Rosen and Spiegelman, 2006), energy intake is subject to an external locus of control, which is relatively unpredictable from a physiological perspective. Conversely, the other components of the equation, energy storage and energy expenditure, have a greater capacity to be modified intrinsically (Müller and Geisler, 2017) and thus anticipated. Only the need for activity arising from food procurement and other essential activities

of survival (e.g. evading predation) reside externally (Waterson and Horvath, 2015), but even this can be made more efficient (Muller *et al.*, 2015). As such, although the first law of thermodynamics clearly applies, the principal components of the equation are not static; energy use is typically modified to align with energy intake in a way that would be conducive to survival (Zera and Harshman, 2001; Hall *et al.*, 2012; Westerterp, 2017; Pan and Myers, 2018).

This principle is exemplified by Muller *et al.* (2015). In response to a chronic shortfall in energy supply, resting energy expenditure and sympathetic activity decrease whilst muscular work becomes more efficient, changes which seem to arise after just three days of energy restriction. Interestingly, in this study the restriction phase was preceded by one week of overfeeding with a 50% energy surplus, which was not accompanied by adaptive increases in any indicator of energy use (Muller *et al.*, 2015). This illustrates the disparity that exists between intake and expenditure, which most likely reflects that energy expenditure is predominantly dictated at the level of the organism, whilst intake is far more contingent on the environment, particularly in the evolutionary context in which many physiological processes developed (Konner and Eaton, 2010). Clearly, upon placing this regulatory bias in the energy-abundant environment of many Western societies, the likelihood of over consumption and energy storage is increased, whilst the adaptive changes to prevent declines in fat mass remain unaltered.

2.1.1 – Hypothalamic Energy Homeostasis

Numerous factors influence energy intake, storage and expenditure, which are coordinated by neuroendocrine pathways in the hypothalamus, the proposed hub of energy homeostasis (Waterson and Horvath, 2015; Timper and Brüning, 2017; Kim, Seeley and Sandoval, 2018). The hypothalamus benefits from selective permeability of the blood-brain-barrier at the medial eminence, meaning it can receive peripheral signals pertaining to energy homeostasis within the system (Rodríguez, Blázquez and Guerra, 2010; Schaeffer *et al.*, 2013). This includes key hormones implicated in the regulation of energy balance such as ghrelin, peptide YY, leptin and insulin (Rodríguez, Blázquez and Guerra, 2010; Schaeffer *et al.*, 2013). Within the arcuate nucleus of the hypothalamus are distinct neuronal clusters which are characterised

according to the neuropeptides they release. Neuropeptide Y- (NPY) and agouti-related peptide- (AgRP) secreting neurons are associated with anabolic effects, whilst preopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) neurons stimulate predominantly catabolic pathways (Lanfray and Richard, 2017). The latter primarily achieve these effects by secreting isoforms of the melanocyte-stimulating hormone, which can stimulate melanocortin receptors to exert their effects (Wilson and Enriori, 2015; Lanfray and Richard, 2017). Comparatively, NPY and AgRP neurons exert their effects by antagonising these signalling pathways (Lanfray and Richard, 2017; Kim, Seeley and Sandoval, 2018). The activity of these neurons therefore stimulates or suppresses the hypothalamic-melanocortin system in the paraventricular nucleus (Kim, Seeley and Sandoval, 2018), which projects to the brainstem and other regions to modify sympathetic and parasympathetic outflow (Rabasa and Dickson, 2016).

2.1.2 – Energy Intake

Determining the role of energy intake in the aetiology of obesity is less straightforward than is often anticipated (Butland *et al.*, 2007). Such complexities are alluded to when discussing energy intake from a purely physiological perspective. For instance, energy intake is typically described as the chemical energy contained within food and drink items that are ingested by an individual within a given timeframe, which in humans is primarily accounted for by carbohydrate, fat, protein and alcohol (Hall *et al.*, 2012). However, within this there will be a small fraction of each macronutrient that may be indigestible or metabolically unavailable, and as such the energy contained within it is excreted rather than absorbed (Krajmalnik-Brown *et al.*, 2012). The study of Southgate and Durnin (1970) observed across different dietary conditions that 4.5% and 3.6% of ingested energy consumed by healthy adults was lost through faecal and urinary excretion, respectively. Upon manipulating dietary composition to increase the energy contribution from plant polysaccharides, an indigestible carbohydrate, the faecal losses increased by approximately 2%, whilst urinary excretion remained largely unchanged. Similar effects of high fibre diets have been reported by others, in that a higher fraction of consumed energy is not absorbed from the gastrointestinal tract (Jumpertz *et al.*, 2011).

Further to this, absorption of ingested energy can also be influenced by other factors such as food preparation. This notion is well-illustrated by Burton and Lightowler (2008), who examined glycaemic responses to ingestion of white bread exposed to different storage and cooking methods. Specifically, freezing and toasting of bread were found to reduce the incremental area under the blood glucose curve, both alone and in combination. This was attributed to these processes enhancing the recrystallisation of resistant starch, which reduces the fraction of available carbohydrate within the food and therefore the amount of glucose (i.e. energy) that could be liberated to enter the circulation (Robertson, 2012; Sullivan *et al.*, 2017). Similar observations have also been made in the context of protein digestion, with consumption of beef mince resulting in greater systemic amino acid availability than beef steak (Pennings *et al.*, 2013).

Adding an extra layer to the complexity, recent advances in the understanding of the gut microbiome have also begun to uncover inherent differences between lean and obese individuals. Such differences could modify the energy harvesting capacity of the gastrointestinal tract and therefore influence energy balance (Turnbaugh *et al.*, 2006; Krajmalnik-Brown *et al.*, 2012). However, whether this is a cause or consequence of obesity remains a matter of debate, as the differences in the microbiome could instead be a biomarker of diet quality (Bäckhed, 2009; Menni *et al.*, 2017). Therefore, while the term energy intake will be used throughout this work to describe the energy contained within the consumed food and drink items that constitute the diet of a group or individual, it is important to note that research in to energy balance is far from a perfect science in this regard. This is reflected by recent suggestions that attempts to measure free-living energy intake using current methods may be entirely futile (Hall *et al.*, 2012; Dhurandhar *et al.*, 2015; Romieu *et al.*, 2017).

2.1.2.1 – Regulation of Energy Intake

While energy intake can primarily be thought of as an environmentally determined factor, it is also subject to regulation at a physiological level. Across numerous mammalian species, including humans, consuming discrete meals is a characteristic feeding behaviour even in the face of continuous energy availability (Baile and Forbes, 1974; Green, Pollak and Smith, 1987; Martire *et al.*, 2013). These discrete consumption patterns are a consequence of numerous interacting neuronal and

humoral factors, which drive sensations of hunger and satiety that broadly align with meal initiation and termination (Spiegelman and Flier, 2001; de Graaf *et al.*, 2004).

Many of these transient regulatory processes arise from the gastrointestinal tract in response to the presence or absence of substance and nutrients (Delzenne *et al.*, 2010). Afferents from this organ are innervated by both mechanical and chemical stimuli to relay information to the central nervous system on feeding state, particularly the hypothalamus (Wilson and Enriori, 2015). Mechanical distension of the stomach by any substance has been shown reduce appetite independent of nutrient intake (Geliebter, 1988), most likely operating via innervation of the vagus nerve (De Lartigue, 2016). However, these effects are potentiated by humoral signals released in response to nutrients entering various parts of the gastrointestinal lumen (Kissileff *et al.*, 2003). In the pre-prandial state, ghrelin is the principle hormone in question and is broadly described as an orexigenic peptide which plays a prominent role in meal initiation by acting on the hypothalamus (Cummings *et al.*, 2001; Austin and Marks, 2009). In agreement with this proposition, during a 24-hour fast Natalucci *et al.* (2005) observed pulsatile patterns of ghrelin secretion, with marked peaks that aligned with habitual meal times. Furthermore, exogenous administration of ghrelin has been shown to increase food intake in ensuing meals across a diverse range of human populations, as first demonstrated by Wren *et al.* (2001) and more recently verified by Garin *et al.* (2013).

Comparatively, several anorexogenic peptides have been identified which are also secreted from various sections of the gastrointestinal tract, regulating aspects of the digestive process and fostering appetite suppression via the hypothalamus (Delzenne *et al.*, 2010). A full discussion of these hormones is beyond the scope of this work, yet cholecystokinin, glucagon-like peptide 1, peptide YY, pancreatic polypeptide and oxyntomodulin are worthy of note due to their proposed role in modifying energy intake (Chaudhri, Small and Bloom, 2006). They are typically secreted in response to specific nutrients entering the small intestine (Crespo *et al.*, 2014), which – along with differences in energy density and rates of gastric emptying (Thomas, 1957) – is part of the reason why certain nutrients are cited as have a greater satiating effect than others (Karhunen *et al.*, 2008; Brennan *et al.*, 2012; Chambers, McCrickerd and Yeomans, 2015). To unravel their respective impacts on energy intake, several studies have

employed an approach in which the anorexigenic peptides are administered exogenously and indices of consumption are measured at an ensuing *ad libitum* meal. Employing such a method, reductions in energy intake have been reported with infusion of pancreatic polypeptide (Batterham *et al.*, 2003; Jesudason *et al.*, 2007), oxyntomodulin (Cohen *et al.*, 2003; Bagger *et al.*, 2015), glucagon-like peptide 1 (Flint *et al.*, 1998; Gutzwiller *et al.*, 1999; Näslund *et al.*, 1999; Bagger *et al.*, 2015), peptide YY (Degen *et al.*, 2005) and cholecystokinin (Brennan *et al.*, 2008), with the latter two exhibiting a dose-response association. However, it should be noted that some conflicting findings have been observed, particularly for glucagon-like peptide 1 (Flint *et al.*, 2001; Steinert *et al.*, 2014). This may be due to the use of supra-physiological concentrations by some of the aforementioned studies, or perhaps the infusion of several gut hormones simultaneously which has been proposed to elicit synergistic effects (Tan *et al.*, 2017). This shows that although energy intake is often a product of environmental influences, human physiology is able to drive this process by integrating a complex array of endocrine cues to control the initiation, rate and termination eating, thereby regulating energy intake on a short-term basis.

Shifting to focus on the long-term regulation of energy intake, leptin is often cited as being the key hormonal regulator of chronic energy balance (Rosenbaum and Leibel, 2014). Contrary to the appetite-related gut hormones described previously, leptin is secreted primarily from white adipose tissue, and as such the circulating concentration typically correlates with various indices of fat mass (Lean and Malkova, 2016). Current understanding suggests that leptin acts centrally by binding to receptors on neurons in the hypothalamus to regulate numerous functions (Seufert, 2004; Pan and Myers, 2018). These functions include suppression of appetite, as shown by studies of human leptin deficiency, which presents with obesity and hyperphagia. However, restoration of leptin levels within the physiological range through chronic exogenous administration has been shown to lower energy intake (Licinio *et al.*, 2004). Further work has revealed that this may be due to inhibition of brain regions relating to hunger and stimulation of those related to satiety, as measured by functional magnetic resonance imaging (Baicy *et al.*, 2007). Although these studies are lacking in statistical power due to the rare nature of the condition (Blüher, Shah and Mantzoros, 2009), the conclusions drawn are reinforced by animal models (Zhang *et al.*, 2007; Ottaway *et al.*, 2015). Interestingly, leptin can also bind neurons in the brainstem, which animal

models have shown may mediate sensitivity to the shorter-term regulatory pathways discussed previously, in a way that could facilitate meal termination (Morton *et al.*, 2005; Huo *et al.*, 2007). Unfortunately, whilst low leptin can serve as a signal to stimulate appetite, hopes of treating common obesity by elevating leptin above the normal range have not successfully suppressed appetite (Flier and Maratos-Flier, 2017), possibly indicating that leptin functions as a negative feedback signal more in relation to negative than positive energy balance.

2.1.2.2 – Measuring Energy Intake

Despite the plethora of regulatory elements, measuring energy intake may be considered the most challenging aspect of energy balance research in human participants (Romieu *et al.*, 2017). In fact, it has been described previously as one of the most challenging measures in human physiology (Garrow, 1978). This is because almost all validated methods rely on self-report data, which is confounded by misreporting due to observational, reporting and recall biases (Stubbs *et al.*, 2014). These methods can be broadly grouped in to prospective methods, which reside in the present and are therefore more likely to represent future intake, whilst others provide retrospective data, in so far as they collect information on prior intake (Stubbs *et al.*, 2014).

a) Retrospective Methods

The food frequency questionnaire is one such retrospective method and involves answering questions to provide frequency and portion size data for a predetermined list of foods over a given time period (Thompson *et al.*, 2010). However, it should be noted that this name encompasses a huge number of variants, with differences in the number and range of foods, as well as timescales (Hackett, 2011; Stubbs *et al.*, 2014). Courtesy of its low burden to participants, this method is frequently employed in large cohort epidemiological studies, such as the National Health and Nutrition Examination Survey (NHANES) (Ahluwalia *et al.*, 2016). However, there are widespread critiques of this approach, with a limited list of foods and low resolution of data, particularly at the individual level (Tucker, 2007; Shim, Oh and Kim, 2014). Other methods involve direct interaction with an interviewer, an approach that is characteristic of the diet history method and the 24-hour recall. In the former, the interviewer asks participants

to describe their habitual food intake over a defined period (e.g. 1 week), whilst in the latter information on consumption over the last 24 hours is gathered (Shim, Oh and Kim, 2014). It is common practice for a multiple pass approach to be employed in which layers of enquiry are structured to capture finer details, such as cooking method and beverages (Moshfegh *et al.*, 2008). These methods do hold certain advantages over the food frequency questionnaire in that they introduce a trained individual into the process (Shim, Oh and Kim, 2014). However, as with all retrospective methods, they are undermined by recall bias and they rely on estimated portion sizes, which can be a substantial source of error in of themselves (Almiron-Roig *et al.*, 2013).

b) Prospective Methods

To overcome the issue of recall bias and portion size estimation, weighed records of food and fluid intake ask participants to list all food and drink items consumed alongside a weighed portion size over a given time frame (Livingstone, Prentice, Strain, *et al.*, 1990). The timescale most often cited as the reference standard is 7 days, as this accommodates day-to-day variability in intake across the week (Ortega, Pérez-Rodrigo and López-Sobaler, 2015; Fuller *et al.*, 2017). However, the associated participant burden can lead to under-reporting or under-eating, therefore a 3-day record is frequently utilised to ease this demand (Trabulsi and Schoeller, 2001; Whybrow, Horgan and Stubbs, 2008; Fyfe *et al.*, 2010). Although such methods overcome the issue of recall bias, it is likely that some modification of intake will occur in order to ease the burden as far as possible (Ortega, Pérez-Rodrigo and López-Sobaler, 2015). For instance, participants may choose to consume more pre-prepared food due to the ease compared against weighing individual ingredients. The same is also true of the duplicate diet method, in which a duplicate portion of each food and drink item consumed is retained to be analysed for energy and nutrient parameters using laboratory tests (Abdulla *et al.*, 1981). However, this is accompanied by high costs and is still prone to the same risk of dietary modification. Observation bias is also a substantial consideration for all these measures, as participants may modify their responses based on perceived social desirability (Hebert *et al.*, 1995).

c) Validity

Courtesy of these various confounding factors, even validating these approaches remains a challenge, as there is no measure that can provide a value for energy and macronutrient intake without some form of observational or recall bias (Livingstone and Black, 2003). Perhaps the best attempt to assess the validity of these differing approaches to measuring energy intake undertaken thus far was that of Stubbs *et al.* (2014). This study employed a direct observation method as their reference standard and compared four widely used methodologies against it, namely weighed records, diet history, 24-hour recall and food frequency questionnaires. To achieve this, the researchers used a bespoke suite which was designed to allow covert but precise monitoring of energy and macronutrient intake throughout a period of 12 days. This time was separated in to covert phases in which participants were asked not to monitor their intake, and overt phases, in which participants completed 3-day weighed records and a series of 24-hour recalls whilst researchers continued with their covert monitoring. To confirm the validity of estimates obtained from the covert observation method, energy balance was monitored through daily measurements of body mass and doubly-labelled water was used to determine energy expenditure throughout all 12 days. The energy balance equation dictates that any change in energy intake should be reflected by changes in the amount of energy either stored or expended (Hill, Wyatt and Peters, 2012). As such, if measured energy intake is found to be above or below energy expenditure with no change in stored energy (i.e. body mass), it can be considered invalid. Naturally, the subjects were not informed of the purpose of the study due to the bias this would have introduced.

Over the 12 days, no change in body weight was observed and there was no difference between covertly-measured energy intake and energy expenditure, affirming the observed method as a valid approach. In comparing these observed values between the covert and overt windows, researchers identified a significant 5.3% reduction in intake, which can be considered as the observation effect. A similar observation effect has been reported under laboratory conditions as well (Robinson *et al.*, 2015, 2016). In comparing the overt observed intake with the self-reported methods during the same periods, the effect of reporting and assessment method can be isolated. This was -5.1% for the weighed dietary records and -10.1% for the 24-hour recalls, whilst the 7-day

diet history collected at the outset and the food frequency questionnaire completed at the end both under-estimated by approximately 15%. Therefore, the best self-report measure of those widely practiced is still likely to be under-reported by approximately 10%. Employing a similar method of covert observation, Whybrow *et al.* (2016) suggest that weighed dietary records underestimate energy intake by at least 5%, whilst Poppitt *et al.* (1998) observed significant under-reporting of energy intake through the 24-hour recall relative to covert observation in the order of 12.5%, both of which show good agreement with the findings of Stubbs *et al.* (2014). Interestingly, this deficit was accounted for almost entirely by under-reporting of snacks rather than meals. Other studies employing concomitant measurement of all three aspects of the energy balance equation have also established under-reporting as an issue across most self-report methods, with the scale varying based on numerous factors including adiposity, dietary restraint and socioeconomic status (Cook, Pryer and Shetty, 2000; Hill and Davies, 2001; Trabulsi and Schoeller, 2001; Singh *et al.*, 2009).

Drawing from the validation method deployed by Stubbs *et al.* (2014), there is a growing trend for biomarkers, which are less prone to these sources of bias, to be employed as a means of estimating energy intake and more recently the intake of specific food items (Yin *et al.*, 2017). The former is based on a rearrangement of the energy balance equation, wherein the sum of energy expenditure and any changes in energy stores must equal energy intake (Shook *et al.*, 2018). Although they do not stand alone as a means of dietary intake, providing no information on macronutrient consumption, they do provide an indication of the degree of error in traditional methods (Hedrick *et al.*, 2012). This frequently takes the form of the Goldberg Cut-off, which establishes the 95% confidence intervals within which discrepancies between intake and expenditure are attributable to chance, as opposed to misreporting in a state of energy balance (Livingstone and Black, 2003). Although several approaches have been developed for quantifying energy needs in this scenario, including estimation of basal metabolic rate and physical activity level, doubly-labelled water remains the reference standard (Livingstone and Black, 2003; Shook *et al.*, 2018). However, a recent study by Shook *et al.* (2018) utilised more accessible metrics to evaluate energy expenditure in this context and found comparable results to doubly-labelled water derived estimates with just 3 $kcal \cdot day^{-1}$ between them, which makes such approaches more viable at the population level.

Due to the widely accepted role of diet in the aetiology of numerous prevalent health conditions, developing more valid measures of dietary intake remains a core objective in nutrition research. Technological advances within fields such as wearable sensors (Fontana *et al.*, 2015) and image recognition, together with the uptake of smartphones, do offer some promising avenues for at least reducing the associated burden for participants and the confounding effects that this incurs. However, a biochemical marker of energy and nutrient intakes remains the elusive Holy Grail (Hedrick *et al.*, 2012), as emphasised by the inclusion of improving methodology for robust measurement in the field of human nutrition within the remit of the Global Challenges Research Fund (Medical Research Council, 2017). Objective measurements of energy expenditure and changes in stored energy are currently the closest attempt, however, these metrics in of themselves are prone to error.

2.1.3 – Energy Expenditure

The other side of the energy balance equation is energy expenditure, which can be thought of as the chemical energy utilised to perform integral biological processes and physical work (Hall *et al.*, 2012). In much the same way as energy intake is accounted for by the main macronutrients, total daily energy expenditure is typically ascribed to the summation of three components, namely basal metabolic rate, physical activity energy expenditure and diet induced thermogenesis (Hills, Mokhtar and Byrne, 2014; Lam and Ravussin, 2016). Beyond these three core components, the concept of adaptive thermogenesis describes the potential for these dimensions of energy use to be adjusted in order to maintain homeostasis in response to various stimuli. This includes extreme models of starvation or overfeeding, changes in ambient temperature (e.g. cold-induced thermogenesis) or, more commonly, sustained weight loss (Camps, Verhoef and Westerterp, 2013; Muller *et al.*, 2015).

Basal metabolic rate reflects the chemical energy required to fuel the fundamental biological processes necessary for the survival of an organism (Hills, Mokhtar and Byrne, 2014). This includes the energy required to fuel the sodium/potassium pumps that allow neuronal firing in the brain (Forrest, 2014), the chemical energy used to permit contraction of the cardiac tissue (Tuomainen and Tavi, 2017), and the energy used to fuel integral processes within each cell (Lynch and Marinov, 2015). The energy

cost associated with these will also vary throughout the lifecycle of the organism, during adolescence or pregnancy for instance (Müller and Geisler, 2017), and as such there is capacity for variation in basal energy use both between and within individuals (Hills, Mokhtar and Byrne, 2014). In a review of 197 studies, McMurray *et al.* (2014) propose a mean value of 0.863 $kcal \cdot kg^{-1} \cdot h^{-1}$, but that this varied in accordance with numerous factors such as sex, BMI and age. As the units reflect, a key determinant of this resting component is body size, with fat-free mass associating closely (Johnstone *et al.*, 2005; Hopkins *et al.*, 2016; Lam and Ravussin, 2016), and more specifically skeletal muscle metabolism (Zurlo *et al.*, 1990; Illner *et al.*, 2000; Wang *et al.*, 2010).

Physical activity energy expenditure on the other hand describes the energy invested to perform voluntary muscular work, from maintaining posture to formal exercise sessions (Lam and Ravussin, 2016). This can be primarily considered as a function of behavioural and environmental determinants, although changes in the ratio of glycolytic to oxidative enzymes have been shown to modify the efficiency of muscle metabolism, which shows that the same physical work could have differing energy costs (Goldsmith *et al.*, 2010). Similarly, both human and animal models have noted that decreases in the energy cost of low-to-moderate intensity activity may also stem from reductions in sympathetic outflow, which also improves efficiency (Muller *et al.*, 2015; Almundarij, Gavini and Novak, 2017). As such, while physical activity thermogenesis is primarily accounted for by behavioural factors, there appears to be some capacity for intrinsic regulation as well.

Lastly, diet-induced thermogenesis is the energy invested to digest, absorb and metabolise nutrients contained within the foods and fluids an organism consumes. This is often approximated to 10% of energy intake for Western diets (Westerterp, 2004), however, there is substantial variability across the four macronutrients. The energy invested to digest and absorb fats, carbohydrates, alcohol and proteins is suggested to be 0-3%, 5-10%, 15-27% and 20-30%, respectively (Tappy, 1996). In addition to the impact of dietary composition on this parameter, there also seems to be differences with time of day, as the thermogenic effect of feeding appears to be greater in the morning relative to the evening despite matching meals for energy and macronutrient content (Bo *et al.*, 2015).

2.1.3.1 – Regulation of Energy Expenditure

Given the number of components that contribute to total daily energy expenditure, and the capacity for variation within each of these, the question of how energy expenditure is regulated is imperative in understanding how energy balance is regulated. Numerous authors suggest that a regulatory system operates within human physiology, which seeks to align energy intake and energy expenditure (Spiegelman and Flier, 2001; Wilson and Enriori, 2015; Pan and Myers, 2018). In keeping with this notion, the brain regions that are implicated in the regulation of energy intake are also implicated in the regulation of energy expenditure. In animal models, lesions of the hypothalamus have been shown result in obesity, even under conditions of consistent energy intake, which points to a regulatory role in energy expenditure (Vilberg and Keesey, 1984). This appears to be coordinated through signalling to metabolic tissues via endocrine factors and outflow to the sympathetic and parasympathetic nervous systems (Schwartz and Seeley, 1997; Münzberg *et al.*, 2016).

Focusing on the hypothalamic-pituitary-thyroid axis, in response to changes in energy needs there are changes in the activity of neurons controlling the secretion of the neuropeptide thyrotropin-releasing hormone. Specifically, leptin stimulates these neurons whilst fasting inhibits them, leading to changes in the secretion of thyroid hormone isoforms which positively regulate resting metabolic rate (RMR) (Mullur, Liu and Brent, 2014). This is achieved through binding to receptors on peripheral tissues, including skeletal muscle, to stimulate metabolism and energy expenditure (Münzberg *et al.*, 2016). This is reinforced by strong positive correlations between basal metabolic rate, to which skeletal muscle is a major contributor, and thyroid hormone levels (López *et al.*, 2013). In response to prolonged fasting, increases in acylated ghrelin and decreases in leptin reduce the activity of thyrotropin-releasing hormone releasing neurons, which provides an avenue through which the two sides of the energy balance equation could be coupled (Kluge *et al.*, 2010; Mullur, Liu and Brent, 2014).

The hypothalamic-pituitary-adrenal axis has also been implicated in humoral regulatory pathways, which aim to modify energy use as part of a reactive or anticipatory response (Herman *et al.*, 2016). In response to stressors, corticotropin-releasing hormone is secreted from hypothalamic neurons at the medial eminence

(Rabasa and Dickson, 2016). This increases the release of adrenocorticotropic hormone from the pituitary gland, which in turn stimulates synthesis and secretion of cortisol from the adrenal cortex (Nieuwenhuizen and Rutters, 2008; Rabasa and Dickson, 2016). This is part of the stress response which elicits a catabolic state to mobilise energy stores and leads to increases in energy expenditure (Rabasa and Dickson, 2016). Infusion studies, and supplementary withdrawal in cases of adrenocortical failure, suggest that cortisol increases energy expenditure in agreement with this proposition (Brillon *et al.*, 1995; Christiansen *et al.*, 2007). Interestingly, cortisol also acts in a negative feedback loop to suppress the hypothalamic-pituitary-adrenal axis, which may explain some of the conflicting associations between cortisol and energy expenditure, with acute administration showing effects whilst more chronic exposure shows no effect (Nieuwenhuizen and Rutters, 2008). Nonetheless, this pathway is suggested to be regulated to some extent by energy balance, with hormones such as insulin and leptin acting to suppress the activity of this axis (Heiman *et al.*, 1997; Schwartz and Seeley, 1997; Morton *et al.*, 2015).

2.1.3.2 – Measuring Energy Expenditure

Compared to energy intake, there are a multitude of objective measurement techniques that are available for measuring energy expenditure (Galgani and Ravussin, 2008), each of which carries its own strengths and weaknesses. Many techniques adopt the approach of measuring aspects, be they reactants or products, of the chemical reactions that lie at the heart of energy metabolism (Levine, 2005). These fundamental processes are shown in equations 1-3, which are adapted from Frayn (1983), and reflect the chemical reactions that proceed during metabolism of a single unit of carbohydrate, fat and protein:

$$1\ g\ Glucose + 0.746\ L\ O_2 \rightarrow 0.746\ L\ CO_2 + 0.6\ g\ H_2O + Energy \quad (1)$$

$$1\ g\ Lipid + 2.029\ L\ O_2 \rightarrow 1.430\ L\ CO_2 + 1.09\ g\ H_2O + Energy \quad (2)$$

$$1\ g\ Protein + 0.966\ L\ O_2 \rightarrow 0.782\ L\ CO_2 + 0.45\ g\ H_2O + Energy \quad (3)$$

a) Direct Calorimetry

Direct calorimetry measures the thermogenic effect of metabolic processes (Kenny, Notley and Gagnon, 2017). This is because the oxidation reactions shown in equations 1-3 are not 100% efficient, in so far as approximately a third of the energy liberated is lost as heat rather than being transferred to the intermediary adenosine triphosphate (ATP) (Ferrannini, 1988), with net efficiency estimated to be in the region of 50% (Nath, 2016). As such, the heat lost by an organism to its environment is directly proportional to the energy expended by the organism (Kenny, Notley and Gagnon, 2017). The challenge in this approach lies in reliably capturing the principle in question.

Direct calorimeters use a closed system which allows heat exchange to occur freely within it, but not between it and the environment in which it is placed (Snellen, Chang and Smith, 1983). Building upon the original work of Lavoisier and Leplace, discussed by Lodwig and Smeaton (1974), water and air are used as mediums for heat transfer. The known specific heat capacity of each allows changes in temperature to be equated to an energy parameter (Kenny, Notley and Gagnon, 2017). One such system is extensively described by Webster *et al.* (1986), however, direct calorimetry is scarcely used due to the operational challenges it presents relative to the alternatives (Kenny, Notley and Gagnon, 2017). A key limitation in this regard is the constraints it places on physical activity. Although larger chambers have been devised to allow measurements in more diverse and representative scenarios, they are not free-living and therefore exert confounding influences on behaviours (Webster *et al.*, 1986; Kenny, Notley and Gagnon, 2017).

b) Indirect Calorimetry

A more widely practiced technique based around a similar principle is indirect calorimetry. Oxidation of a given mass of glucose, fat or protein will consume a known quantity of oxygen and produce a known quantity of carbon dioxide (Frayn, 1983; Ferrannini, 1988), as shown in equations 1-3. As such, the oxygen uptake of an organism reflects the oxygen required to oxidise the three substrates in sufficient quantities to fulfil the energy needs of said organism. The same is also true of carbon

dioxide production, in that it reflects the amount of carbon dioxide produced as a sum of all these oxidative processes. Therefore, by measuring rates of oxygen uptake and carbon dioxide production, it is possible to derive estimates of energy metabolism from expired gas samples (Douglas, 1911; Weir, 1949; Frayn, 1983; Lam and Ravussin, 2016), as described in Section 3.4.2.

Although this holds the advantage of not only estimating energy expenditure but also providing indications of substrate metabolism, it relies on a number of assumptions. Of particular note is the assumption that all oxygen and carbon dioxide kinetics reflect oxidation reactions in that timescale (Weir, 1949; Frayn, 1983; Ferrannini, 1988). This means that non-metabolic processes which consume or produce oxygen and carbon dioxide, including the interconversion of different substrates, introduce a degree of error (Jéquier and Felber, 1987; Schutz, 1995). Nonetheless, there are a number of methods utilised to capture the necessary data for this technique. Respiratory chambers allow oxygen uptake and carbon dioxide production to be measured with a high degree of accuracy over prolonged periods, however, the confined space limits their use to predominantly resting tasks (Levine, 2005; Schoffelen and Plasqui, 2018). Douglas bags offer a similar level of data without the impracticalities of cost and space (Douglas, 1911; Levine, 2005), but this is better suited to short periods and still constrains the type of activity that can be undertaken. This is overcome to some extent by automated and portable systems, which employ continuous monitoring of oxygen and carbon dioxide concentration alongside an inline flow sensor to equate this to a volume (Kenny, Notley and Gagnon, 2017). However, while this works well in permitting a greater variety of tasks, it is still constrained to laboratory and field-based simulations, as opposed to prolonged monitoring of free-living scenarios (Levine, 2005).

c) Doubly-Labelled Water

As discussed above, what these measures offer in terms of precision and resolution, they lack in terms of introducing confounding influences on behaviour (Levine, 2005). Whilst this is not a problem when quantifying RMR and diet-induced thermogenesis, in so far as the equipment available allows these measurements to be captured in representative scenarios, it does hinder free-living assessments of physical activity

energy expenditure. The criterion method in this regard is doubly-labelled water (Westerterp, 2017), which uses isotope tracing to estimate carbon dioxide production as an indicator of cellular respiration (Schoeller and van Santen, 1982). When combined with a value for respiratory exchange ratio (RER), which is usually assumed to be 0.85, this can then be used to quantify energy use over several days (Schoeller and van Santen, 1982).

Following the ingestion of water composed of hydrogen and oxygen isotopes, several hours are allowed for the isotopes to reach equilibrium in the body water pool before a sample is obtained to provide a starting value (Speakman, 1990). Over time, hydrogen isotopes are lost predominantly through water excretion, and while the same is also true of the isotopic oxygen atoms, a fraction will also be incorporated into carbon dioxide molecules that are produced during cellular respiration (Westerterp, 2017). As such, the oxygen isotope is depleted at a faster rate than the hydrogen isotope and this difference is proportional to the amount of carbon dioxide that has been produced, an indicator of the amount of cellular respiration that has taken place (Speakman, 1990). As such, measuring the rate of depletion of the two isotopes in subsequent samples of the body water pool allows energy expenditure to be estimated for the interval between samples (Westerterp, 2017).

Although this is both a precise and non-invasive approach to measuring energy expenditure across multiple scenarios, there are a number of assumptions within the method which can compromise the accuracy under certain scenarios (Speakman, 1990; Butler *et al.*, 2004). For instance, the method does not account for the incorporation of isotopes into molecules other than water and carbon dioxide (Speakman and Hambly, 2016), and nor does it consider changes in enrichment arising from dietary modification (Bhutani *et al.*, 2015). Furthermore, the accuracy of measurements at the individual level is questionable, the acquisition of isotopes remains costly, and without sampling at frequent intervals the resolution of data is low, thereby overlooking key components of physically active behaviours, such as temporal distribution and timing (Butler *et al.*, 2004; Brage *et al.*, 2005; Westerterp, 2017). Perhaps most importantly however, is that any intervention that may impact substrate selection (e.g. fasting, exercise and/or adjusting dietary macronutrient balance) could shift RER from the assumed value of 0.85. This would systematically bias calculated values for total

energy expenditure between treatments. For example, a higher rate of fat metabolism relative to carbohydrate metabolism (i.e. a decrease in RER) would underestimate energy expenditure.

d) Wearable Sensors

Owing to the various logistical challenges associated with doubly-labelled water, wearable sensors are often employed to measure physical activity using surrogate indices. Such approaches offer dynamic temporal free-living data over extended periods for a lower cost, but this must be weighed against the accuracy of the measurement.

Accelerometry – Accelerometers are often utilised to quantify physical activity energy expenditure, as evidenced by their widespread incorporation into commercial devices (Chowdhury *et al.*, 2017). In measuring accelerative forces across different movement planes, they provide an indication of the duration, frequency and intensity of bodily movements, and in doing so begin to capture some key facets of physically active behaviours (Troiano *et al.*, 2014). However, upon comparing accelerometers against the free-living reference standard of doubly-labelled water, only 40% of the variance in overall physical activity energy expenditure is explained in most instances (Plasqui and Westerterp, 2007; Jeran, Steinbrecher and Pischon, 2016). Although the authors highlight that this could be improved with advancements in technology, some variance is likely to reside outside of the measurement technique given that such devices only capture data on movement, when energy expenditure incorporates a plethora of physiological determinants as well (Tudor-Locke *et al.*, 2002).

Heart Rate – To bridge the gap between measuring indices of movement (such as those gleaned from pedometers and accelerometers) and physiological measures (such as heat production and respiratory gas exchange) heart rate is often employed. The advantage here is that heart rate shows a linear relationship with oxygen uptake under diverse scenarios (Bot and Hollander, 2000; Freedson and Miller, 2000), which is inherently linked to energy expenditure (Section 2.1.3). Although there is substantial inter-individual variation in the relationship owing to factors such movement efficiency, age and fitness (Brage *et al.*, 2007), an individual calibration procedure can

be employed to accommodate such factors more effectively (Hills, Mokhtar and Byrne, 2014). When calibrated, heart rate shows good agreement with doubly-labelled water, particularly when using a defined threshold to differentiate between rest and activity (Livingstone, Prentice, Coward, *et al.*, 1990; Brage *et al.*, 2004). However, heart rate is prone to various confounding influences, with temperature, hydration status and stress amongst the most prominent (Turner and Carroll, 1985; Achten and Jeukendrup, 2003). In these scenarios heart rate is modified without a proportionate change in oxygen uptake, which is likely to lead to under- or over-estimation of energy expenditure in certain situations.

Combined Heart Rate and Accelerometry – Although the above two approaches are often adopted separately due to offering a reasonable estimate without the logistical challenges and low resolution of doubly-labelled water, their accuracy limits their application, particularly under the myriad influences that exist in free-living scenarios (Brage *et al.*, 2004). In a novel approach, researchers began to overcome these shortfalls by combining outcomes from these wearable sensors. Coupling data obtained from accelerometry with that of heart rate allows differentiation between a broader range of scenarios (Haskell *et al.*, 1993; Luke *et al.*, 1997; Rennie *et al.*, 2000). This is usually achieved through branched-equation modelling, as described by Brage *et al.* (2004). This system uses quantitative thresholds to classify each minute of activity in to one of four categories: low movement/low heart rate, low movement/high heart rate, high movement/low heart rate and high movement/high heart rate. The allocation across the different branches determines the weighting that is applied to each of the sub-metrics in the estimation of physical activity energy expenditure (Brage *et al.*, 2004). In applying this method, estimates were closer to energy expenditure determined via whole-body calorimetry than either metric in isolation, and even the combined metric without the branched-equation system (Brage *et al.*, 2004). This led to the development of Actiheart™ monitors, which feature a chest-worn heart rate sensor and accelerometer, coupled with in-built memory and a rechargeable battery for long-term monitoring (Brage *et al.*, 2005).

In an initial validation step, both the movement and heart rate sensors showed strong correlations with mechanical and electrical simulations (Brage *et al.*, 2005). Subsequently, estimates of physical activity intensity during treadmill walking and

running using the Actiheart™ devices were compared against estimates derived from concurrent indirect calorimetry. The combined heart rate and movement model (standard error of the estimate = 65.7 $j \cdot min^{-1} \cdot kg^{-1}$) performed better than the movement and heart rate models in isolation and improved further when a basic individual calibration was included to accommodate differences in the relationship between heart rate and physical activity intensity (standard error of the estimate = 57.7 $j \cdot min^{-1} \cdot kg^{-1}$) (Brage *et al.*, 2005). Employing more sophisticated calibration procedures has lowered the standard error of the estimate further to 26 $j \cdot min^{-1} \cdot kg^{-1}$ (Brage *et al.*, 2007). Upon contrasting against doubly-labelled water, calibrated measurements using these combined sensors did not differ significantly from estimates obtained from reference standards (Brage *et al.*, 2015). This suggests that the Actiheart™ provides similar precision and variability in measuring physical activity energy expenditure relative to the reference standard, offering improvements over heart rate and accelerometry in isolation. However, this method also adds valuable information about the dynamic patterns through which energy is expended in terms of time and intensity. Ultimately, the use of one or a combination of these metrics will depend on the objectives of the study and a consideration of the logistical challenges they present.

2.1.4 – Energy Storage

In scenarios where energy intake and energy expenditure are not aligned, the imbalance is reflected by changes in stored energy within the body. Even small, sustained discrepancies in the order of 24 $kcal \cdot day^{-1}$, the equivalent of one square of chocolate, have been suggested to amount to a kilogram of weight gain over a year, even after accounting for the accompanying increases in energy requirements (Hall *et al.*, 2011). However, given that excess energy can be stored in the form of carbohydrate, fat or protein, which vary markedly in their impacts on metabolic health, it is important to explore how the partitioning of surplus energy into energy stores is regulated.

2.1.4.1 - Carbohydrate

Carbohydrate is primarily stored in the form of glycogen, a polysaccharide featuring branched chains of α-1,4- and α-1,6-linked glucose residues (Adeva-Andany *et al.*, 2016). The human body can typically store 500-1000 grams in total, with

approximately 80% localised to skeletal muscle reserves and a further 20% residing in the liver, with just small depots in the brain, heart, kidneys and adipose tissue (Acheson *et al.*, 1988; Flatt, 1995; Wasserman, 2009; Jensen *et al.*, 2011; Ørtenblad, Westerblad and Nielsen, 2013; Adeva-Andany *et al.*, 2016). These reservoirs primarily serve to maintain blood glucose concentrations, which collectively amount to approximately 4 grams in a 70 kg individual and are vital to numerous organs, not least the brain (Wasserman, 2009).

In the post-absorptive state, glucagon is secreted from the pancreas and the resultant balance between elevated glucagon and fasted (i.e. low) insulin encourages the degradation of glycogen stores, a reaction catalysed by glycogen phosphorylase (Adeva-Andany *et al.*, 2016; Röder *et al.*, 2016). In the liver, the glucose is secreted into the portal circulation to help match oxidation rates by various tissues, whilst in skeletal muscle it is phosphorylated to glucose-6-phosphate by hexokinase II, which creates a negative feedback loop that lowers rates of glucose uptake into skeletal muscle (Wasserman, 2009; Adeva-Andany *et al.*, 2016). In the postprandial state, appearance of glucose from the gastrointestinal tract increases circulating glucose concentrations. Glucose transporter (GLUT) 2 proteins spanning the membrane of hepatocytes allow facilitated uptake of glucose from the portal circulation independent of insulin (Adeva-Andany *et al.*, 2016). Similarly, GLUT2-facilitated uptake by pancreatic beta cells stimulates secretion of insulin (Thorens, 2015; Röder *et al.*, 2016). Upon binding of insulin to receptors on skeletal muscle and adipocytes, an intracellular signalling cascade initiates GLUT4 translocation to the plasma membrane (Shepherd and Kahn, 1999; Karim, Adams and Lalor, 2012; Adeva-Andany *et al.*, 2016). These transporters are rate limiting for glucose uptake, and insulin stimulation has been proposed to increase the membrane content of these proteins by up to 40 times the fasted level (Brewer *et al.*, 2014). Whilst GLUT2 is not insulin sensitive, hepatic glucose metabolism is nonetheless regulated by insulin at the level of phosphorylation and glycogen synthesis (Adeva-Andany *et al.*, 2016). Consequently, post-prandial insulin secretion helps to buffer the influx of ingested glucose by stimulating uptake and/or storage across tissues including liver, muscle and adipose. This buffering therefore maintains euglycaemia to avoid toxicity, whilst simultaneously replenishing glycogen reserves through insulin-stimulated activity of glycogen synthase (Wasserman, 2009; Han *et al.*, 2016).

2.1.4.2 - Protein

Comparatively, protein stores have been suggested to account for up to a third of stored energy in a lean male (Galgani and Ravussin, 2008). Synthesised from varying combinations of amino acids, proteins fulfil a wide variety of functional roles, which include being the principle component of enzymes, hormones and antibodies (Frayn, 2010). Storage is predominantly in the form of skeletal muscle tissue, which accounts for up to half of whole-body protein content (Milan *et al.*, 2015; Abdulla *et al.*, 2016). Under conditions of protein balance, wherein skeletal muscle mass remains unchanged, muscle protein turnover is approximately 350 grams per day (i.e. 1-2%), which means that protein balance itself reflects a constant interaction between synthesis and breakdown (Rostom and Shine, 2018). Atherton and Smith (2012) refer to this as a dynamic equilibrium, wherein synthesis exceeds breakdown in the fed state whilst the converse is true in the fasted state, resolving itself to balance over a 24-hour period.

In structural terms, skeletal muscle can be sequentially broken down in to fascicles, muscle fibres, myofibrils and sarcomeres, the latter of which are composed of overlapping filaments of actin and myosin which primarily constitute the contractile apparatus (Meyers *et al.*, 2008; Gillies and Lieber, 2011). High-throughput proteomics methods suggest that much of the protein turnover in muscle tissue is in the contractile proteins (Shani *et al.*, 1981; Uhlen *et al.*, 2015; Uhlen *et al.*, 2010), which is supported by a decrease in myosin and actin protein concentration following bed-rest induced atrophy (Borina *et al.*, 2010). Although the precise mechanisms regulating synthesis and breakdown remain elusive and are beyond the scope of this review, mammalian target of rapamycin (mTOR) and the fork head box O isoforms are believed to be central to synthesis and breakdown, respectively (Atherton and Smith, 2012; Milan *et al.*, 2015).

Amino acid availability and exercise have also been consistently implicated in determining rates of muscle protein synthesis and breakdown (Atherton and Smith, 2012; Sanchez, Candau and Bernardi, 2014). This is because increases in extracellular amino acid concentration are sensed by membrane-bound proteins, such as sodium-coupled neutral amino acid transporter 2, which traffic them in to the cell and prompt the activation of mTOR (Dickinson and Rasmussen, 2013). This signalling path also

upregulates the production of amino acid transporters, further enhancing intracellular delivery of amino acids for use in protein synthesis during times of abundance (Dickinson and Rasmussen, 2013). Interestingly, resistance exercise can also elicit prolonged upregulation of these transporter proteins relative to amino acid ingestion, giving an enhanced sensitivity to nutrient availability in the post-exercise period and fostering an anabolic state (Drummond *et al.*, 2011; Dickinson and Rasmussen, 2013). Achieving a long-term positive protein balance therefore requires adequate intake of dietary amino acids together with a growth stimulus, such as exercise or an increase in sex hormones (Dideriksen, Reitelseder and Holm, 2013). In times of shortage however, the constant pattern of synthesis and breakdown will shift in favour of catabolic pathways, allowing energy to be liberated from muscle proteins in the form of glucose and ketone bodies to provide a vital auxiliary fuel (Grabacka *et al.*, 2016; Luo and Liu, 2016; Rostom and Shine, 2018).

2.1.4.3 - Fat

Lastly, fat stores account for the largest fraction of stored energy in most individuals (Galgani and Ravussin, 2008). This predominantly takes the form of adipose tissue, which is composed of cells known as adipocytes, housed within a matrix of blood vessels, immune cells, collagen and fibroblasts (Ahima and Flier, 2000). Fat is deposited in lipid droplets within adipocytes in the form of triglycerides; a glycerol base bound to three fatty acid molecules (Frayn *et al.*, 2003). The delivery of fatty acids is achieved via chylomicrons from the intestine and very low-density lipoproteins (VLDL) from the liver, both of which are hydrolysed by lipoprotein lipase (LPL) (Mead, Irvine and Ramji, 2002). The glycerol base on the other hand can be obtained in the form of glycerol-3-phosphate via glycolysis, or from oxaloacetate via glycerolneogenesis (Nye *et al.*, 2008).

The uptake and storage of fatty acids within adipose tissue is primarily controlled by insulin. Insulin stimulates the enzyme LPL, which extracts fatty acids from circulating chylomicrons and low-density lipoproteins (LDL) for use in triglyceride synthesis (Dimitriadis *et al.*, 2011). These extracted fatty acids are then trafficked in to cells by CD36 (Cluster of Differentiation 36), a protein that facilitates cellular uptake of fatty acids in multiple tissues (Goldberg, Eckel and Abumrad, 2009). In addition, insulin is also believed to stimulate enzymes implicated in the esterification of these fatty acids

to the glycerol base, including glycerol-3-phosphate acyltransferase, via effects on sterol regulatory element binding protein-1c, which catalyses the proposed rate limiting step in triglyceride synthesis in adipose tissue (Horton, Goldstein and Brown, 2002; Coleman and Lee, 2004; Palou *et al.*, 2008; Wendel, Lewin and Coleman, 2009). Comparatively, in the lower plasma insulin concentrations associated with the post-absorptive state, these lipogenic pathways are suppressed by the absence of insulin (Saltiel and Kahn, 2001). Instead, Adipose Triglyceride Lipase (ATGL) and Hormone Sensitive Lipase (HSL), which are usually suppressed by insulin, stimulate lipolysis to liberate fatty acids from the glycerol base, thereby allowing them to be secreted into the circulation (Coleman and Mashek, 2011; Thompson *et al.*, 2012). Overall, this suggests that insulin acts to encourage energy storage in the postprandial period but drops in the post-absorptive period to allow stored energy to be liberated and utilised (Birsoy, Festuccia and Laplante, 2013).

It is also worthy of note that small triglyceride depots have been identified in the pancreas, heart, liver and skeletal muscle tissue, which have been implicated in the metabolic syndrome which accompanies obesity (van Herpen and Schrauwen-Hinderling, 2008). Intramuscular triglycerides seem to localise around the mitochondria in healthy individuals, suggesting a functional role in substrate metabolism, perhaps buffering the shortfall in fatty acid uptake early in exercise (van Loon, 2004; Roepstorff, Vistisen and Kiens, 2005). By comparison, the role of intrahepatic triglycerides is less clear, with some suggesting that it is simply a consequence of high rates of lipid trafficking (van Herpen and Schrauwen-Hinderling, 2008). However, it also seems plausible that these intrahepatic reserves fulfil the same role as intramuscular pools in offering a more proximal supply of substrate, only in this instance it would be for use in ketogenic and gluconeogenic pathways (Wasserman, 2009; Grabacka *et al.*, 2016; Luo and Liu, 2016).

2.1.4.4 – Nutrient Balance

Given the parallels between the main macronutrients ingested in the diet and their corresponding storage depots, it would be reasonable to suspect that overfeeding of each macronutrient is associated with a proportional increase in the size of that energy reserve. However, this is not the case. In a landmark study, Abbott *et al.* (1988) explored the relationship between energy balance and carbohydrate, protein and fat

balance using 24-hour indirect calorimetry in a respiratory chamber. Participants were prescribed a weight maintenance diet and were required to abstain from vigorous exercise, but differences in energy homeostasis arising from factors such as body composition and low-level physical activity provided a spectrum of energy balance values, spanning approximately 1200 kcal·day^{-1}. Simple correlations revealed that changes in energy balance were positively associated with fat balance in both sexes, whereas no correlation was observed with either carbohydrate or protein balance. This suggests that fat buffers energy imbalance, being oxidised during energy shortage and stored during energy surplus (Galgani and Ravussin, 2008).

A popular misconception is that this increase in fat mass despite protein or carbohydrate overfeeding is courtesy of *de novo* lipogenesis, wherein triglycerides are synthesised from non-lipid substrates (Hellerstein, 1999; Solinas, Borén and Dulloo, 2015). Although this capacity is one that human physiology retains, it is only seen under exceptional circumstances. This is well illustrated by Acheson *et al.* (1988), who depleted glycogen stores via dietary restriction before prescribing a 7-day carbohydrate overfeeding regimen. This required a daily 1500 kcal overfeed alongside a progressive increase in carbohydrate delivery from 737 to 981 grams·day^{-1}. The onset of carbohydrate overfeeding was initially accommodated by increases in carbohydrate oxidation and glycogen storage. However, as carbohydrate intake continued to increase, oxidation plateaued at approximately 500 grams·day^{-1}, whilst partitioning into glycogen stores progressively decreased as they became saturated. The imbalance that resulted between intake and disposal in the latter days of the overfeed was instead accommodated primarily by an increase in *de novo* lipogenesis; this only commenced once glycogen stores had increased above basal levels by approximately 500 grams. Consequently, under normal conditions, the contribution of this pathway is likely to be minimal, whilst any *de novo* lipogenesis that does occur would actually help to buffer the net gain in fat mass. This is due to the relative inefficiency of this pathway as a means carbohydrate disposal (i.e. incorporation of glucose into glycogen requires just 2 mol or 5% of the 36 ATP available, whereas *de novo* lipogensis would result in 25-30% of the available energy being lost from the system) (Solinas, Borén and Dulloo, 2015).

In agreement with the observations of Acheson *et al.* (1988), *de novo* lipogenesis is suggested to only make a meaningful contribution to whole-body energy homeostasis when the energy intake fraction derived from carbohydrate exceeds total energy expenditure under conditions of inactivity (Hellerstein, 1999). As such, this is unlikely to contribute substantially to the positive fat balance seen with overfeeding in the typical Western diet (Hellerstein, 1999). This has been further reinforced by the application of tracer techniques, such as mass isotopomer distribution analysis, which directly measures lipogenesis within specific tissues by scaling the incorporation of labelled compounds (Hellerstein, Schwarz and Neese, 1996). As such, this pathway is sometimes referred to as a last resort in conditions of chronic carbohydrate over-feeding (Hellerstein, 2001). Nonetheless, whilst *de novo* lipogenesis is unlikely to play a quantitatively important role in fat balance, it does appear to play an important role in metabolic regulation and can contribute to hypertriglyceridaemia (Vedala *et al.*, 2006).

The reason as to why discrepancies in energy balance are mirrored by changes in fat balance are essentially two-fold. First is the immense plasticity in this storage medium relative to other energy stores; both carbohydrate and protein stores are tightly regulated, meaning that once the respective storage capacity has been saturated any surplus is utilised (Cuthbertson *et al.*, 2017). Fat stores, however, are large and have a much more flexible capacity (McArdle *et al.*, 2013; Rutkowski, Stern and Scherer, 2015). As such they serve as an energy buffer with the excess being stored rather than oxidised, so even minimal caloric excess or deficit is reflected in body fat content (Abbott *et al.*, 1988; Galgani and Ravussin, 2008). Second is the unidirectionality of any inter-conversion of macronutrients; humans have the capacity to convert carbohydrates and proteins into lipids but lack the enzymes necessary to achieve the reverse, so fatty acids can only be oxidised or stored. Therefore, in cases of positive energy balance, the preferential oxidation of carbohydrate and protein reduces the contribution of fat to whole body energy metabolism, directing more fatty acids to storage in adipose tissue (Flatt, 1995; Horton *et al.*, 1995; Hellerstein, 1999; Galgani and Ravussin, 2008; Cuthbertson *et al.*, 2017).

Work by various authors has verified this finding through the use of an overfeeding model. When energy ingested in the form of proteins exceeds the anabolic

requirements of the body there appears to be an accompanying increase in protein oxidation to achieve protein balance (Abbott *et al.*, 1988; Westerterp-Plantenga *et al.*, 2006; Moore *et al.*, 2008; Frayn, 2010). Similarly, once glycogen stores are saturated, carbohydrate overfeeding results in enhanced carbohydrate oxidation to maintain substrate balance within the body (Abbott *et al.*, 1988; Shulman *et al.*, 1990; Flatt, 1995; Minehira *et al.*, 2004). The same is also true for alcohol, wherein approximately 97% of the ingested fraction is suggested to be oxidised, reducing fat oxidation by up to 87% whilst carbohydrate and protein oxidation remain largely unaltered (Shelmet *et al.*, 1988; Suter, Schutz and Jequier, 1992; Siler, Neese and Hellerstein, 1999). However, with high fat overfeeding there is little change in fat oxidation, leading to greater storage of the ingested fraction (Horton *et al.*, 1995; Galgani and Ravussin, 2008). Consequently, to create a state of negative fat balance and mobilise the stored fraction, a state of negative energy balance must be created.

2.1.5 – Metabolic Consequences of Obesity

As discussed previously, obesity reflects a state of chronic positive energy balance, wherein energy intake exceeds energy expenditure resulting in lipid deposition within adipocytes, assuming the saturation of glycogen stores and protein synthesis (Galgani and Ravussin, 2008). This is of importance because, in addition to its historical role as a storage medium for surplus energy (Coelho, Oliveira and Fernandes, 2013), adipose tissue is also recognised as an endocrine organ capable of synthesising and secreting a number of compounds (Frayn *et al.*, 2003). Current understanding suggests that it is disturbances to this secretory profile that arise as a result of increased adiposity, which may link obesity to the associated co-morbidities, courtesy of a collective influence on energy balance, insulin sensitivity and inflammation (Frayn *et al.*, 2003; McArdle *et al.*, 2013; Rutkowski, Stern and Scherer, 2015; Wensveen *et al.*, 2015).

2.1.5.1 – Adipose Tissue Expansion

When excess energy is stored within adipose tissue there are two means of accommodating it (de Ferranti and Mozaffarian, 2008). The first of these is hyperplasia, which requires the coordinated action of several pathways to recruit adipocyte precursors, thereby increasing the number of specialised cells in which surplus lipids can be deposited (Arner *et al.*, 2010; Choe *et al.*, 2016). This is also

accompanied by remodelling and vascularisation to minimise cellular stress within the tissue (Sun, Kusminski and Scherer, 2011; Rutkowski, Stern and Scherer, 2015). The second expansion method is adipocyte hypertrophy, in which triglycerides are incorporated into pre-existing mature adipocytes to increase adipocyte size (Arner *et al.*, 2010). Although the exact pathways regulating these two scenarios are largely unknown, adipocyte hypertrophy seems to respond primarily to nutrient load, lending credence to the suggestion that it constitutes the predominant expansion method in obesity (Sun, Kusminski and Scherer, 2011).

Within this increase in adipocyte size, there is so-called "healthy" and "unhealthy" expansion (Rutkowski, Stern and Scherer, 2015), giving rise to the concepts of metabolically healthy obesity and metabolically unhealthy normal weight (Sun, Kusminski and Scherer, 2011; Huang, Loos and Kilpeläinen, 2018; Stefan, Häring and Schulze, 2018). Although the determinants of these phenotypes remain elusive, evidence suggests that there may be a genetically or developmentally determined limit of adipocyte expandability (Huang, Loos and Kilpeläinen, 2018; Nedelec *et al.*, 2018), which leads to the onset of insulin resistance and the accompanying dysfunction at different levels of absolute adiposity (Vukovic *et al.*, 2015). In support of this, the notion of adipocyte lipid content surpassing a threshold to trigger the onset of dysfunction is a consistent theme throughout the literature (Dali-Youcef *et al.*, 2013; Wensveen *et al.*, 2015; Cuthbertson *et al.*, 2017; Stefanowicz *et al.*, 2018).

2.1.5.2 – Adipocyte Dysfunction

Hypertrophic adipocytes are suggested to lie at the heart of the metabolic dysfunction that accompanies obesity. With increases in adipocyte size and the continued influx of metabolites in states of chronic overnutrition (Cho and Lumeng, 2011; Stefanowicz *et al.*, 2018), cellular stress signals are presented on the surface of the adipocyte (Wensveen *et al.*, 2015). These stress signals initiate a pro-inflammatory signalling cascade, which disrupts insulin signalling within adipocytes in partnership with an accumulation of lipid intermediaries, resulting in reduced cellular insulin sensitivity (McArdle *et al.*, 2013; Wensveen *et al.*, 2015). Courtesy of this impaired insulin signalling, lipid metabolism in hypertrophic adipocytes becomes dysregulated (McArdle *et al.*, 2013; Wensveen *et al.*, 2015). The net effect of this is reduced lipid retention, resulting in a steady efflux of non-esterified fatty acids (NEFA) from

adipose tissue (Rutkowski, Stern and Scherer, 2015). As seen in the fasted state, these compounds can readily enter non-adipose tissues, leading to ectopic lipid deposition and lipotoxicity, which lowers insulin sensitivity in these peripheral tissues as well (Rutkowski, Stern and Scherer, 2015; Spalding *et al.*, 2017).

In partnership with this dysregulation of lipid metabolism and storage, the initiation of pro-inflammatory signalling cascades by adipocytes also fosters increases in local and systemic inflammation (Maachi *et al.*, 2004; Berg and Scherer, 2005). Not only does this further disrupt insulin signalling within the aforementioned peripheral tissues (Trujillo *et al.*, 2004; Plomgaard *et al.*, 2005; Ahima, Qi and Singhal, 2006; Eder *et al.*, 2009; Kwon and Pessin, 2013), but it also alters the adipose tissue secretome (Frayn *et al.*, 2003; Fuster *et al.*, 2016). For instance, this pro-inflammatory environment has been suggested to suppress adiponectin secretion (Fasshauer and Paschke, 2003; Trujillo and Scherer, 2006), which under normal conditions acts as an insulin sensitising agent for several metabolic tissues (Esmaili, Xu and George, 2014; Lee and Shao, 2014). In part, this is ascribed to its ability to suppress hepatic glucose production and stimulate fatty acid oxidation, thereby helping to rectify peripheral lipotoxicity and preserve glucose and lipid homeostasis (Ruan and Dong, 2016). Consequently, the insulin resistance and inflammatory response which accompanies obesity can contribute substantially to the development of a hyperglycaemic and hyperlipidaemic environment (Martyn, Kaneki and Yasuhara, 2008; Jung and Choi, 2014).

2.1.5.3 – Adipose Tissue Dysfunction and Chronic Diseases

High circulating glucose concentrations and insulin resistance are both symptomatic of type 2 diabetes (Alberti and Zimmet, 1998), the treatment of which accounts for approximately 10% of NHS spending (McKinsey Global Institute, 2014). However, in addition to these issues, inflammatory factors are also suggested to play a role in atherosclerosis formation (Rocha and Libby, 2009). This is a key characteristic of coronary heart disease, a condition which accounted for 13% of deaths in the UK in 2012 (Townsend *et al.*, 2014). Consequently, although obesity is rarely in of itself the cause of ill-health and premature mortality (Duncan *et al.*, 2010), it contributes heavily to the emergence of two of the most prevalent health concerns in the UK, making weight loss a potent therapeutic target (Hamman *et al.*, 2006; Wing *et al.*, 2011).

2.2 – Current Interventions

In order to lower rates of obesity and redress the accompanying dysfunction, it is necessary to return to the energy balance equation. As discussed previously, obesity occurs as a result of a chronic energy surplus, resulting in fat accretion in adipose tissue and ectopic depots which leads to disturbances in metabolism. As such, creating a state of negative energy balance, in which energy expenditure exceeds energy intake, draws upon energy stores to fill the energy shortfall that results (Hill, Wyatt and Peters, 2012). This is most commonly achieved via lipolysis of adipose tissue, wherein stored triglycerides are broken down to glycerol and NEFA which are secreted in to the circulation (Coleman and Mashek, 2011), an ability suggested to only be possessed by adipocytes (Kolditz and Langin, 2010). This is predominantly driven by ATGL, HSL and monoglyceride lipase, a pathway which is activated in response to falling insulin levels during fasting, coupled with increases in the secretion of glucagon and sympathetic catecholamines (Saltiel and Kahn, 2001; Nielsen *et al.*, 2014; Luo and Liu, 2016).

Upon entering the circulation, glycerol is of particular relevance in the liver where it can be converted to glucose via gluconeogenesis to help maintain glucose homeostasis (Wasserman, 2009; Luo and Liu, 2016). Fatty acids on the other hand can be oxidised by various tissues to provide energy, including skeletal muscle (Luo and Liu, 2016). During prolonged fasting, the influx of fatty acids to hepatocytes can also saturate the tricarboxylic acid cycle, which allows surplus acetyl coenzyme A to be channelled into the ketogenic pathway, which is regulated by peroxisome proliferator-activated receptor-α and fibroblast growth factor 21 (Grabacka *et al.*, 2016). This process of ketogenesis forms ketone bodies such as acetone, acetoacetate and beta-hydroxybutyrate, which can serve as auxiliary fuels for both peripheral and central tissues, with uptake facilitated by monocarboxylate transporter 1 (Grabacka *et al.*, 2016; Luo and Liu, 2016). However, even following an overnight fast, ketone bodies are only suggested to account for 4-6% of energy requirements, meaning beta oxidation remains the primary fate of lipolytic products.

2.2.1 – Daily Energy Restriction

Reducing energy intake is the primary nutritional intervention for managing obesity and type 2 diabetes (Bray *et al.*, 2016; Most, Tosti and Redman, 2017). Such reductions generally take the form of daily calorie restriction, wherein energy intake is reduced by 15-40% without malnutrition (Klempel *et al.*, 2010; Smith *et al.*, 2018). A wealth of literature reflects the benefits of this approach in terms of improving markers of metabolic health, which has recently been extensively reviewed elsewhere (Most, Tosti and Redman, 2017). However, a discussion of how these interventions operate in the context of human metabolism is warranted, in order to highlight the complex challenge that tackling obesity presents.

The CALERIE study (Comprehensive Assessment of the Long-term Effects of Reducing Intake of Energy) was set up as a two-phase, multi-site intervention to explore the long-term impacts of daily calorie restriction on human health. The first phase of the trial, referred to as CALERIE-1, served as pilot work to establish the short-term effects of daily energy restriction and identify the most efficacious approach for the ensuing long-term study, known as CALERIE-2 (Rochon *et al.*, 2011). The second phase of the study randomised 220 non-obese volunteers to receive either 25% calorie restriction or an *ad libitum* control for two years (Rickman *et al.*, 2011; Rochon *et al.*, 2011). Energy requirements were determined from measurements of daily energy expenditure by doubly-labelled water, and adherence to the intervention arm was encouraged by a myriad of strategies. These included counselling sessions with qualified dieticians and psychologists, meal provision for the first four weeks, and portion size training, all of which have been shown to be efficacious in their own right (Jeffery *et al.*, 1993; Wadden, Butryn and Byrne, 2004; Rolls, 2014)

The outcomes of this trial were reported by Ravussin *et al.* (2015). Interestingly, despite the rigorous approach taken in the design of the intervention, energy intake was only reduced by 11.7% on average in the calorie restriction condition, peaking at 19.5% in the initial 6 months before receding to 9.1% in the ensuing 18 months. Nonetheless, this restriction translated to significant weight losses of 7.1 (± 0.2) kg, 8.3 (± 0.3) kg and 7.6 (± 0.3) kg at 6, 12 and 24 months, respectively. Approximately 70% of the observed weight losses were attributed to declines in fat mass, with

decreases in lean mass reaching a peak of 2.0 (± 0.2) kg at 24 months. A key determinant of lean mass retention was physical activity (Das *et al.*, 2017). Comparatively, the control condition succeeded in maintaining their habitual energy intake, with no change seen in body mass or composition. This pattern was mirrored by significant improvements in all components of the cholesterol profile with calorie restriction relative to controls, as well as improvements in insulin sensitivity, glucose control, tumour necrosis factor (TNF) -α concentration and c-reactive protein concentration (Ravussin *et al.*, 2015). Interestingly, this improvement in insulin sensitivity was also associated with reductions in urinary markers of systemic oxidative stress, which suggests that weight loss through calorie restriction may be an effective means of alleviating both obesity and the accompanying adipocyte dysfunction (Il'yasova *et al.*, 2018).

A series of ancillary studies have attempted to further such mechanistic insights. In a subset of 53 participants from the CALERIE-2 cohort, Most *et al.* (2018) assessed cardiometabolic risk alongside indices of hepatic and intramyocellular lipid content. Those assigned to the calorie restriction condition averaged a 14.8% reduction in intake over 2 years, which yielded significant reductions in weight, visceral adiposity and subcutaneous abdominal adiposity, relative to controls. Improvements in blood pressure and cholesterol profile were also seen to a degree consistent with a 30% reduction in cardiovascular disease risk. Perhaps most interestingly however, were the observed decreases in intrahepatic (~50%) and intramuscular lipid content (~15%) at 12 and 24 months, respectively. This was accompanied by reductions in insulin resistance, as assessed by the homeostasis model, which agrees with the mechanistic view of metabolic dysfunction discussed earlier. However, it should be noted that this reduction in insulin resistance was only present during weight loss, which plateaued after 12 months. In a separate study, Sparks *et al.* (2017) obtained *in vivo* measurements of intramuscular lipid content and mitochondrial function from 51 participants after 12 months of calorie restriction. The reductions in weight and improvements in body composition seen with calorie restriction were not accompanied by improvements lipid content or mitochondrial function, however, it should be noted that a time effect in the control group and baseline differences did confound these associations. The authors also argue that the largely healthy nature of the participants

at baseline may have made improvements more difficult to observe, particularly given the outcome in question.

As such, another interesting study in this context is the look AHEAD (Action for Health in Diabetes) trial, which explored the impact of calorie restriction and physical activity on cardiovascular disease risk amongst adults with type 2 diabetes (Pi-Sunyer *et al.*, 2007). A total of 5,145 overweight and obese participants with type 2 diabetes were randomised to receive either an intensive lifestyle intervention aimed at decreasing energy intake through calorie restriction, or a control group of standard diabetes support and education. Specifically, the lifestyle intervention employed meal provision to decrease portion size and improve macronutrient composition, together with both individual and group counselling sessions to enhance adherence. This was also supplemented by a surrogate goal of increasing physical activity levels to align with current recommendations. Over one year this resulted in mean weight losses of 8.6% and reductions in waist circumference of over 6 cm on average, alongside an improved blood lipid profile and decreases in glycated haemoglobin concentration. This latter outcome was particularly impressive considering the decreased use of glucose-lowering medications in the experimental group. As such, this study lends further support to the use of energy restriction as a strategy for lowering rates of obesity and managing the accompanying dysfunction. However, although the physical activity dimension of the intervention only targeted increases in walking, improvements in fitness were seen which is likely to have contributed to these favourable outcomes. Furthermore, the precise degree of caloric restriction attained in the two groups was not quantified.

Lastly, in a randomised controlled trial of 20 obese, sedentary participants, Johnson *et al.* (2016) explored the impact of 16 weeks of caloric restriction on insulin sensitivity. The calorie restriction intervention targeted a reduction in energy intake of 1000 kcal per day through reducing carbohydrate and fat consumption, with meal provision and support from dieticians to maximise compliance. The control condition by comparison were asked to maintain their habitual dietary patterns. In agreement with prior studies, calorie restriction resulted in average weight losses of 10.5 kg, which were predominantly accounted for by decreases in fat mass. These improvements were partnered by increases in glucose infusion rate during a euglycaemic-

hyperinsulinaemic clamp, reflecting enhanced insulin sensitivity with calorie restriction relative to controls.

Collectively, these studies suggest that calorie restriction is an effective strategy for reducing fat mass and improving metabolic health across a broad range of metabolic phenotypes. In non-obese cohorts, calorie restriction has resulted in weight loss and has produced improvements in a number of parameters implicated in the metabolic disturbances associated with obesity, including the potential for reduced oxidative stress. Whilst such mechanistic perspectives are lacking in obese populations, calorie restriction has been shown to induce weight loss and improve symptoms of type 2 diabetes, although studies reporting longer-term effects are still necessary.

2.2.2 – Macronutrient Manipulation

Other nutritional approaches to managing obesity and the associated dysfunction have focused upon manipulating dietary composition, which differs from calorie restriction by adjusting the balance of specific macronutrients. Although this is typically coupled with a reduction in energy intake (Stubbs *et al.*, 1995, 1998), eucaloric dietary restriction studies have also been undertaken by compensating for reduced contributions to intake from one nutrient with increases from another (Trepanowski *et al.*, 2011). The rationale for this stems primarily from the subtleties of nutrient and energy balance described earlier (Section 2.1.4), as well as the proposed role of insulin in obesity aetiology. Insulin is a hormone that is most readily secreted in response to dietary carbohydrate, with only some forms of protein eliciting an insulinotropic effect and fat in particular only negligibly increasing circulating insulin (Kahn and Flier, 2000; Acheson *et al.*, 2011; Röder *et al.*, 2016).

A short-term study conducted by Hall *et al.* (2015) compared the impact of six days of 30% calorie restriction when achieved via reductions in either carbohydrate or fat intake. The impact on overall fat balance was the primary focus as assessed by differences between intake and oxidation, but this was supplemented by fasting measures of blood-based metabolic parameters. The diets were matched for energy, sugar and protein content, and were consumed by obese participants in a randomised crossover design. Carbohydrate restriction resulted in decreased insulin secretion and

enhanced fat oxidation, culminating in an overall fat balance of -53 (± 6) grams per day. However, with fat restriction this reached -89 (± 6) grams per day, with the discrepancy accounted for by a degree of negative carbohydrate balance in the carbohydrate restricted trial. This highlights the rationale for this approach as changes in macronutrient balance appear to induce differential metabolic outcomes, even when matched for calorie content. However, whilst these insights are useful, their relevance to the long-term regulation of energy balance is less clear, particularly given the logistical challenges associated with such restrictions in free-living scenarios (Hall *et al.*, 2015).

A study addressing these issues was undertaken as part of the first phase of the CALERIE study, which aimed to isolate the most effective approach to calorie restriction. Specifically, 34 overweight adults were randomised to receive one of two daily calorie restriction diets, as reported by Das *et al.* (2007). Both interventions provided all foods for the study duration and targeted a 30% reduction in energy intake for 6 months but coupled this with either a high glycaemic load (60% carbohydrate, 20% fat, 20% protein) or a low glycaemic load (40% carbohydrate, 30% fat, 30% protein). Time effects from pre- to post-intervention were established for both groups, indicating improvements in body mass, body fat percentage, insulin concentration and cholesterol profile, yet no trial or interaction effects emerged. The changes observed were consistent with an improved metabolic phenotype which was conditional upon the imposition of calorie restriction, not modifications in macronutrient balance.

Extending the timescale further, Sacks *et al.* (2009) randomised 811 overweight adults to one of four dietary conditions for two years. Briefly, the four diets targeted a 750 $kcal \cdot day^{-1}$ reduction in energy intake from baseline requirements but derived differing fractions of total energy intake from carbohydrate (35%, 45%, 55% or 65%), fat (40% and 20%) and protein (15% or 25%). As in previous studies, regular counselling sessions, meal plans, and self-monitoring were employed throughout to maximise compliance. After six months, body mass was reduced but did not differ across the four groups. Over the following 18 months, there was some regression toward baseline values but once again this did not vary across levels of carbohydrate, fat or protein consumption. Some subtle differences did emerge when examining markers of cardiometabolic risk, with low fat diets resulting in improved cholesterol profile and

lower carbohydrate levels decreasing insulin concentration. However, despite these discrepancies, the overall prevalence of metabolic syndrome was reduced to a similar extent across the groups. Whilst this study does offer several strengths in terms of the number of participants and the range of manipulations attempted, it should be noted that the energy restriction achieved was only 225 kcal·day^{-1} on average. More concerningly, the range of macronutrient balances achieved was around half of those targeted, with energy contributions of 43-58%, 26-35% and 17-23% achieved for carbohydrate, fat and protein, respectively. This therefore can be considered to highlight the logistical challenges associated with maintaining macronutrient manipulation in the long term, as it seems the tendency is to regress towards habitual levels whilst sustaining weight loss. This issue is also compounded by, or perhaps a reflection of, the need for knowledge of the macronutrient composition of different foods in order to deploy it effectively outside a research setting.

Numerous studies of macronutrient manipulation have drawn similar conclusions in that there do not seem to be any additional benefits beyond those arising from calorie restriction (Hession *et al.*, 2009; Trepanowski *et al.*, 2011; de Souza *et al.*, 2012). Whilst in some instances this pattern can be ascribed to a failure to achieve the desired macronutrient balance, the study of Song *et al.* (2016) compared eucaloric diets which differed in their macronutrient balance but provided all foods to ensure compliance. Briefly, the diets prescribed were a moderate-fat diet (46% carbohydrate, 36% fat and 18% protein) and a low-fat/high-carbohydrate diet (64% carbohydrate, 18% fat and 18% protein). Once again, after 6 weeks there were no differences in weight or fat mass between the groups, which extended to a range of biochemical parameters, including leptin and inflammatory markers.

Collectively, current understanding of macronutrient manipulation in terms of obesity and metabolic health is well summarised by Hall and Chung (2018). They stipulate that while low-carbohydrate diets can be equally effective for weight loss as other approaches, findings for further gains in terms of glycaemic control and lipid profiles are inconsistent.

2.2.3 – Exercise and Physical Activity

Other conventional approaches to obesity management centre upon the other side of the energy balance equation, increasing energy expenditure through physical activity and exercise to encourage the deficit needed to mobilise lipid stores. Returning once again to the first phase of the CALERIE study, Racette *et al.* (2006) compared the efficacy of reducing fat mass via calorie restriction relative to endurance exercise over 12 months. This study recruited both lean and overweight individuals, with 48 randomised to either 20% calorie restriction, a control group who received information on healthy diets, or an endurance exercise programme tailored to increase energy expenditure by 20%. In broad agreement with prior studies, the accompanying decreases in body mass were approximately 10.7% for calorie restriction, 8.4% with exercise, and 1.7% for controls. This was accompanied by similar reductions in fat mass and visceral adipose tissue mass with both calorie restriction and exercise relative to controls. However, once again, the attained energy deficit fell below the target, with 11.5% calorie restriction in the diet condition and only a 12.6% increase in expenditure in the exercise condition.

At the Pennington Biomedical Research Centre (Heilbronn *et al.*, 2006), 48 overweight participants were randomly assigned to one of four treatment groups for six months: a control group (100% of weight maintenance requirements), a 25% calorie restriction group (75% of weight maintenance requirements), a very low-energy diet group (890 $kcal \cdot day^{-1}$ until 15% weight loss followed by weight maintenance diet), and a calorie restriction with exercise group (87.5% of weight maintenance requirements with 12.5% increase in energy expenditure through prescribed exercise). Decreases in body mass, fat mass, visceral fat mass and fat-free mass were observed across all three intervention groups relative to controls, which were accompanied by reductions in fasting insulin concentration, fat cell size and hepatic lipid content, suggesting that daily calorie restriction could reverse the insulin resistance and ectopic lipid accumulation associated with adipocyte hypertrophy (Heilbronn *et al.*, 2006; Larson-Meyer *et al.*, 2006; Redman *et al.*, 2007). However, beyond improving fitness, there were no detectable benefits to the combined diet and exercise intervention relative to calorie restriction alone (Redman *et al.*, 2007).

A wealth of research supports this notion. Coker *et al.* (2009) compared the effects of exercise with and without weight loss as a means of improving obesity-related health outcomes over 12 weeks. Sedentary, overweight and obese adults were recruited for the study and undertook 4-5 supervised aerobic exercise sessions per week on a cycle ergometer at 50% of maximal oxygen uptake ($\dot{V}O_2max$). However, the exercise without weight loss group were prescribed caloric compensation for the exercise to maintain a stable weight. Weight losses and reductions in body fat were seen in the exercise with weight loss group only, indicating the compensation was adequate. Despite this, both groups experienced a reduction in glucose appearance and an increase in glucose disposal during a multistage insulin infusion test, however, these changes were two-fold greater in the weight loss group. Cumulatively therefore, this study suggests that weight loss through exercise is feasible and can provide improvements in insulin action over and above those resulting from exercise alone.

Tsukui *et al.* (2000) employed a 5-month exercise protocol to examine the effects of exercise on body weight and the associated health markers. The exercise protocol employed involved completing instructional sessions of brisk walking or swimming on a weekly basis together with a supervised, home-based program. The goal was to perform 30-45 minutes of exercise at 40-50% $\dot{V}O_2max$ on 4-5 days a week and the sample was composed of healthy females. Following the exercise intervention, both body mass and body fat percentage decreased by 1.0% and 0.7%, respectively. This was accompanied by reductions in TNF-α concentration which correlated with improvements in diabetes risk, as evaluated by glycated haemoglobin concentration. This reinforces the findings of Coker *et al.* (2009), highlighting that exercise training is a viable means of losing weight and may also help to improve associated markers of disease risk.

2.2.4 – Energy Restriction in the Long-Term

These studies serve to highlight that creating a state of negative energy balance, either through calorie restriction or exercise, is an effective strategy for managing the metabolic dysfunction that accompanies obesity. Although there are arguments pertaining to the cost-effectiveness of these various approaches (World Health Organization, 2009), in principle it does suggest that disrupting the energy balance

equation to create an energy deficit can lower obesity rates and improve metabolic health. Yet if this is the case, why have there been no reductions in obesity prevalence over the last 15 years (Ng *et al.*, 2014)? Numerous societal changes have been implemented to encourage states of negative energy balance, which have been identified as effective in terms of both cost and health outcomes (World Health Organization, 2009; Gortmaker *et al.*, 2011), yet the prevalence of obesity remains largely unperturbed (Swinburn *et al.*, 2011).

This could be explained by the conventional reductionist view of obesity as a simple case of energy imbalance, which overlooks the myriad factors that conspire to foster this state. Butland *et al.* (2007) identified over 100 variables which influence the presence of obesity and associated disorders, including evolutionary, physiological, psychological, environmental, and societal dimensions.

2.2.4.1 – Weight Maintenance

A key critique of these approaches is that the aforementioned weight losses and improvements in metabolic outcomes are not sustainable in the long term. If we return to the two randomised-controlled trials discussed for daily calorie restriction, the look-AHEAD trial conducted a follow-up after a mean interval of 9.6 years. Although weight remained lower in the intervention condition relative to controls, the difference was only 2.6%, as opposed to the 7.9% reduction observed at the initial 1-year follow-up (The Look AHEAD Research Group, 2014). This pattern of regression to baseline was also seen for several other variables, including improvements in glycated haemoglobin, high-density lipoprotein (HDL) cholesterol and triglycerides (The Look AHEAD Research Group, 2013). Similarly, Marlatt *et al.* (2017) conducted a follow-up of participants in the CALERIE-2 study from the Pennington Biomedical Research Centre, two years after the intervention had concluded. Of those eligible, 39 participants opted to enrol which represented 60% of the original Pennington cohort. Much alike the look AHEAD trial, weight loss in the intervention group remained higher relative to *ad libitum* controls, yet the absolute level decreased from 9.0 kg at the end of the intervention to 4.1 kg after two years of follow-up.

Interestingly, ratings of cognitive restraint and hunger remained similarly enhanced throughout the intervention and follow-up periods, which suggests that this is not

simply a case of regression to pre-intervention dietary practices (Marlatt *et al.*, 2017). Conversely, during the initial intervention period, sleeping energy expenditure was reduced by 7% with calorie restriction relative to controls, which was associated with changes in leptin concentration and thyroid activity (Redman *et al.*, 2018). Sleeping energy expenditure also remained reduced relative to baseline values in the intervention group throughout follow-up, although the between-group difference subsided, implying that an adaptive decline in energy use may play a role in the regressive pattern seen. This is in keeping with the proposed hypothalamic regulation of energy expenditure discussed in Section 2.1.3, with falling leptin levels reducing sympathetic outflow and the activity of the hypothalamic-pituitary-thyroid axis to elicit modifications in energy metabolism in peripheral tissues.

2.2.4.2 – Metabolic and Behavioural Adaptation

One of the challenges in weight loss studies, either through diet or exercise, is compensatory adaptations. As discussed earlier in Section 2.1, the physiology of obesity involves tightly controlled regulation of energy intake and expenditure in accordance with body fat levels. From a dietary perspective, reductions in energy intake decrease levels of fat mass which by extension reduce circulating leptin concentrations (Reseland *et al.*, 2001). This reduction then drives appetite and reduces expenditure when losses of fat mass occur, which collectively hinder sustainability. For example, before and following weight loss, Rosenbaum, Sy, *et al.* (2008) examined neural activity in response to visual food cues in obese subjects. They observed changes in brain regions linked to the regulatory, emotional and cognitive control of appetite consistent with increased desire to eat, however, these changes were ameliorated with exogenous leptin administration. This is reinforced by Morton *et al.* (2006), who propose that the lower levels of leptin that accompany weight loss enhance food reward and diminish perceptions of satiety to boost energy intake.

Focusing instead on energy expenditure, Rosenbaum *et al.* (2005) examined a range of parameters related to energy expenditure before and after a period of weight loss and a period of weight loss with exogenous leptin administration. Weight loss was sufficient to reduce circulating plasma leptin concentrations, which was accompanied by significant increases in skeletal muscle work efficiency and resultant reductions in energy expenditure. However, when leptin was replaced to physiological levels in

these weight-reduced subjects, these changes were ameliorated. In line with this proposed function of leptin, in studying 10 obese participants, Kissileff *et al.* (2012) suggest that energy expenditure per kilogram of lean mass is significantly reduced following weight loss. Furthermore, perceptions of satiety, as measured by visual analogue scales following a prescribed test meal, were also reduced, both of which were ameliorated with exogenous leptin administration. Lastly, prolonged fasting that exceeds 12 hours (Kolaczynski *et al.*, 1996) appears to initiate a decline in leptin concentrations which precedes a decline in fat mass (Boden *et al.*, 1996). When energy restriction continues leading to weight loss, the decline in leptin levels is disproportionately large compared to the loss of mass (Weigle *et al.*, 1997). Following adjustment for changes in body composition, the reductions in RMR which partner these changes are suggested to be in the region of 100-200 $kcal \cdot day^{-1}$ (Leibel, Rosenbaum and Hirsch, 1995; Rosenbaum, Hirsch, *et al.*, 2008; Müller and Bosy-Westphal, 2013; Muller *et al.*, 2015).

These observations suggest the role of leptin is to defend against losses of body fat (Weigle *et al.*, 1997), which is reinforced by the finding that leptin administration during fasting ameliorates some of the accompanying hormonal changes which are implicated in energy conservation (Chan *et al.*, 2003; Boelen, Wiersinga and Fliers, 2008). Furthermore, in the context of energy expenditure there is also evidence to suggest that these adaptations extend beyond metabolic factors and into behavioural measures. It is frequently documented that physical activity levels decline in response to caloric restriction. In a review of the three pilot studies that formed the first phase of the CALERIE study, Martin *et al.* (2011) examined the effect of varying degrees of caloric restriction on physical activity energy expenditure, as measured using doubly-labelled water. Across all three trials, despite vast differences in the approach to caloric restriction, there was a significant reduction in activity energy expenditure of up to 500 kcal per day, which they argue could be due to enhanced skeletal muscle work efficiency or reduced fidgeting behaviour.

Such compensatory changes have also been reported in response to exercise-induced weight loss. This is best demonstrated by Turner *et al.* (2010), who randomised a group of sedentary men to receive either a 24-week, individually-tailored exercise program with adherence monitoring, or a control condition which required the continuation of

a sedentary lifestyle. A mean adherence rate of 94% was achieved in the exercise group, which was accompanied by significant reductions in body mass and proportionate decreases in leptin concentration, without a compensatory reduction in non-prescribed physical activity. However, the resultant weight losses only reflected approximately 40% of those predicted by the prescribed exercise, which was attributed to a compensatory increase in energy intake. In agreement with this, Martins *et al.* (2010) observed increases in appetite following exercise-induced weight loss, as measured by changes in the concentrations of several appetite hormones in both the fasted and postprandial state. Cumulatively, this suggests that in cases of weight loss through both diet and exercise there are compensatory mechanisms operating to preserve body fat levels (Schwartz *et al.*, 2003). In light of these suggestions, the trends in obesity prevalence are not surprising, as attempts to reduce fat mass and the associated dysfunction are hampered by this adaptive response in both behaviour and metabolism.

2.2.4.3 – Persistence of Adaptation

Perhaps more concerning is that these adaptations to reduced levels of body fat are not a short-term phenomenon. This is highlighted by studies such as that of Rosenbaum, Hirsch, *et al.* (2008), which used indirect calorimetry to examine the energy expenditure of those maintaining their body weight, those who recently reduced their body weight, and those who had maintained a weight reduction for over a year. Decreases in energy expenditure were seen in the recent weight loss group over and above those predicted by the changes in body composition, and they remained similarly low even after a year of weight loss maintenance. In support of this, Sumithran *et al.* (2011) examined appetite related measures before and immediately after a 10-week weight loss program in 50 overweight and obese participants. Weight losses of 13.5 kg were accompanied by reductions in the satiety hormone peptide YY and increases in the hunger hormone ghrelin, which was reflected by an increase in subjective appetite. In accordance with the findings of Rosenbaum, Hirsch, *et al.*, (2008), these changes remained even after a year of weight maintenance. Consequently, these metabolic adaptations to weight loss not only hamper the initial efforts to lose weight but may also affect the long-term success of those overcoming them.

A meta-analysis by Franz *et al.* (2007) suggests that, in most conventional weight loss studies, improvements plateau after approximately six months before regressing toward baseline over the ensuing years, although some degree of weight loss is usually retained. Another meta-analysis of 22 interventions in healthy and overweight participants suggests a similar trend, with approximately 50% of the losses being regained after 1 year of unsupervised follow-up (Barte *et al.*, 2010). It is generally reported that those who succeed in maintaining weight loss engage in high levels of physical activity, consume a low-calorie, low-fat diet, and continue to monitor their weight (Wing and Phelan, 2005). Similarities can be drawn with the study of Elfhag and Rössner (2005), who also suggest that a physically active lifestyle and continued self-monitoring are key, together with intrinsic motivation for weight management. Thomas *et al.* (2014) also highlight the importance of continued application of dietary restraint, physical activity, macronutrient modification and self-monitoring in successful weight maintenance. Lastly, Hall *et al.* (2012) highlight that while an adaptive decline in metabolic rate does contribute to the plateau in weight loss that typically follows an initially successful period of dieting, modelling studies based on this alone suggest that it would occur after years of restriction, not months. Consequently, poor compliance and behavioural adaptation to continuous dietary restriction are likely to drive this earlier plateau.

This proposition is supported by studies which suggest that adherence to conventional dietary programs over one year is in the region of 65%, with commonly cited drop-out reasons focusing on difficulty and dissatisfaction with weight losses (Dansinger *et al.*, 2005). However, Ahern *et al.* (2011) highlight that when taken outside of a research setting adherence rates are generally lower. In an analysis of the NHS weight watchers referral scheme, even when commercial weight management services are subsidised by the NHS on a large scale, attendance is only 54% to a three month course, with only one third achieving a clinically meaningful weight loss of 5% or more (Ahern *et al.*, 2011).

Del Corral *et al.* (2011) looked at weight-regain following a calorie restricted weight loss protocol which elicited 12.2 kg of weight loss after which they were followed up for several years. Those with low adherence to the initial weight loss protocol regained virtually all of the lost weight over the two-year follow up, while those with high

adherence regained approximately 50% of the initial losses. In both cases there is plainly some recovery toward baseline values. This finding is emphasised by Pekkarinen, Kaukua and Mustajoki (2015), who randomised 201 overweight and obese adults to receive a 17-week weight loss program involving behaviour change strategies and a low calorie diet, or the same but with a one-year maintenance program. After the one year of weight maintenance, 52% of participants were still at their weight loss goal with the maintenance program compared to 44% without. However, one year following the completion of the maintenance program, only a third of participants had maintained their weight loss in both groups.

Cumulatively therefore, while in principle the conventional approaches to obesity management are effective, the resultant weight losses and health benefits are hampered by compensatory reductions in energy expenditure and poor long-term compliance. These adaptations are shown to persist at least one year into the weight maintenance phase, and together with the poor long-term adherence rates may explain why weight regain is generally seen. Overall, these findings suggest that human physiology acts to defend an energy surplus and maintain a set body weight, even if this is to the detriment of health. Although this seems paradoxical in the present context and the energy-abundant environment of Western cultures, when considered in an evolutionary setting it is not overly surprising that our physiology functions in this manner (Chakravarthy and Booth, 2004; Cordain *et al.*, 2005; Prentice, Hennig and Fulford, 2008).

2.2.5 – Evolutionary Discordance

The evolutionary discordance hypothesis (Cordain *et al.*, 2005) postulates that the reason for the growing prevalence of obesity and its comorbidities is the disparity between the patterns of intake and expenditure throughout human evolutionary history and current energy-abundant environments. Such suggestions were initially presented by the 'Thrifty Genotype Hypothesis' (Neel, 1962), which proposes that the reason for the growing prevalence of obesity and type 2 diabetes is the hunter-gatherer subsistence that shaped human evolution, a lifestyle characterised by cyclic periods of plenty and shortage. Consequently, genetic variations which permitted the enhancement of energy storage during times of abundance, and energy conservation

during times of shortage, would have favoured survival and been propagated throughout the human genome.

However, given the impact of Western cultures on our patterns of intake and expenditure, these genes are now maladaptive, as periods of shortage are rare and the periods of abundance are approaching perpetual, while physical activity thermogenesis is in decline (Chakravarthy and Booth, 2004). This provides an explanation for the shortcomings of the conventional approaches to obesity management and the tendency to regain lost weight which is reinforced by several other authors (Eaton, Konner and Shostak, 1988; Chakravarthy and Booth, 2004; Cordain *et al.*, 2005; Prentice, Hennig and Fulford, 2008; Konner and Eaton, 2010). Furthermore, as the detriment to metabolic health rarely undermines genetic propagation, there is no selective advantage to favourable genotypes, meaning that such issues are unlikely to resolve naturally (Budnik and Henneberg, 2017). As such, there is a need to explore novel approaches to managing obesity and the accompanying dysfunction in order to tackle the issue more effectively.

2.3 – Nutrition and Time

In an evolutionary setting, activity and feeding were behaviours confined predominantly to daylight hours, whilst rest and fasting were synonymous with the night (Gerhart-Hines and Lazar, 2015). This represents a considerable challenge to energy homeostasis, yet endogenous circadian rhythms offer a means of anticipating these cyclical states and martialling energy reserves accordingly (Longo and Panda, 2016; McGinnis and Young, 2016). Although the potential of these rhythms in managing obesity and the accompanying dysfunction has been overshadowed by manipulation of the principle components of the energy balance equation (Arble *et al.*, 2009), in recent years research has begun to reveal the extent to which human physiology is contingent on time (Dibner and Schibler, 2018), be that in terms of clock time or the frequency and consistency of key behaviours (Ekmekcioglu and Touitou, 2011). To understand how this temporal regulation of physiology may impact upon obesity and its comorbidities, an exploration of the underpinning physiology is necessitated.

2.3.1 – Circadian Rhythms

Circadian rhythms refer to the oscillations in mammalian behaviour and physiology seen throughout the solar day (Johnston, 2014; Hirano, Fu and Ptáček, 2016). With up to 10% of the human transcriptome suggested to characterise such undulations (Mohawk, Green and Takahashi, 2012; Buhr and Takahashi, 2013; Archer *et al.*, 2014; Brown, 2014) they span a diverse range of processes, from respiration (Spengler, Czeisler and Shea, 2000) and wound healing (Cable, Onishi and Prendergast, 2017; Hoyle *et al.*, 2017), to the cell cycle itself (Brown, 2014). Given the preservation of such rhythms across species (Eckel-Mahan and Sassone-Corsi, 2013; Albrecht, 2017), it is likely that they confer an evolutionary advantage, which fits with the hypothesis that they serve to align physiological processes with anticipated environmental cues to optimise the response (Mohawk, Green and Takahashi, 2012; Brown, 2014). Such rhythms have also been established in key metabolic tissues using animal models (Mohawk, Green and Takahashi, 2012). It seems that, in this context, such rhythms exist to anticipate energy availability and modify physiological processes to maintain homeostasis (McGinnis and Young, 2016).

2.3.1.1 – The Molecular Clock

The importance of circadian rhythms to human physiology is illustrated by the expression of the core clock machinery in almost all cell types (Albrecht, 2017; Dierickx, Van Laake and Geijsen, 2018). The foundation of this intricate system is composed of a transcription-translation feedback loop involving roughly 10 genes, one cycle of which takes approximately 24 hours and therefore broadly corresponds to a single solar period (McGinnis and Young, 2016). The positive limb of this loop involves the proteins CLOCK (Circadian Locomotor Output Cycles Kaput) and BMAL1 (Brain and Muscle ARNT-Like 1), which dimerise and bind to the promoter region of target genes to initiate transcription (Kwon *et al.*, 2006; Hirano, Fu and Ptáček, 2016). These target genes include clock-controlled output genes, which contribute to myriad physiological processes, as well as the principal components of the negative limb of the feedback loop (Mohawk, Green and Takahashi, 2012).

The negative limb involves the PER (Period) and CRY (Cryptochrome) isoforms (Mohawk, Green and Takahashi, 2012). Once transcribed, these proteins can then dimerise themselves to create a second heterodimer, which must accumulate in sufficient quantities in order to localise to the nucleus (Schmutz *et al.*, 2010; St John *et al.*, 2014; Hirano, Fu and Ptáček, 2016). Once this threshold is exceeded and nuclear localisation occurs, the CRY protein can bind the CLOCK:BMAL1 heterodimer to shut down further transcription of the negative proponents (Ye *et al.*, 2014), and that of the other clock-controlled genes (Chiou *et al.*, 2016). Degradation of the components of the negative limb is then required to terminate the repression phase and restart the loop (Sahar and Sassone-Corsi, 2012; Buhr and Takahashi, 2013; St John *et al.*, 2014).

2.3.1.2 – The Master Synchroniser

As discussed previously, the function of circadian rhythms is to align physiological processes and behaviours with anticipated events in light/dark cycles (Mohawk, Green and Takahashi, 2012; Brown, 2014). A key feature of this therefore is the ability to sense photic stimuli, a property that most cells throughout the body lack (Kwon *et al.*, 2011). As such, circadian rhythms are organised in a hierarchical manner, wherein a master clock which resides in the brain receives photic input and translates this into

the molecular rhythm described previously, before disseminating this information to all other clocks throughout the body (LeGates, Fernandez and Hattar, 2014). In recent years, the term 'master clock' has been called in to question, as this implies an intrinsic rhythm which is misleading (Buhr and Takahashi, 2013). Arguably, the rotation of the Earth upon its axis represents the master clock, in producing the cycling of light and dark around which everything is arranged, with this central clock adopting the role of a master synchroniser (Kwon *et al.*, 2011). Nonetheless, the suprachiasmatic nucleus (SCN) of the hypothalamus is widely acknowledged as fulfilling this role, as reinforced by its position just above the optic chiasma and its proximity to the retina (Dibner, Schibler and Albrecht, 2010).

2.3.1.3 – Signalling Pathways

Circadian rhythms have been established in most major tissues and organs (Mohawk, Green and Takahashi, 2012; Eckel-Mahan and Sassone-Corsi, 2013). However, in order to synchronise their actions with the 24-hour photoperiod across which physiology is coordinated, pathways are needed to relay the central circadian clock in the SCN to these peripheral tissues (Albrecht, 2012). This is achieved via modifications in body temperature, endocrine actions and autonomic stimulation, thereby entraining a broad spectrum of metabolic processes to the light/dark cycle (Albrecht, 2012; Mohawk, Green and Takahashi, 2012). For instance, Cailotto *et al.* (2005) used an animal model to show that severing the sympathetic pathways between the SCN and the liver disrupts circadian oscillations in glucose concentration, leading to higher average glycaemia throughout a solar day. Similarly, the impacts of temperature are well-demonstrated by *in vitro* studies, wherein the circadian rhythms of cultured fibroblasts are blunted when temperature is held constant at 37^{o}C, as opposed to oscillating between 35-38^{o}C, as would be expected *in vivo* (Saini *et al.*, 2012).

The hormone melatonin is also worthy of mention in this regard, as it is often characterised as a key humoral entraining factor for peripheral tissues, including pancreatic islets and the gastrointestinal tract (Slominski *et al.*, 2012). It is not only synthesised and secreted from the pineal gland in response to direct autonomic stimulation by the SCN (Reiter, 1991), but it also acts as a sleep-inducing agent to produce key behavioural rhythms, which can be an entraining agent in of themselves

(Cipolla-Neto *et al.*, 2014). Owing to this direct central entrainment and systemic secretion, melatonin acts as a useful marker of circadian rhythms (Wyatt *et al.*, 1999), and disturbances in melatonin have been implicated in the development of metabolic syndrome (Maury, Ramsey and Bass, 2010; Richards and Gumz, 2012; Cipolla-Neto *et al.*, 2014).

2.3.1.4 – The Local Clock

The molecular clock has been characterised in a several metabolic protagonists, including the liver, pancreas, skeletal muscle and white adipose tissue (Mohawk, Green and Takahashi, 2012; Eckel-Mahan and Sassone-Corsi, 2013). Although, much of what we know about the resultant rhythms is extrapolated from animal models. For example, Coomans *et al.* (2013) used an SCN ablation model in mice which resulted in severe hepatic insulin resistance, whilst Zhang *et al.* (2014) developed a liver-specific BMAL1 knockout model in which lipogenesis was impaired. However, *in vitro* studies have begun to address this by utilising culture techniques to identify cell-autonomous rhythms in various human tissues. Perrin *et al.* (2015) were the first to show a cell-autonomous clock operating in human skeletal muscle tissue, finding that these rhythms were necessary for basal secretion of myokines, such as interleukin-6. A similar approach was employed by Pulimeno *et al.* (2013), isolating human pancreatic beta cells to establish the presence of a cell autonomous clock. Further work by Saini *et al.* (2016) revealed that disruption of this particular peripheral clock suppressed the basal rhythm seen in insulin secretion, as well as hindering glucose-stimulated insulin secretion, highlighting the importance of circadian rhythms in glucose homeostasis (Wasserman, 2009).

While these studies of isolated human cells provide compelling evidence for a role of timing in good health, they remove the complex regulation seen *in vivo* and as such should be applied cautiously (Gotlieb *et al.*, 2015). Due primarily to the logistical challenges associated with obtaining serial tissue biopsies from human donors (Otway *et al.*, 2011), very little translational work has been undertaken. One exception to this is the study of Otway *et al.* (2011), which examined oscillations in gene expression using adipose tissue biopsies collected at 6-hour intervals over 24 hours. In accordance with the findings from animal models, rhythms were established in the expression of core clock elements and metabolic interfaces. Recently, we have shown similar

rhythms human skeletal muscle via transcriptomics and lipidomics approaches (Loizides-Mangold *et al.*, 2017; Perrin *et al.*, 2018). Moreover, the regulation of GLUT4 and membrane lipids *in vitro* was altered by disruption of the core clock genes, which suggests a functional role for these peripheral clocks in regulating tissue insulin sensitivity and glucose uptake (Loizides-Mangold *et al.*, 2017; Perrin *et al.*, 2018).

2.3.1.5 – Feeding Behaviour as a Zeitgeiber

Whilst understanding of these clocks will continue to advance, research is also beginning to shed light upon the interplay between these endogenous rhythms and behavioural cues. Amongst the most cited studies in this regard is that of Damiola *et al.* (2000), which employed a time-restricted feeding approach to modify peripheral rhythms in a mouse model. Mice were fed within a 12-hour window for nine days, which aligned with either the dark phase (control) or the light phase (misaligned). Upon re-examination of rhythmic patterns of expression it was found that the molecular clock in peripheral tissues, including the liver and pancreas, had undergone a 12-hour phase shift to accommodate the revised feeding opportunity. Interestingly, however, this phase shift was seen in the periphery only, with the light-entrained oscillation in the SCN being unaffected. The authors argue that this shift represents an uncoupling of the periphery from the central rhythm. However, the pattern is more consistent with a realignment of the two clocks, as feeding behaviour can still be anticipated by light/dark transitions in this model. Nonetheless, what this study does show is that changes in feeding pattern can shift rhythms in peripheral clocks and a plethora of metabolic processes as a result. Of particular interest, Dang *et al.* (2016) suggest that BMAL1 can be regulated at a post-translational level by insulin in mouse models, which provides a clear avenue through which nutrient timing could impact upon obesity.

2.3.2 – Rhythms in Energy Metabolism

In terms of energy balance, circadian rhythms are suggested to accommodate the variability in energy flux throughout a single 24-hour period (McGinnis and Young, 2016). In an evolutionary context, this arises due to diurnal variations in behaviour, with feeding and activity being synonymous with the light cycle, whilst rest and fasting are usually confined to the dark cycle (Longo and Panda, 2016). As such, human

physiology evolved rhythms that would drive energy acquisition and consumption primarily during daylight hours to an extent that would fulfil the energy demands of the active phase, whilst also allowing partitioning into energy stores to sustain the metabolic demands associated with the rest phase (Mattson *et al.*, 2014; Longo and Panda, 2016).

The intake of nutrients triggers increased systemic concentrations of glucose, lipids and amino acids. This fosters an anabolic state by encouraging the uptake and storage of nutrients (Section 2.1.4), whilst also stemming the degradation of pre-existing endogenous substrate stores (Saltiel and Kahn, 2001; Dimitriadis *et al.*, 2011). In myocytes, insulin encourages the uptake of glucose and triglycerides; glycolytic pathways predominate in energy metabolism whilst glycogen and triglyceride synthesis are upregulated (Dimitriadis *et al.*, 2011). Although insulin also affects protein kinetics in skeletal muscle, uptake and storage are dictated primarily by amino acid availability and sensitivity (Fujita *et al.*, 2006; Dickinson and Rasmussen, 2013). In hepatocytes, facilitated uptake of exogenous glucose by GLUT2, coupled with increased insulin concentrations, suppresses hepatic glucose output and increases glycogen synthase activity (Agius, 2015). Furthermore, glycolytic products are channelled into *de novo* lipogenesis, the products of which are either stored within the liver itself or packaged into lipoproteins alongside exogenous triglycerides (Dimitriadis *et al.*, 2011; Rui, 2014). Lastly, in adipocytes, insulin and raised triglyceride concentrations drive lipid uptake, increasing triglyceride deposition whilst also inhibiting lipolysis, leading to adipose tissue expansion (Samra *et al.*, 1998; Evans, Clark and Frayn, 1999; Czech *et al.*, 2013).

Conversely, as the post-prandial period concludes, circulating concentrations of insulin and metabolites return to fasting levels while glucagon concentration increases; lipolytic pathways begin to predominate in adipocytes leading to net efflux of NEFA into the circulation (Duncan *et al.*, 2007). These secreted lipids can then be taken up into skeletal muscle to fuel ATP production via beta oxidation, alongside NEFA sourced from intramuscular triglyceride pools (Halberg *et al.*, 2005). Hepatocytes on the other hand will more readily degrade stored glycogen to maintain blood glucose levels. This is essential to meet the energetic requirements of the brain, which accounts for up to 60% of glucose disposal in the fasted state (Wasserman, 2009). If the fasting

period extends and glycogen stores become depleted then adipose-derived NEFA are used to saturate the tricarboxylic acid cycle, thereby opening up ketogenic pathways to provide ketone bodies as an auxiliary substrate for energy metabolism (Rui, 2014). However, even after an overnight fast, this pathway is estimated to account for just 4-6% of total energy requirements (Laffel, 1999).

Consequently, during fasting the body relies predominantly upon endogenous triglyceride stores to meet energy demands, whilst in the fed state metabolism shifts to buffer the exogenous influx of nutrients through either oxidative or anabolic pathways (Lambert and Parks, 2012). This diurnal pattern of substrate metabolism under conditions of energy balance is reflected by a rhythm in RER, with a nadir in the rest phase and a peak in the active phase to reflect predominant fat and carbohydrate oxidation, respectively (van Moorsel *et al.*, 2016). This also suggests that in states of energy balance, bodily energy stores still oscillate over a 24-hour period, with positive energy balance in the active phase and negative energy balance in the rest phase, which is supported by the diurnal variation in leptin concentration (Dallongeville *et al.*, 1998; Scheer *et al.*, 2009; Lecoultre, Ravussin and Redman, 2011). However, with the advent of artificial light, food procurement and preparation are now less confined by the solar day in humans, meaning that energy intake is similarly unconfined, which can lead to disordered rhythms in energy metabolism (Eckel-Mahan and Sassone-Corsi, 2013).

2.3.2.1 – Observational Data

Observational studies have consistently indicated a link between nutrient timing and metabolism, with a higher prevalence of metabolic disorders seen amongst those with temporally disordered eating (Johnston, 2014). For instance, workers who frequently undertake night shifts are consistently shown to be at a higher risk of type 2 diabetes than their day shift colleagues (Skene *et al.*, 2018). Drawing data from two cohort studies which collectively surveyed in excess of 175,000 women, Pan *et al.* (2011) explored the relationship between working rotating night shifts (≥3 nights per month) at baseline and the incidence of type 2 diabetes over 18-20 years of follow up. Pooled hazard ratios showed a 5% increase in the risk of developing type 2 diabetes amongst those who had worked rotating nightshifts for 1-2 years, increasing up to 58% in those working rotating night shifts for at least 20 years. Although such designs clearly show some interplay between inconsistencies in daily rhythms and metabolic health, it is

impossible to isolate disturbances in behaviours (i.e. fasting/feeding, rest/activity, sleep/wake) as the cause, due to myriad confounding influences (Health and Social Care Information Centre, 2013). However, experimental studies in which simulated shift-work is imposed on acute basis highlight how such temporal disturbances may adversely affect energy metabolism (Skene *et al.*, 2018).

2.3.2.2 – Misaligned Feeding and Postprandial Metabolism

Adverse metabolic responses have been consistently observed in response to misaligned feeding using models of simulated shift-work, which may underpin the observational findings discussed previously. For instance, Al-Naimi *et al.* (2004) exposed eight lean males to a randomised crossover design, which compared the metabolic responses to meals and snacks consumed during simulated day- and night-shift work. All meals and snacks were provided to ensure the two arms were matched for energy and macronutrient intake, whilst pre-trial intake was also standardised. During the day shift arm (12:00-20:00), meals were consumed at 13:00 and 19:00, with a snack at 16:00. Conversely, during the night shift condition (00:00-08:00), meals were consumed at 01:00 and 07:00, with the snack falling at 04:00. Over the 8-hour measurement window, the night shift condition elicited greater increases in postprandial triglyceride concentration relative to the day shift condition. This became apparent after peak values were reached following the first meal, alluding to an impaired ability to clear triglycerides and/or increased hepatic VLDL secretion. This discrepancy was then amplified following the snack before returning toward daytime values around habitual waking time. A similar pattern emerged for post-prandial glucose and insulin concentrations, whilst NEFA remained unaltered, reflecting the profound suppression of lipolysis. This pattern is consistent with a nocturnal reduction in insulin sensitivity, which has been attributed to the anticipation of fasting by endogenous clocks (Carrasco-Benso *et al.*, 2016).

The above demonstrates a causal role of nutrient timing in the metabolic disturbance that arises with temporally disordered eating. Although a nocturnal reduction in insulin sensitivity may appear favourable by dampening the lipogenic environment, infusion studies have shown that high circulating triglycerides can suppress lipolysis even in the absence of insulin (Samra *et al.*, 1998; Evans, Clark and Frayn, 1999). Consequently, eating out of phase with anticipated rhythms in feeding and fasting

results in an exaggerated postprandial response that enhances fat retention via suppressed lipolysis, thereby contributing to obesity. This notion is well illustrated by the study of Bray *et al.* (2010), which acclimated mice to a 12-hour light/dark cycle and restricted food intake to the first and last 4 hours of the active phase for 12 weeks. In the experimental condition, mice received a normal chow diet in the first 4 hours followed by a high fat diet in the final 4 hours, whilst the control group received the diets in the opposing order. Despite consuming identical quantities of both fat and calories, high fat feeding at the end of the active phase resulted in increased RER, body mass and adiposity, together with hyperinsulinemia and hypertriglyceridemia, relative to controls. Although longer-term human trials are lacking, in a study of 52 healthy adults, Dattilo *et al.* (2011) explored the relationship between temporal characteristics of nutrient consumption, as assessed by 3-day food records, and body composition. In agreement with rodent models, higher adiposity associated positively with consumption of a higher fraction of daily calories in the evening.

Consequently, in states of temporally disordered eating, there appears to be a greater risk of obesity and the associated comorbidities. Studies of simulated shift work highlight that such effects may be mediated through the induction of an exaggerated postprandial response when calories are consumed out of phase with anticipated rhythms in feeding and fasting. Such an exaggerated response results in prolonged elevations in insulin and triglycerides, which suppress lipolysis and reduce opportunities for lipid-derived substrates to predominate in energy metabolism, thereby encouraging adipose tissue retention. This suggests that modifications in nutrient timing could be used to extend the fasting window and induce a more favourable balance between energy influx and efflux in adipose tissue.

2.3.2.3 – Eating Frequency

Perhaps the most widely researched dimension of nutrient timing in the context of obesity in humans is eating frequency. Early work by Fabry *et al.* (1964) deployed a cross-sectional approach to explore the relationship between intake frequency and metabolic health. Interestingly, in a group of 440 men, higher eating frequency broadly corresponded to lower body weight, improved cholesterol levels and enhanced glycaemia. Contrary to this, employing data from the NHANES cohort, Murakami and Livingstone (2015) found that those eating on more than four occasions per day were

approximately 50% more likely to be overweight or obese by BMI relative to those eating on less than three occasions per day. Such discrepancies are a consistent theme throughout these cross-sectional studies; a recent systematic review by Canuto *et al.* (2017) analysed data from 31 such studies containing a collective sample of over 130,000 participants. Of these 31 studies, 14 established an inverse association, 10 showed no association, and 7 revealed a positive association, which the authors ascribe to the spectrum of approaches employed.

Upon shifting to prospective methodologies, the pattern appears to be largely the same; two recent systematic reviews conclude that the majority of studies show no association between eating frequency and obesity (Raynor *et al.*, 2015; St-Onge *et al.*, 2017). The review of Raynor *et al.* (2015) makes a particularly strong case, given that only human studies in which food was provided or intake monitored in a laboratory setting were included, thereby reducing the confounding influence of misreporting (Leech *et al.*, 2017). However, of the studies analysed, most evaluated the impact of increased meal frequency on metabolic health, using three meals per day as the reference for reduced intake, which does not necessarily increase the fasting period relative to more frequent meals.

2.3.2.4 – Daily Plasma Metabolite Profiles

In Western cultures, a pattern of three meals per day is generally accepted as a societal norm (Lhuissier *et al.*, 2013; Yates and Warde, 2015). However, upon considering the time-course of the resultant postprandial glycaemic, lipaemic and insulinaemic responses, this is likely to lead to an anabolic state predominating over the course of each day (Frayn, 2016).

The typical postprandial response to a mixed-macronutrient meal in healthy subjects is characterised by a spike in blood glucose within the first hour followed by a steady return to fasted glycaemia over the ensuing two hours (Coppack *et al.*, 1990; Frayn *et al.*, 1993). This is paralleled by an accompanying spike in insulin secretion within the first hour followed by a decrease over the ensuing 4 hours (Frayn *et al.*, 1993). In a more gradual response, plasma triglyceride concentrations rise steadily to a peak after 4 hours and remain 50% higher than baseline even after 6 hours (Coppack *et al.*, 1990). When a subsequent meal is ingested, approximately five hours later as is common in

Western diets, glucose peaks at a similar time after feeding, albeit an attenuated absolute peak (Gonzalez, 2014), but takes slightly longer to return to baseline as the day progresses, a pattern that is largely mirrored by insulin (Ahmed, Gannon and Nuttall, 1976). Plasma triglycerides on the other hand reach their first meal peak shortly after the second meal is ingested, fall rapidly due to the effect of the insulinaemic response to the second meal on LPL, before peaking again around five hours after the second meal (Ahmed, Gannon and Nuttall, 1976).

Based on these postprandial responses, consuming just two meals per day could be sufficient to elevate plasma triglyceride concentrations for over 12 hours. Given that such increases can stimulate lipid uptake and suppress lipolysis in adipose tissue, even in the absence of accompanying surges in glucose and insulin concentration (Samra *et al.*, 1998; Evans, Clark and Frayn, 1999), this means that in temporal terms there are more opportunities for fat storage and fewer opportunities for fat mobilisation within each 24-hour period. This imbalance is then amplified when a third meal is consumed several hours after the second (Ruge *et al.*, 2009). In fact, the findings of McQuaid *et al.* (2011) suggest that the uptake of triglycerides into adipose tissue is elevated for over 16 hours by Western feeding schedules. Consequently, the majority of each day is spent in a postprandial and lipogenic state, which is conducive to positive fat balance (Frayn, 2016; Travers *et al.*, 2017). This may therefore explain the conflicting results emerging from meal frequency studies; as they centre upon exploring the effects of increasing meal frequency from 3 meals per day, they are unlikely to have overcome the underlying bias toward fat storage. Consequently, reducing meal frequency from 3 meals per day could prove to be a more efficacious strategy for improving metabolic health, by offering more opportunities for energy efflux from adipose tissue and the predominance of lipid-derived substrates in energy metabolism (Anton *et al.*, 2018).

Of the studies reviewed by by Raynor *et al.* (2015) and St-Onge *et al.* (2017), only one reduced meal frequency below two meals per day. This was the study of Stote *et al.* (2007), which explored the impact of reducing meal frequency to 1 meal per day under conditions of energy balance. Briefly, 15 normal-weight subjects completed two 8-week intervention periods in a randomised crossover design with an 11-week washout interval. In one treatment, all calories were consumed in a single meal between 17:00 and 21:00, whilst the other treatment separated the same foods into three meals as a

conventional breakfast, lunch and dinner format. To facilitate compliance, the dinner in both conditions was consumed under supervision and all foods were provided. The diets were matched for both energy and macronutrient content and targeted weight maintenance, with daily adjustment of prescribed intake based on body weight measurements, which were then mirrored in the opposing trial.

No differences in body mass, body composition or health markers were apparent at the outset of each treatment, suggesting that the washout period was sufficient. Furthermore, no differences in energy intake, macronutrient balance or physical activity were noted between the two conditions. The result of this was an isocaloric comparison of repeated exposure to either a typical overnight fast or an extended fast of 20 hours per day. Interestingly, weight and fat mass were both lower following the one meal per day condition relative to the three meals per day condition, suggesting favourable effects on fat balance. However, this was not accompanied by improvements in lipid profile or fasting glycaemic control; on the contrary, oral glucose tolerance tests completed before and after the respective conditions showed an exaggerated glycaemic response following the one meal per day arm (Carlson *et al.*, 2007). Yet it should be noted that the glucose tolerance test was completed in the morning, which is likely to have negatively impacted upon the one meal per day format due to circadian misalignment with the revised feeding schedule (Scheer *et al.*, 2009).

This suggests that, in much the same way as extending the lipogenic window is conducive to energy surplus, prolonged fasting on a routine basis, even in an energy balance context, could be an effective strategy to counter fat accretion by encouraging net efflux of lipids from adipocytes and a shift toward fat metabolism (Mattson *et al.*, 2014). In addition, it has been argued that the increased reliance on lipid-derived substrates from endogenous stores, which usually only occurs after 12-14 hours of uninterrupted fasting, acts as a potent metabolic signal that induces several benefits, which may be independent of net energy balance (Anton *et al.*, 2018). Beyond these mechanistic points, it has also been postulated that temporal restrictions in energy intake may be easier to implement and maintain than continuous alternatives (Varady, Bhutani, *et al.*, 2009; Scheer, Morris and Shea, 2013). Johnstone (2015) argues that such a strategy is implicitly associated with lower motivational demands, as well as being a simpler concept by negating the need to continually quantify energy intake.

2.4 - Intermittent Fasting

This notion that routine extension of fasting intervals may be beneficial in the context of managing obesity and the associated morbidities has given rise to several therapeutic interventions (**Table 2.1**). The umbrella term used to describe these approaches is *intermittent fasting* (Patterson and Sears, 2017), which involves complete or partial restriction of energy intake during defined time intervals. The rationale behind this stems in-part from the impact of fasting on energy metabolism, but arguments also centre upon the proposed ease relative to the demands of continuous energy restriction (Heilbronn, Smith, *et al.*, 2005; Varady, Bhutani, *et al.*, 2009; Klempel *et al.*, 2010) and better alignment with patterns of fasting and feeding that are believed to have shaped metabolic regulation in humans (Halberg *et al.*, 2005). Irrespective of the rationale, such approaches have been subject to growing popularity in recent years, yet experimental data to support their application is comparatively sparse (Johnstone, 2015; Patterson and Sears, 2017).

Table 2.1: Intermittent fasting modalities and the typical characteristics of each

Name	Nominal Definition
5:2 Diet	- Fasting on two days per week - 400-600 kcal permitted during fasted periods, typically consumed in a single meal at lunch - *Ad libitum* intake maintained on the other five days
Modified Alternate-Day Fasting	- Fasting on every other day from midnight - 300-800 kcal (~20-25% of energy needs) permitted during fasted periods as a single meal - Habitual intake on non-fasting days
Time-Restricted Feeding	- Daily fasting for at least 12-14 consecutive hours - Complete fasting during defined periods (i.e. unsweetened energy-free drinks only) - *Ad libitum* consumption during designated feeding windows
Complete Alternate-Day Fasting	- Fasting on every other day - Complete fasting during defined periods (i.e. unsweetened energy-free drinks only) - *Ad libitum* consumption during non-fasting periods

Adapted from (Patterson and Sears, 2017)

2.4.1 – The 5:2 Diet

Amongst the most coveted forms of intermittent fasting in the public domain is the 5:2 diet, wherein severe energy restriction is imposed on two days per week with *ad libitum* consumption on the remaining five. In a study of 24 obese men, Conley *et al.* (2018) compared such an approach to daily calorie restriction over 6 months. Participants were randomly allocated to reduce their energy intake to 600 kcal per day for two days a week, or to reduce their energy intake by 500 kcal every day. Accordingly, energy and macronutrient intake were reduced in both groups from baseline at 3 and 6 months. This resulted in a similar degree of weight loss between the intermittent and continuous groups, 5.5% and 5.4% respectively, whilst fasting levels of glucose and lipids were unaffected.

A similar study was conducted by Carter, Clifton and Keogh (2016), who randomised 63 overweight and obese adults with type 2 diabetes to 12 weeks of either daily calorie restriction or a 5:2 approach. The 5:2 group were required to reduce their intake to 400-600 kcal for two days per week and follow their habitual diet on the remaining five, whilst the daily restriction group reduced their daily intake to 1200-1550 kcal per day. Although the attained restriction was not reported for either group, in agreement with Conley *et al.* (2018) main effects of time but not group were seen for reductions in body mass, fat mass and fat-free mass. Improvements were also seen in both mean glycated haemoglobin concentration and the use of diabetic medications, suggesting improved glycaemic control in response to both conditions. Similar conclusions were also drawn by the study of Sundfør, Svendsen and Tonstad (2018), which compared this 5:2 approach (i.e. 400-600 $kcal \cdot day^{-1}$ on two non-consecutive days) against daily restriction over 6 months.

This would appear to suggest a broad equivalency between the metabolic impacts of the 5:2 diet and daily calorie restriction. However, in a randomised controlled trial of 107 overweight and obese women, Harvie *et al.* (2011) observed differential changes in fasting insulin and insulin resistance when comparing the two approaches. For six months, participants undertook two consecutive days of 75% calorie restriction per week or continuously restricted calories by 25% daily. Once again, the resultant decrease in body mass was not different between groups. A similar pattern also

emerged for decreases in fat mass, fat-free mass, inflammatory markers and fasting leptin concentration, as well as improvements in lipid profile. Both interventions also resulted in modest reductions in fasting insulin concentration and insulin resistance, as assessed by the homeostasis model. However, these were significantly greater with the 5:2 method when compared to continuous restriction. Although this may reflect a more potent influence of using two consecutive days of severe energy restriction, as opposed to non-consecutive, there were also greater reductions in energy and carbohydrate intake in this group which confound this observation.

Using a broadly comparable approach, Antoni, Johnston, *et al.* (2018) sought to compare the effects of intermittent energy restriction against daily calorie restriction when matched for weight losses, to minimise the confounding influence of such factors on metabolic health outcomes. Furthermore, this study featured dynamic indices of nutrient metabolism, building upon the prior studies which only featured fasted measures. Briefly, 27 overweight and obese participants were randomised to undertake either an intermittent or a continuous energy restriction diet. The intermittent diet followed a 5:2 format, restricting participants to ~630 kcal per day for two consecutive days each week with a self-selected eucaloric diet on the remaining five. Comparatively, the continuous restriction implemented a self-selected diet which aimed to reduce energy intake by 600 kcal every day.

As opposed to returning to the lab after a fixed period, participants were instead reassessed upon achieving a 5% weight loss. These post-intervention measurements also followed a week free from energy restriction, thereby matching for weight loss and eliminating any acute effects of the dietary modifications. Despite larger reductions in energy intake in the intermittent group, the design meant that changes in body mass were similar between groups. Body composition and fasting biochemical outcomes were also similarly affected by the two diets, showing good agreement with previous studies. However, the intermittent diet resulted in significant reductions in postprandial triglyceride concentration relative to daily calorie restriction. Furthermore, postprandial C-peptide concentration followed a similar pattern, suggesting reduced insulin secretion following intermittent but not continuous restriction. The authors concluded that this highlights a potential superiority of intermittent energy restriction relative to continuous.

However, while these latter observations do suggest some benefit to shorter and more intense periods of calorie restriction when applied on an intermittent basis, the interventions do not confine the permitted intake to a specific time window. Consequently, they do not necessarily extend the fasting interval relative to continuous restriction, and as such may not fully capture the therapeutic potential of intermittent fasting (Section 2.3.2).

2.4.2 – Alternate-Day Fasting

The majority of human studies which examine intermittent fasting have centred upon a strategy referred to as alternate-day fasting (Patterson and Sears, 2017). It differs from the 5:2 diet in two key ways; the severe restriction is generally applied during alternating periods of 24 hours and the permitted calories during fasting are provided as a single meal, thereby ensuring an extension of the typical overnight fast. Johnson *et al.* (2007) were amongst the first to apply this approach in a single group design, prescribing 8 weeks of alternate-day fasting to 9 obese participants. The intervention involved alternating between 24-hour periods of fasting and *ad libitum* feeding, with only a single 320-380 kcal meal replacement shake permitted during the 'fasting' period (80% energy restriction). Body mass decreased steadily throughout the intervention, resulting in an 8% reduction at follow-up. Although there was no effect of this on fasting levels of glucose and insulin, the weight loss was accompanied by improvements in fasting lipid profile, reductions in inflammatory markers and reductions in markers of oxidative stress. Furthermore, blood samples collected during fasting cycles characterised 4-fold increases in serum concentrations of beta-hydroxybutyrate relative to fed cycles. This is a ketone body which increases with prolonged fasting, reflecting that such an approach exerts a profound impact on energy metabolism.

Much of the work undertaken in this field originates from the studies of Varady and colleagues, utilising a strategy which is commonly referred to as modified alternate-day fasting (Patterson and Sears, 2017). This is similar to the intervention employed by Johnson *et al.* (2007), except that during 'fasted' periods participants are permitted to consume a single 600-800 kcal meal between 12:00 and 14:00 (75% energy restriction). The impacts of this approach on weight were initially explored by Varady,

Bhutani, *et al.* (2009) in a single-arm trial. A sample of 12 obese participants completed the 10-week study consisting of three components: a two-week control phase; a four-week controlled modified alternate-day fasting phase; and a four-week self-selected modified alternate-day fasting phase. The control phase effectively served to establish stability of body mass before commencing the intervention, whilst the latter two phases differed in that the fasting day meal was provided by the research team during the controlled phase.

Adherence to the fasting protocol remained similarly high throughout the intervention weeks, with energy intake averaging 26% of habitual (Varady, Bhutani, *et al.*, 2009; Klempel *et al.*, 2010). Comparatively, intake on feeding days reached 95% of the habitual level, resulting in a 37% calorie restriction on average. This led to weight losses of 5.6 kg, 5.4 kg of which was accounted for by decreases in fat mass (Varady, Bhutani, *et al.*, 2009). Total cholesterol, LDL cholesterol and triglycerides were also reduced by at least 20%, effects which were associated with improvements in adipokine profile (Bhutani *et al.*, 2010). Subsequent work by the same group has shown that these effects are similar when applied to cohorts of overweight adults (Varady *et al.*, 2013), and that meal timing on the fasting day can be varied (Hoddy *et al.*, 2014). Furthermore, concurrent macronutrient manipulation does not appear to have additive effects, with high-fat and low-fat forms being equally effective in lowering body mass and the risk of coronary heart disease (Klempel, Kroeger and Varady, 2013).

Collectively, this suggests that modified alternate-day fasting is a viable means of improving cardiometabolic health in overweight and obese adults, which is comparable to daily calorie restriction. However, much alike the 5:2 approach and the studies of meal frequency (Section 2.3.2), this equivalency between intermittent and continuous approaches may actually reflect an inadequate fasting interval. In modified alternate-day fasting, the designated 'fasting' period is interrupted by the consumption of the permitted meal. Consequently, it remains to be seen whether omitting this meal and extending the fasting interval beyond ~12 hours may reveal further benefits to intermittent fasting. This highlights the fundamental asymmetry of feeding and fasting in eliciting a metabolic response; feeding disrupts the fasted state within a matter of minutes, which then does not return for a number of hours (Section 2.3.2.4).

This issue is compounded by the single-arm nature of the modified alternate-day fasting diets discussed so far, as without a comparative daily calorie restriction group it is difficult to isolate the effects the fasting periods are having. This was addressed recently by a comparison of the two methods under isocaloric conditions with a no intervention control group (Trepanowski, Kroeger, Barnosky, Klempel, Bhutani, Hoddy, Gabel, *et al.*, 2017; Trepanowski, Kroeger, Barnosky, Klempel, Bhutani, Hoddy, Rood, *et al.*, 2017). Briefly, 69 obese adults were randomised to undertake one year of modified alternate-day fasting or daily calorie restriction, consisting of a 6-month weight loss phase and a 6-month weight maintenance phase. Prior to the diets, energy requirements were determined using measurements of energy expenditure from doubly-labelled water. The alternate day fasting diet restricted participants to a single meal between 12:00 and 14:00 during fasting periods as described previously but prescribed 125% of energy requirements on feeding days which was spread across three meals. The daily calorie restriction diet prescribed a 25% reduction in energy intake every day, resulting in an equivalent reduction in energy intake of 25% over the first six months in both groups. All foods in the two intervention groups were provided for the first three months followed by dietary counselling thereafter to support self-selected adherence.

In the first 6 months, macronutrient balance was preserved in both groups and the achieved calorie restriction when averaged per group was 21% and 24% for alternate-day fasting and daily calorie restriction, respectively. Body mass loss was also similar between the two groups at 6.8%, a pattern which was paralleled by changes in both fat mass and lean mass. Fasted markers of metabolic health were largely unaffected by either intervention, including lipid profile, inflammatory markers, adipokines, fasting glucose concentration and insulin resistance, as assessed by the homeostasis model (Trepanowski, Kroeger, Barnosky, Klempel, Bhutani, Hoddy, Gabel, *et al.*, 2017; Trepanowski, Kroeger, Barnosky, Klempel, Bhutani, Hoddy, Rood, *et al.*, 2017). The only exception to this was HDL cholesterol concentration, which was increased with modified alternate-day fasting relative to both the daily calorie restriction and control conditions.

For the latter 6 months, energy requirements were reassessed and dietary prescriptions were adjusted to maintain body mass whilst continuing with the respective modalities.

In the alternate-day fasting group this was achieved by consuming 50% of energy needs during the single fasting meal and 150% of energy needs during feeding cycles, whilst the continuous group consumed 100% of their measured energy requirements each day. As in the first phase, no differences emerged between the intervention groups with regard to body mass. Slight regain was seen in both groups but it remained reduced relative to controls by -6.0% and -5.3% for alternate-day fasting and daily calorie restriction, respectively. This reflected partial regressions of both fat mass and lean mass toward baseline values, but once again there was no difference between groups. Furthermore, there was no change in most fasting markers of metabolic health, although the augmented HDL concentration seen with alternate-day fasting was ameliorated at month 12 and LDL cholesterol was increased instead.

The above study indicates that modified alternate-day fasting and daily calorie restriction are equally effective in improving most relevant health outcomes, as concluded previously for the 5:2 approach. However, during the modified alternate-day fasting intervention, participants consistently over-consumed on fasting days and under-consumed on fed days, in what the authors describe as *de facto* calorie restriction (Trepanowski, Kroeger, Barnosky, Klempel, Bhutani, Hoddy, Gabel, *et al.*, 2017). Consequently, over 12 months, the difference in reported energy intake between feeding and fasting days was less than 500 kcal on average (Kroeger *et al.*, 2018). Upon stratifying the 34 participants that undertook alternate-day fasting into those who lost more or less than 5% body mass, those closest to the prescribed intake targets showed larger decreases in body mass despite consuming more calories overall (Kroeger *et al.*, 2018). Unfortunately, the mechanisms underpinning this are unclear, it could reflect a more favourable balance of energy flux within adipose tissue or lower levels of adaptive thermogenesis with intermittent methods, or on the other hand it may simply reflect poorer dietary reporting by those with lower adherence.

2.4.3 – Time-Restricted Feeding

The adherence issues in these modified alternate-day approaches may lie in the imposition of a severe restriction as opposed to a complete fast, which in being an absolute could facilitate adherence (Varady, Bhutani, *et al.*, 2009; Scheer, Morris and Shea, 2013; Johnstone, 2015). Drawing from this premise, time-restricted feeding is

another method of intermittent fasting which has emerged recently (Patterson and Sears, 2017) and requires no knowledge of food composition or restraint at eating occasions, only awareness of the time at which eating occasions are permitted. It aims to restrict food intake to a window of 10 hours or less in the waking phase, thereby reducing feeding opportunities and extending the overnight fasting period to at least 14 hours every day (Sutton *et al.*, 2018). In a study by Gabel *et al.* (2018), 23 obese subjects undertook a 12-week time-restricted feeding protocol in which *ad libitum* energy intake was permitted daily between 10:00 and 18:00, with the remaining 16 hours spent fasting. This represented a 3-hour decrease in feeding time on average across the group. Both body mass and energy intake decreased relative to a matched control group by 2.6 ± 0.5% and 341 ± 53 kcal·day^{-1}, respectively, highlighting that fasting may alleviate the associated burdens of conventional approaches to calorie restriction and offer greater compliance.

Similarly, the work of Betts and colleagues, which explored the impact of extending the overnight fast on energy balance and nutrient metabolism, has direct applications in this regard. This is because the intervention randomised 33 lean adults to either consume breakfast, defined as over 700 kcal before 11:00 (with half consumed within 2 hours of waking), or to continue fasting up until 12:00 (Betts *et al.*, 2014). Interestingly, improvements in anthropometric parameters and fasting health markers were not meaningfully different between interventions. In agreement, a panel of hormones implicated in the regulation of energy balance showed little change following the two interventions, along with measures of adipose tissue insulin sensitivity. However, analysis of subcutaneous adipose tissue biopsies taken before and after the intervention did reveal upregulation of genes involved in lipid turnover and insulin signalling with extended fasting (Gonzalez *et al.*, 2018).

These largely null findings relative to prior research could be explained by the non-prescriptive approach taken to dietary intake and physical activity outside the imposition of breakfast or extended fasting. The breakfast group consumed more calories than the fasting group when averaged throughout a 24-hour period, but this was compensated for by increases in physical activity. Upon applying this protocol to a cohort of obese adults (Chowdhury *et al.*, 2016), extended fasting resulted in a compensatory increase in energy intake following fasting, whilst regular breakfast

consumption was associated with greater physical activity energy expenditure in the morning, although this did not result in meaningful changes in body mass or composition between groups. However, breakfast did result in improved insulinemic responses during an oral glucose tolerance test relative to the fasting condition, which is in accordance with the findings of Carlson *et al.* (2007). Unfortunately, as this test was also aligned for circadian cycle rather than feeding cycle, it could simply be a reflection of better alignment with anticipated events in the breakfast condition.

More recent work has explored this proposition by applying time-restricted feeding under eucaloric conditions, much alike the study of Stote *et al.* (2007). Focusing on energy metabolism, Moro *et al.* (2016) randomised 34 men to eight-weeks of time-restricted feeding or a control diet. Diets were matched for energy and macronutrient content and aimed to provide 100% of energy requirements across three meals in both conditions. In the control condition, meals were consumed at 08:00, 13:00 and 20:00, whilst in the experimental condition meals were consumed at 13:00, 16:00 and 20:00 to give a 16-hour fast. The time-restricted approach resulted in reductions in fat mass relative to controls, which were partnered by decreases in RER, indicating a shift toward fat oxidation. Interestingly however, despite accompanying reductions in leptin and hypothalamic-pituitary-thyroid signalling, resting energy expenditure was maintained. This reinforces the notion that nutrient timing affects energy metabolism, given that a more negative fat balance was seen in response to time-restricted feeding despite similar dietary intake. Considering this in light of the typical postprandial nutrient profile discussed previously, the increase in fasting duration may be restoring a more balanced ratio of adipose tissue influx to efflux and providing more opportunities for utilisation lipid-derived substrates. This again points to the possibility that routine extension of the fasting period beyond 12 hours may be key to these benefits, which was not necessarily achieved by the 5:2 or modified alternate-day methods discussed thus far. The pivotal question is whether these improvements are enhanced with even longer durations of complete fasting.

This possibility was recently examined by Sutton *et al.* (2018), who argue that circadian rhythms in energy metabolism mean time-restricted feeding could be potentiated when feeding windows are confined to earlier stages of the waking phase, in what the authors describe as ‘early time-restricted feeding’. This also overcomes the

confounding influence of circadian alignment on key outcomes, as it is the termination of daily eating that differs between conditions rather than the onset. To test this hypothesis, eight pre-diabetic men completed two trial arms of 5 weeks each in a randomised crossover design with a 7-week washout. In the control arm participants consumed their prescribed calories in three meals spread across a 12-hour window to give a 12-hour fast, whilst the experimental condition required the consumption of the same meals within a 6-hour window to give an 18-hour fast. The diets were prescribed based on energy requirements to maintain energy balance and were also matched for energy and macronutrient content. Furthermore, participants standardised the timing of the breakfast meal, which defined the start of the feeding period, across trials.

Compliance to the two conditions was very high and this was mirrored in stable measures of body mass during both trial arms. Glycaemic parameters were unaffected whilst fasted triglyceride concentrations increased with time-restricted feeding relative to controls. However, improvements in fasting insulin were also seen with time-restricted feeding, together with reductions in peak insulin and insulin resistance during an oral glucose tolerance test. Unfortunately, these impacts on insulinaemia (i.e. incremental response above *baseline*) were driven by baseline differences arising from a trial order effect, which may explain the contrasts with the observed stability in triglyceride levels and deteriorations in postprandial glycaemia seen with routine 20-hour fasts (Carlson *et al.*, 2007). Furthermore, the fasting duration preceding post-intervention measurements was not standardised across the trials, which is likely to have acutely impacted upon both glucose and lipid metabolism. Although the authors argue that these issues are likely to have compromised these beneficial effects as opposed to enhancing them, doubt is nonetheless cast, necessitating further research with more robust designs.

Based on these findings, evidence does point to an effect of extended fasting intervals on fat mass independent of energy balance, particularly when the fasting interval is extended to at least 16 hours, as shown by Stote *et al.* (2007) and Moro *et al.* (2016). In both cases, this produced significant reductions in fat mass relative to a routine 12 hour fast, which implicates extended fasting beyond 12 hours as a key factor. However, the importance of such changes for metabolic health are less clear due to a series of confounding influences.

2.4.4 – Complete Alternate-Day Fasting

Thus far, the intermittent fasting strategies discussed typically permit the consumption of calories within each 24-hour cycle to some degree, meaning that the fasting interval is only extended by a few hours (Gabel *et al.*, 2018). This is primarily to facilitate adherence (Heilbronn, Smith, *et al.*, 2005; Varady, Bhutani, *et al.*, 2009), but the disruption of energy efflux from adipose tissue is undermined nonetheless and is profoundly asymmetrical, in that even a short feeding bout would suppress lipolytic activity for several hours (Coppack *et al.*, 1990; Frayn *et al.*, 1993). Comparatively, the 20-hour fasting interval used by Stote *et al.* (2007) is likely to have led to a lipolytic state predominating over the course of 24 hours, which may explain the reduction in fat mass despite eucaloric intake.

Building upon this suggestion, the study of Halberg *et al.* (2005) applied a 20-hour fast on alternate days from 22:00 to 18:00, representing an integration of the strategies employed by Stote *et al.* (2007) and Varady, Bhutani, *et al.* (2009). Fasting prohibited all intake with the exception of water to ensure no disruption of the lipolytic state, whilst during the intervening feeding periods participants were told to double their habitual intake to maintain weight. Although intake was not monitored, blood samples collected in a subset of fasting periods confirmed compliance with the fasting protocol, with corresponding changes in glucose, NEFA, glycerol, adiponectin and leptin concentration. Although both body mass and fat mass remained unchanged, the glucose infusion rate during a euglycaemic-hyperinsulinemic clamp increased significantly in the final 30 minutes of the sampling period, suggesting enhanced insulin sensitivity. Accordingly, this was accompanied by more rapid suppression of adipose tissue lipolysis during the insulin infusion. The lack of an effect on body mass and fat mass relative to prior studies may represent the disparity in cumulative fasting time, with Stote *et al.* (2007) and Halberg *et al.* (2005) imposing 20-hour fasts on 48 and 7 occasions, respectively. Nonetheless, the authors conclude that this approach to intermittent fasting improves metabolic health even in the absence of changes in body mass.

Employing a similar approach, Soeters *et al.* (2009) sought to explore the associated mechanisms more closely using a counterbalanced crossover design. Once again, eight

healthy males were recruited and subjected to two weeks of a standard weight maintenance diet and two weeks of intermittent fasting, using the same format as described previously for the study of Halberg *et al.* (2005), with a washout period of four weeks between the two arms. In this instance, a more prescriptive approach was adopted to the feeding cycles, with liquid meals used to bolster intake and adjustment of prescription in the event of a meaningful weight change. Accordingly, body mass and composition were unaltered, yet there were no significant changes in glucose, lipid or protein kinetics in the basal state, or during a two-stage euglycaemic-hyperinsulinemic clamp. In actuality, the only difference was a slight decrease in resting energy expenditure following intermittent fasting, although these values were not normalised to body mass or fat-free mass and as such should be treated with caution.

To the contrary of Halberg *et al.* (2005) and Stote *et al.* (2007), the above findings suggest that recurrent extension of the fasting period exerts no influence on energy or nutrient metabolism. The only exception to this was a possible decline in resting energy use. Whilst there are some discrepancies in terms of the approach to feeding cycles and the method employed to assess nutrient metabolism under dynamic conditions, attributing to such factors would suggest the effect is unlikely to be clinically meaningful, particularly outside a research context. However, work by Heilbronn and colleagues provides some interesting insights that could explain such stark contrasts between similar approaches (Heilbronn, Civitarese, *et al.*, 2005; Heilbronn, Smith, *et al.*, 2005). The study applied an intermittent fasting intervention to a cohort of 16 non-obese adults. This involved fasting from midnight to midnight on alternating days for 3 weeks, with fasting periods only permitting energy-free drinks and sugar-free gum. Although, it should be noted that chewing has been suggested to stimulate insulin secretion (Suzuki et al., 2005). On the intervening days, food consumption was *ad libitum*. Participants were informed that eating twice as much as normal would be required to maintain body mass, although it is unclear exactly how this information was used as energy intake was not reported. Assessments of body composition, a mixed-meal test and muscle biopsies were carried out at baseline and follow-up, with an additional set of measurements collected after a 36 hour fast to explore the physiological impact of individual fasting periods on energy metabolism.

The intervention reduced body mass by 2.5% on average, approximately two thirds of which was accounted for by reduced fat mass. However, the majority of fasting parameters, including plasma glucose concentration, RMR, substrate oxidation and muscle GLUT4 content showed no notable change (Heilbronn, Civitarese, *et al.*, 2005; Heilbronn, Smith, *et al.*, 2005). Key exceptions were sex-specific alterations in cholesterol profile, with women experiencing an increase in HDL cholesterol concentration and men exhibiting reductions in fasting triglycerides. Values collected after 36 hours of fasting confirmed increased fatty acid oxidation, raising the key question of why the routine upregulation of fat metabolism combined with body mass losses resulted in no consistent changes in metabolic health. However, this pattern of sexual dimorphism continued into postprandial outcomes following a mixed-meal test, with increases in glucose area under curve for females and reductions in insulin area under curve for males (Heilbronn, Civitarese, *et al.*, 2005). Although, it is important to note that the pre-trial fasting duration was not standardised across the pre-intervention and post-intervention meal tests.

This would appear to suggest that males and females respond differently to this form of alternate-day fasting. However, there were a number of baseline differences between men and women which should be considered in this interpretation, with men exhibiting higher glucose, insulin and triglyceride concentrations in the fasted state (Heilbronn, Smith, *et al.*, 2005). Upon contextualising this in the physiology of insulin resistance discussed previously (Section 2.1.5), it seems plausible that the metabolic state of male participants at baseline would stand to benefit more from the routine extension of fasting, introducing some possibility of statistical regression. In these individuals, the shift toward fat oxidation seen in response to prolonged fasting could help to clear lipid intermediaries from non-adipose tissues, thereby enhancing insulin sensitivity. This is supported by the reported increase in CPT1 (Carnitine Palmitoyltransferase 1) protein content in muscle tissue after the intervention (Heilbronn, Civitarese, *et al.*, 2005), which is rate limiting in shuttling long chain fatty acids to the inner mitochondrial membrane for beta-oxidation (Henique *et al.*, 2010).

Extending this premise to the studies of Halberg *et al.* (2005) and Soeters *et al.* (2009), the average body fat percentage of their cohorts was 20.1% and 14.8%, respectively. This may fit with the notion that those with lower levels of adiposity may not stand to

benefit as much from such interventions, with the lower resting energy expenditure reported by Soeters *et al.* (2009) reflecting a more profound adaptive response courtesy of the already low levels of body fat. Consequently, it is imperative to consider the seemingly distinct responses seen between leaner and more overweight cohorts when interpreting the results of such intervention studies. This is not only because the potential for weight loss and health gain may vary, but also because the presentation as either lean or obese at baseline may be symptomatic of a natural predisposition towards various compensatory adjustments that can predict responsiveness to treatment.

Furthering this line of enquiry, Catenacci *et al.* (2016) undertook a randomised controlled trial of complete alternate-day fasting in a sample of obese adults. Briefly, 26 participants were randomised to undertake 8 weeks of either daily calorie restriction, requiring a reduction in energy intake of 400 kcal per day, or a complete alternate-day fast. The intermittent fasting condition imposed a fast on every other day and provided a diet to meet estimated energy requirements on feeding days, with a series of 200 kcal optional food modules to permit *ad libitum* intake. All foods were provided by the study team; however, the diets were only matched for macronutrient balance, not energy intake. Consequently, energy intake across the 8-week intervention was lower with the intermittent fasting approach, averaging 53% of weight maintenance requirements compared to 72% for daily calorie restriction. This was accompanied by a trend for greater reductions in body mass with intermittent fasting relative to calorie restriction, with 8.8% and 6.2% reductions seen in the respective conditions. Despite this, fat mass and lean mass decreased to a similar degree in both groups, a pattern mirrored by improvements in fasted lipid profile. Only intermittent fasting produced improvements in fasted glucose concentration from baseline to follow-up, yet responses to a dynamic test of insulin sensitivity were unaltered. Conversely, RMR was reduced by daily calorie restriction only, following correction for body composition changes, with a trend for a between-group difference. Unfortunately, the discrepancy in energy intake means the effect of this on weight change cannot be ascertained.

Following the intervention, participants were also assessed after 24 weeks of unsupervised follow-up to establish the persistence of these changes. Although daily

calorie restriction and intermittent fasting characterised a similar degree of weight re-gain, +2.8% and +1.9% of baseline weight, respectively, the composition of this differed between groups. The regain seen with daily calorie restriction was accounted for by similar increases in both fat and lean mass, whilst with intermittent fasting only lean mass increased. The pattern for RMR also continued, with the adaptive decline in energy use following daily calorie restriction remaining throughout the unsupervised follow-up period, in agreement with prior studies (Franz *et al.*, 2007).

This once again suggests that intermittent fasting and daily calorie restriction may exert differing effects on energy metabolism, but that extending the fasting period beyond 14-16 hours is key. This is well-illustrated by the study of Catenacci *et al.* (2016), as the weight loss and energy restriction were greater with intermittent fasting, yet there was no accompanying decline in energy use. However, between-group comparisons were compromised by baseline differences, with those in the daily calorie restriction group presenting with higher body mass, fat mass and fasting insulin concentrations on average.

2.4.5 – Outstanding Questions in the Literature

Consistent with the above, intermittent fasting clearly encompasses a broad spectrum of dietary interventions. The defining characteristic is the confinement of calorie restriction to a specified temporal window, be that 16 hours each day (Moro *et al.*, 2016), every other day (Heilbronn, Smith, *et al.*, 2005; Varady, Bhutani, *et al.*, 2009), or just two days per week (Harvie *et al.*, 2011; Antoni, Johnston, *et al.*, 2018). Across these various models, intermittent fasting has been shown to elicit reductions in body mass and improvements in metabolic health, effects which appear to be broadly comparable to daily calorie restriction interventions (Barnosky *et al.*, 2014). However, as the therapeutic potential of these strategies may lie in extending catabolic periods, these similarities could instead reflect a failure to actually extend the post-absorptive period with several forms of intermittent fasting, particularly the 5:2 diet and modified alternate-day fasting. These designs do not give due consideration to the time-course of postprandial responses to acute feeding episodes (Coppack *et al.*, 1990; Frayn *et al.*, 1993).

Time-restricted feeding and complete alternate-day fasting overcome this by routinely applying a fasting duration of 14 hours or more and will be the focus of this review henceforth. This represents a more tangible distinction from typical feeding patterns (Moro *et al.*, 2016; Gabel *et al.*, 2018) and has been associated with reduced fat accretion and enhanced insulin sensitivity (Heilbronn, Civitarese, *et al.*, 2005; Stote *et al.*, 2007; Moro *et al.*, 2016). However, studies deploying these prolonged periods of fasting are relatively scarce, and the true effects are difficult to isolate due to order effects, metabolically diverse samples, and the use of single-arm trials.

Identifying more effective strategies for managing obesity and the accompanying dysfunction is imperative, and intermittent fasting may represent a potent therapeutic tool which capitalises upon circadian rhythms in energy metabolism (Patterson and Sears, 2017). However, the research to support this is scarce whilst a number of potential facets have been largely overlooked, which will be considered in the following sub-sections. Addressing these issues is vital in order to establish whether intermittent fasting is simply an alternative means of achieving calorie restriction (Gabel *et al.*, 2018), or a dietary strategy which is better aligned with proposed evolutionary norms (Halberg *et al.*, 2005).

2.4.5.1 – Intervention Design

Firstly, despite the diversity of fasting protocols employed in previous studies, there is no robust rationale given for the use of any particular format. The initial study of Heilbronn, Smith, *et al.* (2005) utilised a protocol in which participants fasted on every other day from midnight to midnight for 3 weeks. Although this provided the longest fasting duration of any such study to date, the use of a night-time transition point may have encouraged nocturnal eating, which has been associated with adverse metabolic responses (Al-Naimi *et al.*, 2004; Scheer *et al.*, 2009). Halberg *et al.* (2005) addressed this by employing a fast which started at 22:00 and ended at 18:00 the following evening, giving a 20-hour duration while encouraging the consumption of food at normal waking times. However, the selection of this particular format still lacks a clear rationale and gives a reduced fasting opportunity relative to other alternatives.

In most cases, the structure of interventions employed in intermittent fasting studies seems to have been carried over from trials of daily calorie restriction. In doing so they

fail to capitalise on the versatility an intermittent approach can provide; while in continuous restriction the only variables are the intensity of restriction and the duration of the intervention, intermittent approaches can also manipulate the frequency, timing and duration of individual restriction periods. Given that a common rationale for intermittent fasting is reduced motivational demands (Heilbronn, Smith, *et al.*, 2005; Klempel *et al.*, 2010), the arbitrary designs included in most studies to date may fail to truly capture the practical potential of intermittent fasting and could actually undermine improvements in metabolic health.

2.4.5.2 – Changes in Energy Expenditure

Secondly, a key issue with conventional obesity management approaches is compensatory changes in other dimensions of energy balance, particularly decreased energy expenditure with daily calorie restriction (Section 2.2.4). Yet it is not clear from the pre-existing body of intermittent fasting research whether these same compensatory changes are invoked. It can certainly be argued that the short-term and discontinuous nature of energy restriction in these trials minimises adaptation (Byrne *et al.*, 2018); however, if leptin is indeed the key signalling compound in this response, then any decline in adipose tissue mass is likely to induce modifications in energy expenditure (Rosenbaum *et al.*, 2005; Rosenbaum, Hirsch, *et al.*, 2008).

The findings of prior research have been relatively inconsistent in this regard. The initial work by Heilbronn, Smith, *et al.* (2005) reported no decrease in RMR following 3 weeks of intermittent fasting despite reductions in fat mass. Similarly, Catenacci et al. (2016) observed that, following adjustment for body composition, RMR decreased by 110 $kcal \cdot day^{-1}$ in response to 8-weeks of daily calorie restriction, but only by 16 $kcal \cdot day^{-1}$ with intermittent fasting. Moreover, these effects persisted throughout 24 weeks of unsupervised follow-up. Conversely, Soeters *et al.* (2009) observed a decline in resting energy use of 59 $kcal \cdot day^{-1}$ following 2 weeks of eucaloric intermittent fasting. However, as discussed earlier, this may reflect the leaner nature of the participants in the latter study and the lack of adjustment for body composition.

Nonetheless, RMR is but one component of total daily energy expenditure. Physical activity energy expenditure has scarcely been examined in response to such interventions. The study of Klempel *et al.* (2010) observed no changes in daily step

counts during 8-weeks of modified alternate-day fasting, despite clinically meaningful weight losses. Whilst it can be argued that pedometers lack the precision to detect such effects (Crouter *et al.*, 2003; Corder, Brage and Ekelund, 2007), ensuing studies employing accelerometers have verified this outcome (Klempel *et al.*, 2012; Varady *et al.*, 2016). However, it should be noted that both of these studies also employed a modified alternate-day fasting approach as well, which may have differ in its effects relative to complete alternate-day approaches, as discussed previously.

Although Sutton *et al.* (2018) also argue that the stability (or at least lack of detectable *significant* differences) of body mass in their eucaloric time-restricted feeding study suggests that energy expenditure is not affected by temporal restrictions of energy intake, this is not necessarily the case. Dhurandhar *et al.* (2015) highlight that accurate determination of energy balance necessitates measurement of all aspects of the equation. Cumulatively therefore, it can be argued that although there are promising indications that the compensatory mechanisms reported for daily calorie restriction may be blunted by intermittent fasting, the degree of weight loss, the precise form of fasting employed and the examination of isolated dimensions of energy expenditure prevent reliable conclusions being drawn. There remains a definite need to examine the cumulative impact of intermittent fasting on the components of energy expenditure.

2.4.5.3 – Postprandial Nutrient Metabolism

The third issue arising from the pre-existing literature is that the majority of studies have focused on fasting measures of glucose, insulin and triglycerides, with very few studies employing dynamic tests to assess post-prandial responses, which typically predominate throughout waking hours. The issue this presents is well-illustrated by the impact of intermittent fasting on insulin; improvements in fasting insulin have been consistently shown in a number of studies of intermittent fasting, as reviewed by Barnosky *et al.* (2014). They also show that in a subset of these studies fasting indices of insulin resistance such as the homeostasis model generally improve following a period of intermittent fasting. However, it is important to note that while these fasting indices are useful in easing experimental demands, there are several limitations. Specifically, Borai *et al.* (2011) suggest it is possible for a participant to be insulin resistant without demonstrating fasting hyperinsulinaemia.

The same problems emerge when discussing postprandial glycaemia, whilst postprandial lipaemia has been largely ignored. This latter oversight is particularly pertinent in light of the potential impact of postprandial lipaemia in the aetiology of obesity (Section 2.3.2). On an acute basis, Antoni *et al.* (2017) demonstrate that a day of 100% caloric restriction results in enhanced suppression of postprandial triglyceride and NEFA concentrations, relative to habitual intake and partial energy restriction. Extending this to the 5:2 approach, a similar pattern emerged with improvements in postprandial triglyceride levels with the intermittent condition relative to continuous restriction (Antoni, Johnston, *et al.*, 2018). Such effects are also in keeping with the enhanced suppression of adipose tissue lipolysis reported by Halberg *et al.* (2005). Given the importance of these outcomes in the context of obesity and the associated comorbidities, closer examination is warranted.

2.4.5.4 – Comparative Designs

Despite being proposed as an alternative approach to weight loss, few human trials to date have directly compared complete alternate-day fasting against daily calorie restriction. Although it is generally reported that the outcomes are similar, the broad spectrum of cohorts and experimental protocols employed confounds reliable comparisons against the pre-existing literature (Varady, 2011). The study of Catenacci *et al.* (2016) is certainly an exception to this pattern, in so far as it directly compared complete alternate-day fasting and daily calorie restriction; however, the two conditions were not matched for the degree of calorie restriction imposed. For this reason, reaching a consensus on the relative merits of complete alternate-day fasting is not possible at this time.

2.4.5.5 – Fasting-Dependent Effects

Evidence suggests that fasting may exert independent effects on health outcomes over and above those resulting from weight loss. This is supported by Halberg *et al.* (2005), who elicited significant improvements in insulin sensitivity. However, the failure of Soeters *et al.* (2009) to replicate this finding with a near identical fasting protocol calls this attribute into question. This conflict may be driven by methodological contrasts in baseline adiposity and the refeeding protocol employed, but it leaves a pertinent question nonetheless. If fasting-dependent effects on health are established, it may be

that conventional meal patterns are contributing to metabolic disturbances irrespective of calorie content. This would mean that changes in feeding times could constitute a novel dimension of what is considered a healthy diet, as opposed to simply being a vehicle for calorie restriction.

2.5 – Thesis Objectives

Collectively, the literature review in this chapter highlights that obesity is a prevalent health challenge, which arises due to chronic positive energy balance. The energy surplus is deposited as triglycerides within adipose tissue leading to expansion, predominantly as a result of adipocyte hypertrophy. If sustained over time, these hypertrophic adipocytes become dysfunctional, culminating in chronic low-grade inflammation, insulin resistance, hyperglycaemia and hyperlipidaemia, thereby fostering comorbidities such as type 2 diabetes and cardiovascular disease. To remedy this metabolic dysfunction, interventions look to redress the imbalance by reducing energy intake or increasing expenditure, which does typically improve health outcomes. However, these improvements are hampered by compensatory changes in appetite and energy use, as well as poor adherence, resulting in low long-term success rates.

Thus far, the therapeutic potential of nutrient timing in improving metabolic health has been largely overshadowed by the manipulation of the principal components of the energy balance equation. However, advances in the understanding of circadian rhythms suggest that this could be a particularly potent strategy, given that current societal norms result in a lipogenic state predominating in energy metabolism for all but a few hours each day. Not only could interventions that manipulate nutrient timing help to restore diurnal rhythms in substrate metabolism and encourage lipolysis of surplus triglycerides, but these approaches may also be more acceptable in practice than conventional alternatives.

Building upon this premise, several therapeutic interventions have emerged which confine energy restriction to designated temporal windows. However, in many cases these diets do not increase the opportunity for lipolysis as the fasting period is interrupted prematurely by the provision of a small meal to aid motivation and adherence. A subset of these strategies have routinely applied fasting durations of 16 hours and over, which offer promising insights on the potential of this approach. However, these studies are relatively sparse, methodologically inconsistent, and overlook several potentially key facets of intermittent fasting as a strategy for managing obesity and the accompanying dysfunction. Consequently, this thesis will

aim to address the following six objectives, thereby providing greater clarity on the safety and efficacy of these dietary approaches:

1. Establish daily rhythms in subjective hunger and appetite under the influence of diurnal stimuli to inform the design of an intermittent fasting intervention.
2. Clarify how intermittent fasting impacts postprandial glycaemic and insulinaemic responses using a mixed-meal tolerance test.
3. Provide a novel exploration of how intermittent fasting affects the three components of total daily energy expenditure.
4. Compare metabolic impacts of intermittent fasting against an isocaloric daily calorie restriction intervention.
5. Isolate the respective contributions of routine fasting and negative energy balance to any measured changes in metabolism or health.
6. To explore the differential responses to intermittent fasting in independent lean and overweight/obese cohorts.

Chapter 3: General Methods

This Chapter elaborates upon the methodological techniques employed in the ensuing experimental chapters that constitute this thesis. All research activities were conducted in accordance with the principles set out by the Declaration of Helsinki (World Medical Association, 2013). Ethics approval was obtained from the NHS Research Ethics Committee prior to participant recruitment in all cases. The methodology in Chapter 4 was authorised under reference number 14/SW/0123 by the Cornwall and Plymouth delegation, whilst the protocol for Chapters 5 and 6 was approved under reference number 15/SW/0007 by the South West Frenchay committee. In accordance with the terms of this approval, site management permission for both protocols was obtained from the Research Ethics Approval Committee for Health at the University of Bath. In all cases, the University of Bath acted as the research sponsor.

3.1 – Sampling/Recruitment

Recruitment for both studies was achieved by means of advertising at the University of Bath and the surrounding area in South West England. Interested parties were encouraged to contact the research team for more information and were subsequently briefed on what the respective protocols would involve, both verbally and in writing. Those who expressed both a willingness and ability to undertake the protocol were then invited to provide written informed consent for their participation, if deemed eligible according to the criteria specified in each chapter. At all times, participant consent was voluntary and as such could be withdrawn at any time without justification if they so wished. Eligibility was confirmed with a compulsory health screening questionnaire and a battery of validated scales to quantify characteristics, such as chronotype, physical activity readiness and disordered eating, as necessitated by the two studies. To ensure confidentiality, all participants were assigned a unique code which was used on all data collection documentation in place of identifiable information.

3.1.1 – Questionnaires

In Chapter 4, a series of questionnaires were used to assess participant eligibility and characterise the study cohort, including the Morningness–Eveningness Questionnaire (Horne and Ostberg, 1976), the Pittsburgh Sleep Quality Index (Buysse *et al.*, 1989) and the Munich Chronotype Questionnaire (Roenneberg, Wirz-Justice and Merrow, 2003). Whilst full descriptions of these questionnaires are available in the original citations, an overview of each questionnaire and their key outcomes are provided in the ensuing subsections for clarity.

3.1.1.1 – Morningness–Eveningness Questionnaire

The premise of this questionnaire is that differences within circadian rhythmicity can broadly classify an individual as a 'morning type' or an 'evening type' (Horne and Ostberg, 1976). These differences in diurnal preference lead to distinct patterns of energy intake, physical activity and sleep, patterns which need to be considered when examining circadian rhythms *in vivo* (Mota *et al.*, 2016; Maukonen *et al.*, 2017). To this end, the questionnaire posits a number of questions pertaining to factors such as preferred sleep-wake times, diurnal alertness and optimal performance. Respondants are required to provide an answer in all instances, with responses that are consistent with morning preference receiving higher scores and those that are consistent with evening preference receiving lower scores. Once all the questions have been scored, they are summed together to provide a Horne-Ostberg Score, which will fall into one of the following categories: definite evening type (score = 16-30), moderate evening type (score = 31-41), neither type (score = 42-58), moderate morning type (score = 59-69) or definite morning type (score = 70-86).

3.1.1.2 – Pittsburgh Sleep Quality Index

This questionnaire was developed in order to quantify sleep quality over the previous month (Buysse *et al.*, 1989). It poses a series of questions that aim to evaluate seven dimensions of global sleep quality: subjective sleep quality, sleep latency, sleep duration, sleep efficiency, sleep disturbance, sleeping medication use and daytime dysfunction. In this instance, responses that are consistent with higher sleep quality

equate to lower scores, with the cumulative total ranging from 0 (no difficulty) to 21 (severe difficulty in all areas).

3.1.1.3 – Munich Chronotype Questionnaire

Much alike the Morningness–Eveningness Questionnaire, discussed previously, the Munich Chronotype Questionnaire seeks to assess interindividual variation in the temporal organisation of behaviour, particularly the timing of sleep within each day (Roenneberg, Wirz-Justice and Merrow, 2003). The questions centre upon establishing the clock time at which certain sleep-related events occur, which is done separately for work days and free days. These times are then used to establish sleep phase, otherwise known as chronotype, which can be used as a reference point for comparisons between individuals. Specifically, the midpoint between sleep onset and wake up is typically used. Although there are no nominal categories into which individuals can be classified in this instance, earlier midsleep times are consistent with morning preference and later midsleep times are consistent with evening preference.

3.2 – Ambient Conditions

With the exception of free-living measurements (Section 3.6), all further data was collected in the Human Physiology Laboratories at the University of Bath. The ambient conditions were carefully controlled to ensure consistency of metabolic variables both within and between laboratory sessions. Temperature was monitored using a thermometer to maintain it within a range of 20-25ºC in accordance with best practice guidelines for key metabolic outcomes (Compher *et al.*, 2006). Ambient lighting in the laboratory was set at an intensity of 800 lux at all times, unless otherwise stated.

3.3 – Body Composition

3.3.1 – Height

Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Seca; Hamburg, Germany) with a working range of 5-230 cm. For all measurements,

participants were required to remove their shoes and stand with their heels in contact with the baseplate. Researchers ensured that the buttocks, scapula and head were in contact with the stadiometer before lowering the scale to meet the participant's head at the most superior point during deep inspiration.

3.3.2 – Body Mass

For all measurements of body mass participants were required to wear lightweight clothing; where this guidance was not followed, appropriate clothing was provided. All measurements were taken immediately post-void and participants removed their shoes along with any additional layers, heavy items and jewellery. Measurements were taken using a sliding balance scale (424, Weylux; UK) to the nearest 0.1 kg whilst participants stood in a relaxed position with their weight evenly distributed between their feet. For repeated measurements, as in Chapters 5 and 6, participants were required to wear the same lightweight clothing for each session to ensure consistency across observations. Body mass and height measurements were then used to determine body mass index by dividing body mass (kg) by height (m) squared (Keys *et al.*, 1972).

3.3.3 – Dual-energy X-ray Absorptiometry

In Chapters 5 and 6, body composition was assessed using the Dual-energy X-ray Absorptiometry (DEXA) method. The principle of this approach is well described by Andreoli *et al.* (2009), wherein the distribution of bone, fat and other (i.e. lean) tissues can be established by their propensity for radiographic attenuation at two frequencies, hence, 'dual-energy'. This produces a planar image in which each pixel can be classified as either bone, fat, or bone- and fat-free (i.e. lean) (Pietrobelli *et al.*, 1996). This is then extrapolated to provide whole-body measurements of bone mineral density, lean tissue mass and fat tissue mass. However, while this offers a direct measurement and permits the exploration of regional distribution, thereby improving upon indirect methods such as circumferences, BMI and bioelectrical impedance, it makes a number of assumptions which should be considered (Wells and Fewtrell, 2006). For instance, the composition of lean mass is assumed to be constant despite the capacity for substantial variability, particularly with changes in hydration status, although this only impacts toward the extremes of the physiological range (Andreoli

et al., 2009). More concerning, is that the scanner cannot adequately compensate for changes in trunk thickness in the sagittal plane, as the image obtained only provides a cross-section in the frontal plane (Lee and Gallagher, 2008). Consequently, scans are best supplemented by indices of trunk thickness to account for changes that may arise in longitudinal designs (Williams *et al.*, 2006).

Scans were obtained at the end of data collection sessions to provide greater control over hydration status. For the purpose of the scans, participants voided, wore the same lightweight clothing and removed their shoes along with any additional layers, heavy items and jewellery. They were then positioned centrally within the scanning frame (QDR Discovery W, Hologic; MA, USA) in a supine position with the legs slightly abducted and feet inverted (Lorente Ramos *et al.*, 2012). The arms were also slightly abducted and placed equidistant to the trunk (Nightingale *et al.*, 2016). In line with the recommendations of the manufacturer, prior to each exposure a quality control procedure was executed in which a spine phantom with known radiographic attenuation properties was scanned to ensure adequate performance. This was accompanied by a background radiographic uniformity test at regular intervals, in which a whole-body scan was completed whilst the scanning table was empty to ensure proper functioning and monitor changes in background radiation levels.

3.4 – Laboratory Measurements

3.4.1 – Hydration Status

Hydration status was assessed at the outset of each lab session in Chapters 5 and 6. The objective of this was to ensure consistency across the three visits, as even mild dehydration can result in reduced body mass relative to euhydration (Armstrong, 2005). To this end, participants were required to provide a mid-stream urine sample when voiding ahead of the body mass measurement. Samples were then analysed immediately for urine specific gravity using an optical refractometer (SUR-NE Clinical, Atago; Tokyo, Japan) with a working range of 1.000 to 1.030. This technique scales the density of the sample relative to a calibration standard of ultrapure water, with greater degrees of solute relative to solution increasing the density, and therefore refraction of light, which characterises higher levels of dehydration (Armstrong,

2005). To this end, five drops of urine were placed on the stage which was gently closed to ensure there were no air pockets within the confines of the refractory window. This was then held directly below an ambient light source emitting at 1000 lux and a reading was taken to the nearest 0.001. Values greater than or equal to 1.020 were considered indicative of inadequate hydration (Oppliger *et al.*, 2005).

3.4.2 – Indirect Calorimetry

Expired gas samples were collected and analysed throughout the experimental work that constitutes this thesis to quantify rates of energy production and substrate oxidation via indirect calorimetry. The principles underpinning this technique are well described by Frayn (1983) and Weir (1949), from which the ensuing descriptions and equations have been derived, unless otherwise cited.

3.4.2.1 – Principles of Indirect Calorimetry

In basic chemistry, oxidation of a given mass of glucose, fat or protein will consume a known quantity of oxygen and produce a known quantity of carbon dioxide (equations 1-3). As the amount of energy released per unit of oxygen consumed and carbon dioxide released varies by substrate, the cumulative totals of the respective gases alone is not sufficient to determine energy needs. Instead, these values must be used to determine the grams of each substrate that have been oxidised, which can then be used to estimate how many units of energy have been released. However, as the oxygen uptake and carbon dioxide of an organism are each the product of three unknowns (i.e. the amount of glucose, fat and protein consumed), this remains somewhat problematic.

$$1\ g\ Glucose + 0.746\ L\ O_2 \rightarrow 0.746\ L\ CO_2 + 0.6\ g\ H_2O + Energy \quad (1)$$

$$1\ g\ Lipid + 2.029\ L\ O_2 \rightarrow 1.430\ L\ CO_2 + 1.09\ g\ H_2O + Energy \quad (2)$$

$$1\ g\ Protein + 0.966\ L\ O_2 \rightarrow 0.782\ L\ CO_2 + 0.45\ g\ H_2O + Energy \quad (3)$$

Focusing initially on protein metabolism, in addition to carbon dioxide being released as a waste product, nitrogen is also excreted through urine, predominantly in the form of urea (Skogerboe *et al.*, 1990). As nitrogen accounts for approximately 16% of protein by weight, measurement of urinary nitrogen excretion (Section 3.4.3) can be used to estimate protein breakdown (equation 4), along with the accompanying amount of oxygen consumed and carbon dioxide produced via modification of equation 3 (equation 5), thereby removing one of the unknowns.

$$Protein = 6.25\ x\ urinary\ nitrogen\ excretion\ (N) \quad (4)$$

$$1\ g\ Nitrogen + 6.038\ L\ O_2 \rightarrow 4.888\ L\ CO_2 + 2.81\ g\ H_2O + Energy \quad (5)$$

Although no similar method can be adopted for carbohydrate or fat, the ratio of carbon dioxide produced to oxygen consumed during carbohydrate oxidation is 1:1. This means that if urinary nitrogen excretion is known, any discrepancy between oxygen uptake and carbon dioxide production can only be explained by oxidation of fat, which has a ratio 0.695. In knowing this, the amount of oxidised lipid can be determined as a function of oxygen consumption, carbon dioxide production and urinary nitrogen excretion (equation 9), before itself being used to determine the rate of carbohydrate metabolism (equation 11). This is shown mathematically in equations 6-11, wherein equation 7 is subtracted from equation 6 to give equation 8, which is then rearranged for equation 9. Equation 9 is subsequently substituted back in to equation 6 to give equation 10, before rearranging to give equation 11.

$$\dot{V}O_2 = (0.746\ x\ glucose) + (2.029\ x\ lipid) + (6.038\ x\ nitrogen) \quad (6)$$

$$\dot{V}CO_2 = (0.746\ x\ glucose) + (1.430\ x\ lipid) + (4.888\ x\ nitrogen) \quad (7)$$

$$\dot{V}O_2 - \dot{V}CO_2 = (0.599\ x\ lipid) + (1.150\ x\ nitrogen) \quad (8)$$

$$Lipid = 1.67\left(\dot{V}O_2 - \dot{V}CO_2\right) - (1.92\ x\ nitrogen) \quad (9)$$

$$\dot{V}O_2 = (0.746\ x\ glucose) + \left(3.388\ x\ \dot{V}O_2\right) - \left(3.388\ x\ \dot{V}CO_2\right) - (2.144\ x\ nitrogen) \quad (10)$$

$$Glucose = \left(4.542\ x\ \dot{V}CO_2\right) - \left(3.201\ x\ \dot{V}O_2\right) + (2.874\ x\ nitrogen) \qquad (11)$$

Once the amount of each substrate that has been oxidised is determined, multiplying them by their respective caloric equivalents and summing the outcomes will yield a value for energy expenditure over the specific time frame.

3.4.2.2 – Expired Gas Collection and Analysis

In accordance with best practice guidelines for resting samples (Compher *et al.*, 2006), participants were required to rest in a semi-supine position for 20 minutes prior to sample collection. A nose clip was then applied to eliminate nasal breathing and a mouthpiece inserted which was attached to a one-way respiratory valve (Hans Rudolph; MO, USA). This was connected to a fully evacuated 200 L Douglas Bag via falconia tubing, thereby creating a closed system from the point of expiration to allow a sample of expired air to be collected within a desired timeframe, five minutes in the case of a resting sample. The mouthpiece and noseclip were placed at least 30 seconds prior to the collection period to clear ambient air from the system (Compher *et al.*, 2006). During sampling, ambient temperature and barometric pressure were recorded, alongside ambient oxygen and carbon dioxide concentrations via paramagnetic and infrared sensors (MiniMP 5200, Servomex; Sussex, UK), respectively. These sensors were calibrated using a two-point method at the outset of each trial using gases of known concentration (British Oxygen Company; Guildford, UK). Following collection, expired samples were also analysed for oxygen and carbon dioxide fraction, with the flow rate recorded for correction of expired volume. The temperature and remaining volume of the expired gas were then obtained using an in-line thermistor probe (Checktemp 1, Hanna Instruments; Bedfordshire, UK) and a dry gas meter (Harvard Apparatus; Cambridge, UK), respectively. All volumes were then standardised for pressure (760 mmHg) and temperature (0^oC) of a dry gas. Using these parameters, oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$), the principal components of the ensuing calculations (Weir, 1949; Frayn, 1983), were determined using the Haldane Transformation (Haldane, 1912; McArdle, Katch and Katch, 2015).

In situations where a sample of expired air was required during exercise, as in Chapters 5 and 6, a facemask (Hans Rudolph; MO, USA) was attached to the one-way

respiratory valve in place of the mouthpiece. Elasticated straps were fastened around the head and adjusted to ensure an air-tight seal. This was for the purposes of participant comfort when collecting samples during treadmill locomotion.

3.4.3 – Nitrogen Excretion

During Chapters 5 and 6, resting values of energy and substrate metabolism are also corrected for rates of protein oxidation. Throughout the postprandial period, this required collection of total urine output into 500 ml containers prepared with 5 ml of a 10% thymol–isopropanol solution, which acted as a preservative. Following the conclusion of the 3-hour measurement window, the samples were combined to determine urinary volume, and a homogenous 1 ml sample was aliquoted and frozen for subsequent analysis of urinary urea concentration (Section 3.5.1), which is assumed to represent approximately 90% of urinary nitrogen excretion (Skogerboe *et al.*, 1990). The first and last blood samples collected during the measurement window were also analysed for plasma urea concentration (Section 3.5.1). Upon obtaining these parameters, it was then possible to estimate the rate of protein oxidation using the method described by Jéquier and Felber (1987), thereby allowing measurements of energy expenditure and substrate oxidation to be corrected for protein oxidation.

3.4.4 – Blood Sampling

3.4.4.1 – Venepuncture

Venepuncture was used to obtain a blood sample when only a single sample was required in a given laboratory session. To this end, a suitable vein in the antecubital fossa was identified and the surrounding skin sterilised with an alcohol swab. Following a 30 second pause for evaporation, a 21G needle (BD; NJ, USA) was then inserted into the lumen of the vessel and the desired volume of venous blood was drawn into a 10 ml syringe. The needle was then withdrawn and disposed of accordingly whilst pressure was applied to the sampling site. The sample was dispensed immediately across 5 ml sample tubes coated with ethylenediaminetetraacetic acid (EDTA) and centrifuged to separate the supernatant. Unless otherwise stated hereafter, all blood samples were centrifuged (Biofuge Primo

R, Heraeus, Germany) at 3466 x g for 10 minutes at 4ºC, before being aliquoted and frozen at -80ºC pending subsequent analysis.

3.4.4.2 – Cannulation

Where recurrent blood samples were required in the same laboratory session, an indwelling intravenous cannula was used. As before, a suitable vein was identified in the antecubital fossa and sterilised. Following evaporation, a 20G intravenous cannula (Venflon, BD; NJ, USA) was inserted into the lumen and attached to a sampling valve (Octopus Biovalve, Vygon; Swindon, UK) before the cannula was secured with a dressing (Veca-C, BD; NJ, USA). Blood samples were then drawn at the desired intervals into a 5 ml or 10 ml syringe and immediately dispensed into an appropriate sample tube before being centrifuged as described previously. After each draw the cannula was flushed with a matched volume of a 0.9% sodium chloride solution (B. Braun; Hessen, Germany) to keep it patent throughout the trial. Consequently, with the exception of the first draw, the first 3 ml of blood at each sampling interval was discarded to avoid the confounding influence of saline dilution on the outcomes of interest.

3.4.4.3 – Arterialised-Venous Sampling

To glean insights on the exposure of peripheral tissues to plasma metabolites, arterial concentrations are necessary (Copeland, Kenney and Nair, 1992). Owing to the associated risks these methods are scarcely used, causing many to default to venous samples from the antecubital fossa (Edinburgh *et al.*, 2017). However, this assumes that venous concentrations are directly proportional to those in the artery. The arteriovenous difference in glucose concentration varies with tissue sensitivity and hormone concentrations, meaning that venous samples alone provide an incomplete picture of peripheral glucose kinetics (Liu *et al.*, 1992). This is particularly problematic in intervention studies such as those discussed in Chapters 5 and 6, because tissue sensitivity and hormone concentrations can be affected by dietary modification (Johnson *et al.*, 2016).

To overcome this, a heated-air box was used to permit the sampling of arterialised-venous blood, which closely approximates arterial samples for concentrations of oxygen, glucose and lipids (McGuire *et al.*, 1976; Jensen and Heiling, 1991; Liu *et al.*, 1992). The degree of arterialisation has also been shown to be superior to alternative methods of heating, including the heating pad approach (Jensen and Heiling, 1991), in addition to being more practical (Zello *et al.*, 1990). This technique was used in Chapters 5 and 6 in line with the method described by Edinburgh *et al.* (2017). Specifically, for 10 minutes prior to arterialised-venous sampling intervals, participants were asked to place the hand of their cannulated arm into a heated-air box (University of Vermont; VT, USA), the internal environment of which was held steady at 55^{o}C. This method has been shown to be sufficient for arterialisation, with oxygen saturation increasing from 67% to 96% (Kurpad, Khan and Elia, 1994). Following this 10-minute interval, the blood sample was drawn from the cannula and handled as previously described.

3.4.5 – Visual Analogue Scales (VAS)

VAS were employed within Chapter 4 to evaluate subjective ratings of motivation to eat (Stubbs *et al.*, 2000). At the desired measurement intervals, participants were provided with an A4 sheet featuring eight visual scales. Each scale posed a question relating to a specific dimension of appetite (e.g. "how hungry do you feel?"), before presenting a 100 mm horizontal scale with defined extremes (e.g. "not at all hungry" and "as hungry as I have ever been"). Participants were asked to place a vertical line that intersected the scale to denote their respective sensations relative to the two extremes at the time of asking. The scales encompassed, hunger, fullness, prospective consumption, thirst and desire to eat specific food types (sweet, salty, savoury, fatty). The horizontal distance between the start of the scale and the intersecting line was measured to the nearest millimetre.

3.5 – Biochemical Assays

3.5.1 – Spectrophotometry

Concentrations of plasma glucose, cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, non-esterified fatty acids, glycerol and urea were determined using an automated clinical chemistry analyser (RX Daytona, Randox Laboratories; Country Antrim, Northern Ireland). All necessary reagents were obtained from the manufacturer (Randox Laboratories; Country Antrim, Northern Ireland) and the analyser was calibrated using commercially-available standards of known concentration (Randox Laboratories; Country Antrim, Northern Ireland). Furthermore, daily quality controls were performed to ensure consistent performance of the machine (Acusera, Randox Laboratories; Country Antrim, Northern Ireland). Urinary urea concentration was determined using the same approach and assay as for plasma urea concentration, however, samples were diluted using ultrapure water (1+10 ratio) in accordance with the manufacturer's instructions.

3.5.2 – Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA techniques were employed to determine concentrations of insulin (Mercodia; Uppsala, Sweden), leptin (R&D Systems; MN, USA) and unacylated ghrelin (SPI Bio; Yvelines, France). The principles of these techniques have been described previously by Aydin (2015). The absorbance of the final solution was measured using a microplate reader (Spectrostar Nano, BMG Labtech; Buckinghamshire, UK) at the wavelength specified by the assay manufacturer.

3.5.3 – Radioimmunoassay (RIA)

RIA techniques were employed in the quantitation of plasma melatonin concentration in Chapter 4, the underpinning principles of which were recently summarised by Grange, Thompson and Lambert (2014). Specifically, a commercially-available assay was employed (Surrey Assays Ltd., University of Surrey; Guildford, UK), which has been previously described by Fraser *et al.* (1983).

3.5.4 – Assay Performance

The inter- and intra-assay coefficient of variation for each test is shown in **Table 3.1**.

Table 3.1: Performance characteristics of biochemical analytical techniques (mean ± SD)

Analyte	Method	Coefficient of Variation (%)	
		Intra-assay	Inter-assay
Glucose	Photometric	3.0 ± 0.7	3.3 ± 0.3
Cholesterol	Photometric	4.0 ± 0.9	3.8 ± 1.1
HDL Cholesterol	Photometric	4.2 ± 1.8	5.3 ± 0.4
LDL Cholesterol	Photometric	2.1 ± 0.5	2.3 ± 0.5
Triglycerides	Photometric	4.4 ± 1.9	4.9 ± 2.2
NEFA	Photometric	5.6 ± 2.0	8.8 ± 3.9
Glycerol	Photometric	12.4 ± 5.0	17.7 ± 6.1
Urea	Photometric	3.0 ± 0.4	2.9 ± 1.1
Insulin	ELISA	5.5 ± 7.4	10.6 ± 6.1
Leptin	ELISA	3.2 ± 0.2	4.4 ± 1.0
Unacylated Ghrelin	ELISA	5.7 ± 1.0	15.7 ± 2.6
Melatonin	RIA	9.7 ± 4.9	16.5 ± 8.7

Abbreviations: HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein; NEFA = Non-Esterified Fatty Acids.

3.6 – Free-Living Measurements

3.6.1 – Energy and Macronutrient Intake

The merits of differing approaches to dietary monitoring were discussed previously in Section 2.1.2, and as such this discussion will focus only on the methods employed in this programme of research.

Throughout these studies, a weighed inventory of food and fluid consumption was employed as the primary medium for quantifying energy and macronutrient intake. For all measurements, participants were provided with an A5 record booklet and a set of digital scales to create their record. The booklet provided details of how to record their diet accurately, including a worked example and a frequently asked questions section which was explained thoroughly by the researcher. Two sides of A4 were provided per day of monitoring, featuring a recording table and a comments section for recipes and other information participants deemed relevant. Participants were asked to specify the

time of consumption, the brand or restaurant, a full description of the item and a serving size. Where possible, participants were asked to record the cooking method and cooked weight of the item, providing the weights of raw ingredients and recipes in the comments section. In scenarios where a weighed portion size was not possible, participants were asked to provide an approximation based on perception (e.g. small portion) or UK household equivalents (e.g. 3 tablespoons). Spare record tables were provided at the back of the booklet in the event further space was required. Furthermore, where packaged foods were consumed, participants were encouraged to retain the packaging for more precise analysis of energy and macronutrient content.

All records were analysed using Nutritics^{TM} Research Edition (version 5.031; Dublin, Ireland). Where specified, energy and macronutrient content were sourced directly from manufacturers and added to a custom foods database. In situations where the food was fresh, the manufacturer was not given, or the composition was unavailable, a series of databases were used to provide standard vales, including composition of foods integrated dataset which is based on the work of McCance and Widdowson (Public Health England, 2015; Roe *et al.*, 2015). All records for a given participant were analysed by the same researcher to ensure consistency. Total energy intake and the fractions contributed by each of the four main macronutrients were determined and carried forward to subsequent analysis.

Although the weighed inventory is prone to threats such as observational and recording bias relative to the reference standard of covert observation, the logistical challenges associated with the latter limit its utility to validation scenarios (Stubbs *et al.*, 2014). Given that diet records tend to perform better than other free-living metrics, including the food frequency questionnaire, 24-hour recall and diet history methods (Black, Welch and Bingham, 2000; Prentice *et al.*, 2011; Stubbs *et al.*, 2014), this was the most appropriate choice considering the objectives of the research. The decision to utilise a weighed record as opposed to portion size estimates was predicated on the variability in subject capacity to accurately estimate portion size (Williamson *et al.*, 2003; Gibson *et al.*, 2016).

The primary flaw of the weighed inventory method is participant burden (Shim, Oh and Kim, 2014). This can lead to reporting fatigue and greater inaccuracy with longer

durations, yet shorter periods fail to capture the variability in day-to-day intake across the week (Trabulsi and Schoeller, 2001; Whybrow, Horgan and Stubbs, 2008; Fyfe *et al.*, 2010; Ortega, Pérez-Rodrigo and López-Sobaler, 2015; Fuller *et al.*, 2017). Consequently, the duration of recording windows was carefully considered depending on the purpose of the resulting data. In Chapters 5 and 6, wherein habitual intake was evaluated for use in dietary prescription, repeated 3-day diet records were utilised. Participants were instructed to include a representative balance of working and non-working days (where relevant) and to separate each period by at least two days, thereby retaining accuracy whilst minimising the associated burden of continuous monitoring. In the latter stages of the protocol, the purpose of monitoring shifted from establishing intake to monitoring compliance. Consequently, 6-day dietary records were applied at the start and end of all three interventions, with the extended duration serving to capture a representative sample of fasting and feeding cycles across the week in the case of intermittent fasting groups. In Chapter 4, the diet records were used simply to characterise intake and establish compliance with standardisation procedures across the group.

3.6.2 – Physical Activity Energy Expenditure

Data pertaining to physical activity energy expenditure was captured using combined heart rate and accelerometry methods. Actiheart™ monitors (Cambridge Neurotechnology; Cambridge, UK) were worn continuously by participants during periods of dietary recording; they were only removed during these times for activities in which the monitor would be directly exposed to water. Such activities were recorded in an activity log and adjusted for when analysing the data, using information from the Compendium of Physical Activities (Ainsworth *et al.*, 2011) and measurements of resting metabolic rate. The monitors were positioned above the heart at the level of the Xyphoid process, as described by Brage *et al.* (2007), and were secured using either adhesive pads (3M, UK) or a chest-strap (Cambridge Neurotechnology; Cambridge, UK) depending on participant preference.

Upon analysing the data, estimates of physical activity energy expenditure are made using a group calibration setting by default. In this instance, the relationship between heart rate and physical activity intensity, from which estimates are interpolated, is

based on data obtained from a sample of 51 adults (Brage *et al.*, 2007). However, the authors highlight that an individual-calibration procedure can account for over 95% of the variance in the relationship between heart rate and physical activity intensity, providing more precise measurements at the individual level. Consequently, an adapted version of the treadmill protocol employed by Brage *et al.* (2007) was used to calibrate the Actiheart^{TM} monitors on a participant-by-participant basis in Chapters 5 and 6.

To this end, participants were led through an exercise test on a treadmill (Valiant, Lode; Groningen, Netherlands), which involved four incremental stages of 3 minutes each; level walking (3.2 km·h^{-1} +0% gradient), brisk level walking (5.2 km·h^{-1} +0% gradient), brisk uphill walking (5.8 km·h^{-1} +10% gradient) and level running at a self-selected pace (9.0-12.0 km·h^{-1} +0% gradient). During each stage, the first two minutes were designed to elicit a steady state before measurements of heart rate and energy expenditure were taken in the final minute. In situations where steady state was not achieved in the designated window, as evidenced by progressive increases in heart rate, the sampling period was delayed until heart rate stabilised. Heart rate was measured using a heart rate sensor mounted on an ECG chest strap (H7, Polar Electro; Kempele, Finland) which was connected via Bluetooth to a watch (RS400, Polar; Electro; Kempele, Finland). Energy expenditure was determined simultaneously using indirect calorimetry, as previously described in Section 3.4.2. The test was ended prematurely if heart rate exceeded 90% of age-predicted maximum or if participants asked to stop.

The relationship between heart rate and energy expenditure in sequential stages was then plotted as a segmental linear regression. As the manufacturer software only permitted entry of energy expenditure values at increments of 10 bpm, average heart rate readings were rounded accordingly to the nearest increment, and the associated energy expenditure parameter was interpolated using the equation from the relevant segment of the regression. The software classifies the heart rate data from each 1-minute epoch as one of four states; “lost”, “interpolated”, “recovered” or “OK”. Data is considered to be lost or OK depending on the physiological plausibility of the value. The manufacturer defines implausible as a value less than 30 bpm, a change of over 100 bpm relative to the previous epoch, or a 75% increase relative to the average heart

rate from the last four epochs. In these instances, the raw value is replaced with zero and labelled as lost, with activity estimates relying solely on accelerometry data, if available. Recovered data then takes each of these zero points and uses the inter-beat interval to estimate heart rate instead; if this is within 30 bpm of the last valid epoch then the estimated value supplants the lost data point and is labelled as recovered. At this stage, any gaps of less than five sequential epochs are interpolated from the epochs at either side and categorised as interpolated.

Although no guidance is available from the manufacturer or the wider literature on the acceptability of differing degrees of data loss and recovery, the merit of this approach lies in the combination of the two data types (Haskell *et al.*, 1993; Luke *et al.*, 1997; Rennie *et al.*, 2000; Brage *et al.*, 2004). Consequently, in cases where over 10% of the data on a given monitoring day was lost, excluding any missing data arising from water-based activities, that day was removed from further analysis (Farooqi *et al.*, 2013). Inevitably, most days featured a fraction of lost data as the device was removed for routine activities such as bathing. This acceptable fraction of lost data was handled by adjusting daily values for the fraction of missing data in accordance with the recommendations of Catellier *et al.* (2005). Although recovered data was less of a threat to accuracy, given that both heart rate and accelerometery data were available, visual inspection of the trace revealed a tendency to underestimate actual heart rate in most cases. Consequently, a conservative threshold of 25% was set on recovered data, to ensure that this tendency did not lead to excessive underestimation.

The measurement of physical activity in Chapters 5 and 6 served to quantify changes in physical activity energy expenditure in response to the interventions, as well as changes in the manner in which this was accrued. Although the free-living reference standard of doubly-labelled water is well-supported for the former, it lacks the resolution to explore the subtle modifications in the various dimensions of physically active behaviours previously reported in response to dietary modification (Betts *et al.*, 2014; Chowdhury *et al.*, 2016). Furthermore, the assumed respiratory exchange ratio of 0.85 is prone to violation by the extreme dietary patterns applied in Chapters 5 and 6 (Halberg *et al.*, 2005; Heilbronn, Smith, *et al.*, 2005; Schmidt *et al.*, 2013).

Comparatively, Actiheart™ monitors have been shown to provide valid and reliable measurements of both heart rate and activity counts, whilst estimates of physical activity energy expenditure derived from this data show good performance relative to doubly-labelled water (Brage *et al.*, 2015). This is further enhanced with the inclusion of an individual calibration, as was the case in Chapters 5 and 6. Furthermore, Actiheart™ monitors provide data over long periods at 1-minute epochs, providing the necessary resolution to explore the temporal shifts in physically active behaviours that may accompany intermittent fasting.

Chapter 4: Diurnal rhythms in appetite under conditions of semi-constant routine.

4.1 – Introduction

Circadian rhythms describe the rhythmic fluctuations in mammalian physiology and behaviour which occur with approximate 24-hour periodicity across most species (Van Gelder and Buhr, 2016). Such rhythms seek to align physiological processes with anticipated environmental events to optimise the outcome (Johnston, 2014), thereby enhancing survival in an evolutionary context (Panda, Hogenesch and Kay, 2002). As understanding of circadian rhythms has continued to advance, nutritional interventions are beginning to explore their therapeutic potential in treating conditions such as obesity (Mattson *et al.*, 2014; Patterson and Sears, 2017). Given the motivational demands associated with continuous energy restriction (Dansinger *et al.*, 2005; Klempel *et al.*, 2010; Ahern *et al.*, 2011), it has been suggested that structuring interventions around circadian rhythms in appetite could be beneficial (Varady, Bhutani, *et al.*, 2009; Scheer, Morris and Shea, 2013; Carnell *et al.*, 2018).

Scheer, Morris and Shea (2013) were the first to explore circadian rhythms in subjective appetite ratings. The study employed a forced desynchrony protocol in which participants were exposed to a recurring 20-hour behavioural cycle for 13 days. As this temporal rhythm of environmental cues lies outside the entrainable range (Scheer, Morris and Shea, 2013), it uncouples them from the endogenous clock and allows the underlying rhythm to be isolated, as the environmental cues are distributed evenly throughout the endogenous period (Pagani *et al.*, 2010). This revealed that subjective ratings of hunger derived from visual analogue scales (VAS) did indeed characterise a circadian rhythm, peaking in the biological evening at 19:50 and reaching their nadir shortly after waking. This pattern was mirrored in ratings of prospective consumption and food preference, with the only exceptions being fullness and the desire to consume vegetables and dairy products. Comparable rhythms have also been observed by Sargent *et al.* (2016) and Wehrens *et al.* (2017), using similar approaches in order to isolate the underlying oscillations (Duffy and Dijk, 2002).

However, whilst these constant routine and forced desynchrony protocols are useful in unmasking such endogenous rhythms, the utility of these rhythms in intervention design is less clear. This is because they remove the diurnal rhythm of behavioural cues, such as sleep and feeding, which could alter or even subvert this rhythm in an ecologically valid setting (Scheer, Morris and Shea, 2013; McHill *et al.*, 2018). Consequently, if such rhythms are to be effectively applied in intervention design, there is a definite need to establish 24-hour rhythms in subjective appetite ratings under diurnal conditions, which no study has examined to date.

The potential impact of these diurnal behaviours is well-characterised when discussing rhythms in ghrelin, an orexigenic peptide which contributes to sensations of hunger by acting on the hypothalamus (Cummings *et al.*, 2001; Austin and Marks, 2009). To date, only one study has concurrently measured circadian rhythmicity in subjective appetite ratings and ghrelin concentrations, which revealed similarly phased rhythms (McHill *et al.*, 2018). In combination with the wider literature on the role of ghrelin in appetite regulation (Cummings *et al.*, 2001; Garin *et al.*, 2013; Gibbons *et al.*, 2013), this would therefore appear to suggest that the endogenous rhythm in hunger is driven to an extent by endogenous rhythms in ghrelin.

In light of this broad concordance, an interesting observation is that these rhythms in ghrelin concentration have been shown to change in response to diurnal environmental influences. For instance, the observations of Dzaja *et al.* (2004) suggest that sleep modifies the endogenous rhythm in plasma ghrelin concentration by exerting a suppressive effect. Focusing instead on the role of feeding, the findings of Cummings *et al.* (2001) implicate decreasing postprandial suppression of ghrelin secretion with successive meals as the driving factor behind diurnal rhythms in ghrelin concentration. This is also reinforced by the consistent observation that ghrelin concentrations decrease over time in response to prolonged fasting (Espelund *et al.*, 2005; Natalucci *et al.*, 2005). As such, it can be argued that the diurnal rhythm of ghrelin secretion is capable of being modified by both feeding and sleeping, effects which may be able to modify the aforementioned rhythms in subjective appetite as well.

Insights as to how these diurnal and circadian aspects of appetite regulation might interact can be gleaned from the study of Stratton, Stubbs and Elia (2003). This

examined waking profiles of subjective appetite ratings during a typical meal pattern (i.e. breakfast, lunch and dinner) either in the presence or absence of diurnal enteral tube feeding. This showed that the addition of enteral tube feeding, and the resulting steady influx of nutrients, did not reduce subjective ratings of appetite or *ad libitum* consumption throughout the day. Whilst this does support the notion that rhythms in subjective appetite could be robust to changes in feeding pattern, the unphysiological nature of tube feeding means that the possibility that subjective appetite is responsive to the cephalic phase of appetite regulation cannot be ruled out (Stratton and Elia, 1999). More importantly though, no rhythmic analyses were performed, meaning it cannot be ascertained whether there were differences in how acute responses to feeding changed throughout the day.

Consequently, the present study aimed to quantify 24-hour rhythms in subjective appetite under diurnal conditions (i.e. feeding/fasting, waking/sleeping). This was done using hourly isocaloric feedings throughout waking hours in an attempt to suppress the postprandial ghrelin rebound, which may drive previously reported rhythms (Cummings *et al.*, 2001; Spiegel *et al.*, 2011). Collectively, this will establish the utility of rhythms in subjective appetite as a concept around which time-related dietary interventions can be structured. Based on the findings of Stratton, Stubbs and Elia (2003), it was hypothesised that subjective appetite ratings would continue follow the circadian rhythm reported by Scheer, Morris and Shea (2013), despite the revised feeding schedule.

4.2 – Methods

Using a single-group design, rhythms in subjective appetite ratings were quantified under conditions of semi-constant routine, as previously described by Mäntele *et al.* (2012). Briefly, participants were monitored over 24 hours in a laboratory setting, with a designated sleeping opportunity and hourly isocaloric feedings during waking periods to preserve diurnal inflences, as necessitated by the study objectives. VAS were completed hourly during waking periods to measure subjective appetite ratings, whilst hourly blood samples were collected throughout to monitor accompanying rhythms in unacylated ghrelin, leptin and melatonin concentration. Ethics approval for the experimental protocol (detailed below) was obtained from the NHS research ethics committee (reference: 14/SW/0123). It should also be noted that these data were collected within a larger study exploring diurnal rhythms in skeletal muscle lipidomics, gene expression and activity, which has been described elsewhere (Loizides-Mangold *et al.*, 2017; Perrin *et al.*, 2018).

4.2.1 – Participants

A cohort consisting of 9 men and 1 woman (**Table 4.1**) was recruited via local advertising. Participants were screened for any disorder of, or medication known to influence, sleep, circadian timing, metabolism or inflammation. Furthermore, those reporting irregular sleep/wake cycles, extreme diurnal preference, psychiatric/neurological disease, drug/alcohol abuse, smoking, excessive caffeine intake (>4 daily servings), shift-work, or recent travel across two or more time-zones (<3 weeks prior) were excluded. These data were obtained via the completion of a general health questionnaire and validated questionnaires to assess habitual sleep patterns and diurnal preferences (Horne and Ostberg, 1976; Buysse *et al.*, 1989; Roenneberg, Wirz-Justice and Merrow, 2003). All volunteers were fully briefed on the requirements of the study and provided written informed consent for their involvement.

Table 4.1: Participant characteristics of the study cohort. Data are presented as mean ± SD.

Characteristic	Values
Age (years)	30 ± 10
Height (m)	1.808 ± 0.060
Body Mass (kg)	78.7 ± 7.0
Body Mass Index ($kg \cdot m^{-2}$)	24.1 ± 2.7
Resting Metabolic Rate ($kcal \cdot day^{-1}$)	1724 ± 314
Chronotype [1]	03:42 ± 01:13
Horne-Östberg Score	57 ± 11
Pittsburgh Sleep Quality Index	3 ± 2

[1] determined from Munich Chronotype Questionnaire

4.2.2 – Experimental Protocol

In the week leading-up to the data collection participants adhered to a strict routine of feeding and sleeping. Specifically, they were required to wake between 06:00 and 07:00 and go to bed between 22:00 and 23:00, which was confirmed using time-stamped voicemails. Furthermore, each day participants were required to expose themselves to at least 15 minutes of natural light within 1.5 hours of waking, compliance with which was affirmed by wrist actigraphy (Actiwatch^{TM}, Cambridge Neurotechnology; Cambridge, UK). Self-selected meals were also scheduled at 08:00, 12:00 and 18:00, with designated snacking opportunities at 10:00, 15:00 and 20:00. For the final two days of this standardisation period, participants were required to complete a weighed record of their food and fluid intake.

Following this, participants reported to the laboratory the evening prior to the scheduled 24-hour measurement window to acclimatise to the laboratory environment. All laboratory conditions were standardised for the duration of their stay, with blackout blinds to prevent the penetration of natural light and room temperature maintained at 20-25^{o}C. Artificial lighting was set at 800 lux in the direction of gaze during waking hours (07:00-22:00) and turned off (0 lux) during sleeping hours (22:00-07:00), for which participants were asked to wear an eye mask. Participants remained in a semi-recumbent position throughout. Upon arrival, participants were shown to their bed and provided with a prescribed meal composed of a baked potato with butter and cheese, steamed vegetables and a bowl of fresh mixed berries (1245 kcal; 31% carbohydrate, 50% fat, 19% protein). A snack of instant hot chocolate made with whole milk was then provided at 21:30 (242 kcal; 56% carbohydrate, 24% fat, 20% protein) before lights out at 22:00.

Participants were woken at 07:00 and resting metabolic rate was immediately measured over 15 minutes via indirect calorimetry before an intravenous cannula was fitted. From 08:00 onwards, at hourly intervals throughout waking periods participants completed a set of VAS and a 10 ml blood sample was drawn. After each set of measurements, an hourly feeding was also ingested in the form of a nutritionally-balanced meal-replacement shake (1.25 $kcal \cdot ml^{-1}$, 45% carbohydrate, 25% fat, 30% protein; Resource Protein, Nestlé; Vevey, Switzerland). Hourly doses were prescribed to give 6.66% of measured resting metabolic rate, thus resulting in energy balance for the 24-hour sampling period. Water was consumed *ad libitum* and participants had access to mobile devices, on-demand entertainment, music and reading material throughout waking hours only. Toilet and shower breaks were permitted in the first half of each hour as required.

At 21:15, participants were asked to ready themselves for bed before the final set of waking measurements were collected at 22:00 along with ingestion of the final prescribed feed. Following this, the lights were switched off and participants were asked to sleep with the eye mask on. Blood samples continued throughout the night at hourly intervals using a portable lamp as the participant slept. At 07:00, participants were once again awoken and immediately completed a set of VAS before a blood sample was drawn. They were then permitted a bathroom break to prepare for the day with the final set of measurements following at 08:00.

It should be noted that, in accordance with the wider objectives of the study (Loizides-Mangold *et al.*, 2017; Perrin *et al.*, 2018), muscle biopsies were collected at 4-hourly intervals from 12:00 until 08:00 (i.e. 6 in total). These were taken under local anaesthetic from the *vastus lateralis* using the Bergström method (Bergström, 1962; Tarnopolsky *et al.*, 2011). For both night-time samples (i.e. 00:00 and 04:00) participants were woken and dim lighting (200 lux) was permitted but participants continued to wear the eye mask. Each biopsy took ~10 minutes and was taken following the VAS and blood sample but before the prescribed feed in all cases.

4.2.3 – Outcome Measures

Subjective Appetite Ratings – VAS were obtained every hour, on the hour during waking periods to assess subjective appetite ratings. They featured eight scales to assess hunger, desire to eat, fullness, thirst and food preference (sugary, salty, savoury and fatty). Each scale presented a question (e.g. how hungry do you feel?), which participants answered by placing a vertical line on a 100 mm scale to denote their perception relative to the extremes, which were defined as 'not at all/very low' to 'extremely/very high'.

Blood Sampling and Analysis – Blood samples were drawn every hour on the hour throughout both the waking and sleeping periods. On each occasion, 10 ml was drawn and distributed between lithium heparin, serum, and EDTA-coated tubes. The lithium heparin and EDTA tubes were both immediately centrifuged for 10 minutes (3466 x g, 4^{o}C), after which the supernatants were removed and stored at -80^{o}C. The serum tube was left to stand for 30 minutes at room temperature before being centrifuged and stored in the same way.

Plasma melatonin concentration was measured at hourly intervals in the heparinised samples using a radioimmunoassay (Surrey Assays Ltd). Unacylated ghrelin (SPI-Bio) and leptin concentration (R&D Systems) were quantified throughout the protocol at 4-hourly intervals starting at 08:00 (i.e. 7 samples total) using the EDTA plasma samples and commercially-available assays.

4.2.4 – Statistical Analysis

Analysis of rhythmicity in all outcome measures was conducted using the cosine method (Prism 7, Graphpad; CA, USA). In this approach a cosine wave is applied to the dataset with the optimal fit established and compared against a horizontal line through the mean values (null). If a significant difference in amplitude is detected between the cosine and horizontal lines then the dataset characterises circadian rhythmicity, with the mesor (rhythm-adjusted mean), amplitude (magnitude of the difference between mesor and peak/trough values) and acrophase (timing of rhythmic peak) all identified and reported (Refinetti, Lissen and Halberg, 2007; Cornelissen,

2014). Due to the high inter-individual variability in leptin and ghrelin concentration, values were normalised to give a percentage of the 24-hour mean for each participant (Spiegel *et al.*, 2011; Mäntele *et al.*, 2012). The threshold for determining dim light melatonin onset (DLMO) was set at 25% of peak concentration (Benloucif *et al.*, 2008; Sletten *et al.*, 2009), with the precise crossing point within hourly time bins interpolated from linear regression. Where a comparison of two means was required, a paired t-test or a Wilcoxon signed rank test was performed depending on an assessment of normality (SPSS Statistics 23.0, IBM; NY, USA). Significance was accepted at $p \leq 0.05$. All data are presented as mean ± SD unless otherwise stated.

4.3 – Results

4.3.1 – Melatonin

The plasma melatonin rhythm throughout the 24-hour measurement period is shown in **Figure 4.1**. Concentrations remain low throughout waking hours but increase rapidly following lights-out at 22:00. The DLMO when averaged across participants occurred at 23:09 ± 00:43, providing a marker of the circadian cycle. The 24-hour rhythm characterised diurnal variations as verified by cosinor analysis (p<0.01), with the acrophase occurring at 03:59 ± 00:54 and an amplitude of 26.6 ± 6.3 pg·ml^{-1}.

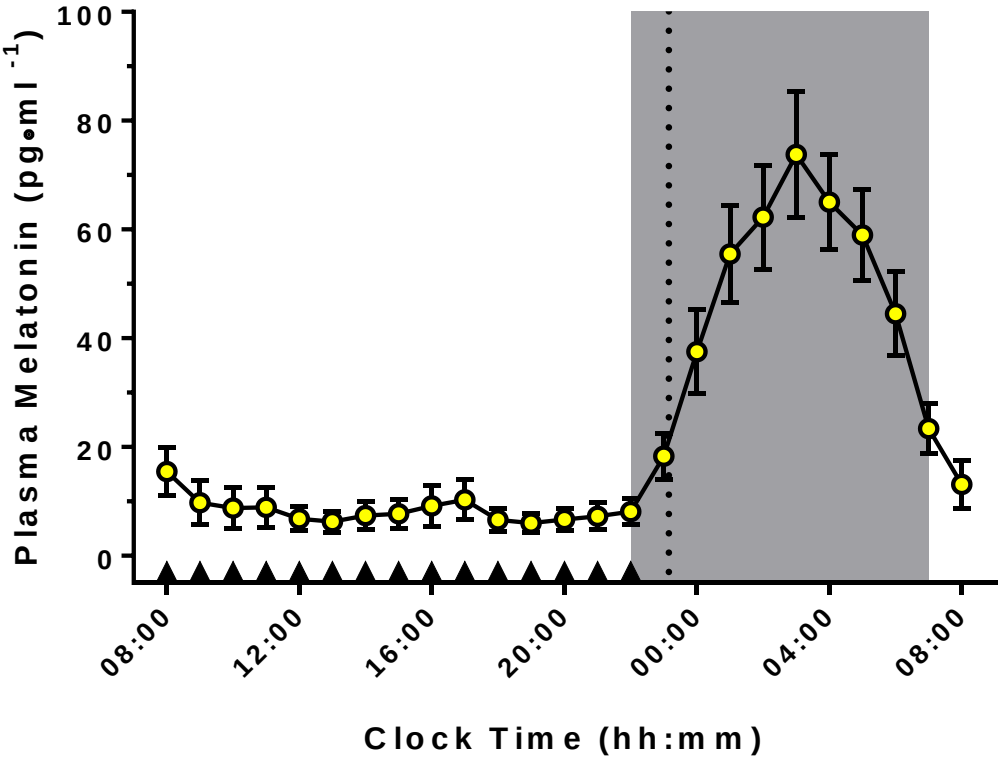

Figure 4.1 – 24-hour profile for plasma melatonin under conditions of semi-constant routine. Values are presented as mean ± SEM. The shaded area represents the 9-hour sleep window (0 lux), the black triangles denote hourly feedings, and the dotted line on the x-axis represents the DLMO.

4.3.2 – Subjective Ratings of Appetite

As shown in **Table 4.2**, diurnal rhythms were established in all dimensions of subjective appetite except for sweet preference. Interestingly, hunger and prospective consumption both oscillated around the centre of the scale, whilst ratings of fullness tended to be lower throughout the 24-hour period. Conversely, rhythms in desire to eat savoury foods returned both the highest mesor and amplitude.

Table 4.2: Mesor, amplitude and acrophase for diurnal rhythms in subjective appetite ratings. Data are shown as mean ± SD.

Scale	Mesor (mm)	Amplitude (mm)	Acrophase (hh:mm)	*p*-value
Hunger	50 ± 6	6 ± 6	20:44 ± 05:47	**<0.01**
Fullness	30 ± 5	7 ± 8	12:05 ± 03:26	**0.01**
Prospective Consumption	55 ± 6	5 ± 6	19:34 ± 07:19	**0.03**
Sweet Preference	48 ± 6	N/A	N/A	0.33
Salty Preference	45 ± 7	7 ± 6	19:45 ± 05:35	**<0.01**
Savoury Preference	63 ± 7	9 ± 7	20:37 ± 04:31	**<0.01**
Fatty Preference	44 ± 8	8 ± 8	21:13 ± 06:07	**0.01**

p-values denote the statistical comparison of amplitude between null and cosinor waves

Shifting to focus on the temporal rhythms in these subjective ratings, both hunger and prospective consumption characterised similar phase relationships, reaching their peak in the evening before falling to their nadirs shortly after waking (**Figures 4.2 and 4.3**). This pattern was mirrored in the desire to eat certain food types, with salty, savoury **(Figure 4.4)** and fatty preference all peaking within a 2-hour window shortly before lights out. Accordingly, fullness characterised a broadly antiphasic rhythm (**Figure 4.5**), peaking shortly after midday and falling to a trough after sleep onset, whilst sweet preference was not rhythmic (**Figure 4.6**)

It is also interesting to note that the 08:00 ratings of appetite were all higher at the end of the 24-hour period relative to the beginning, whilst fullness tended to be lower. These differences were significant for hunger (p=0.04), prospective consumption (p=0.03) and desire to eat savoury foods (p=0.03), approached significance for desire to eat fatty (p=0.06), sweet (p=0.08) and salty (p=0.08) foods, and were not significant for fullness (p=0.12). This may be related to the apparent suppression of appetite seen from 08:00-12:00 in **Figures 4.2** to **4.6**.

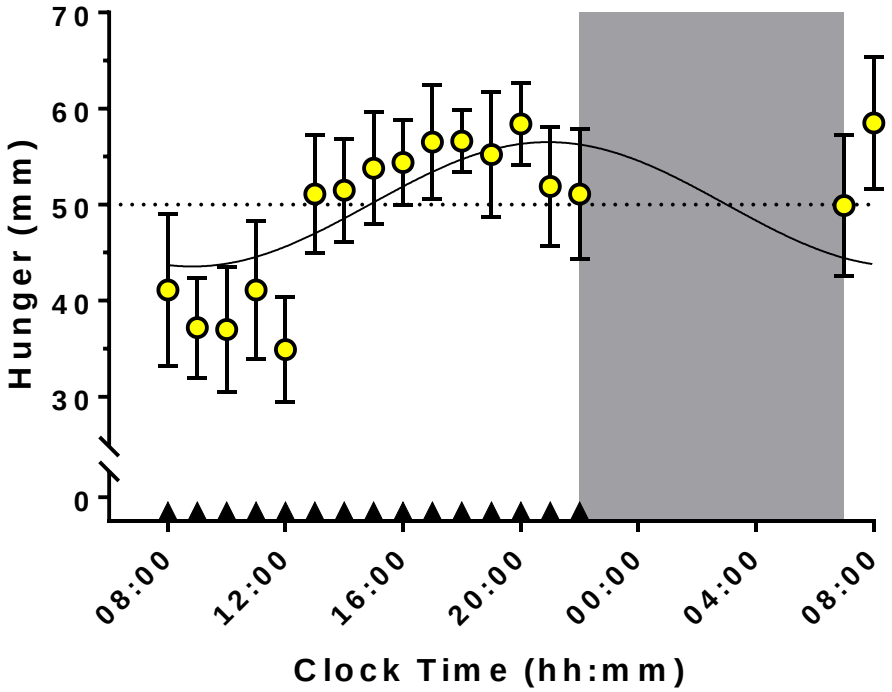

Figure 4.2: 24-hour profile for subjective ratings of hunger, as measured by VAS throughout waking periods. Values are presented as mean ± SEM. The solid line denotes the regression that best fits the data and the dotted horizontal line shows the 24-hour mean concentration used for the null comparison. The black triangles denote hourly feedings and the shaded area represents the 9-hour sleep window (0 lux).

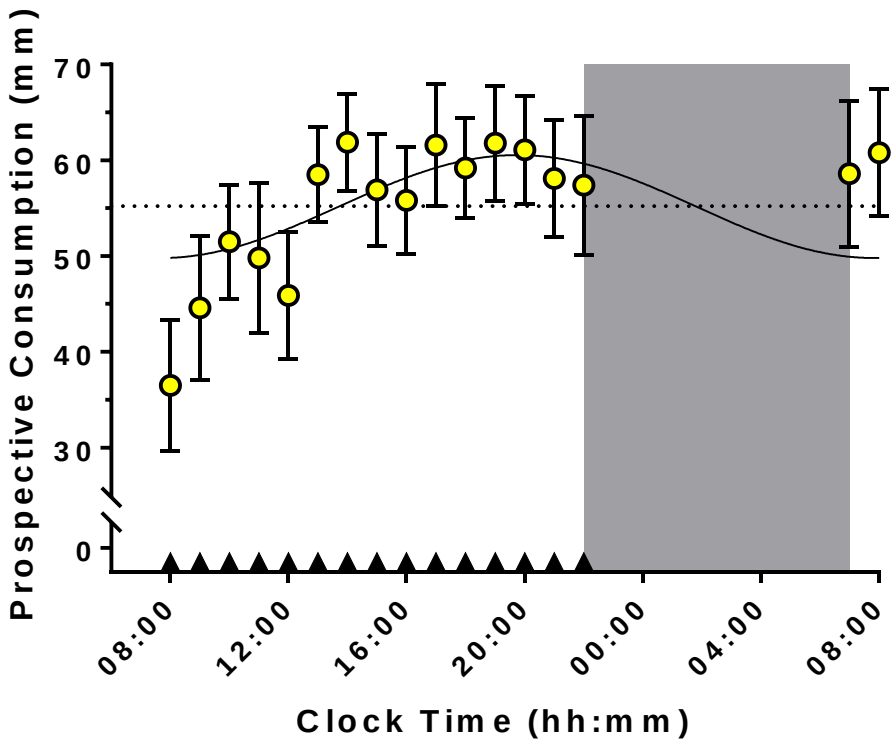

Figure 4.3: 24-hour profile for subjective ratings of prospective consumption, as measured by VAS throughout waking periods. Values are presented as mean ± SEM. The solid line denotes the regression that best fits the data and the dotted horizontal line shows the 24-hour mean concentration used for the null comparison. The black triangles denote hourly feedings and the shaded area represents the 9-hour sleep window (0 lux).

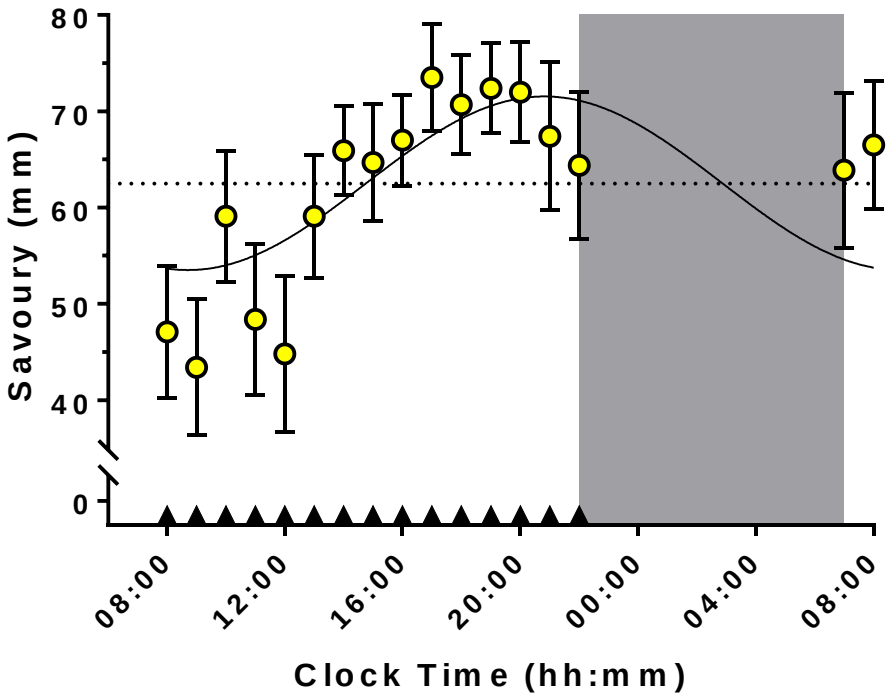

Figure 4.4: 24-hour profile for subjective ratings of desire to consume savoury foods, as measured by VAS throughout waking periods. Values are presented as mean ± SEM. The solid line denotes the regression that best fits the data and the dotted horizontal line shows the 24-hour mean concentration used for the null comparison. The black triangles denote hourly feedings and the shaded area represents the 9-hour sleep window (0 lux).

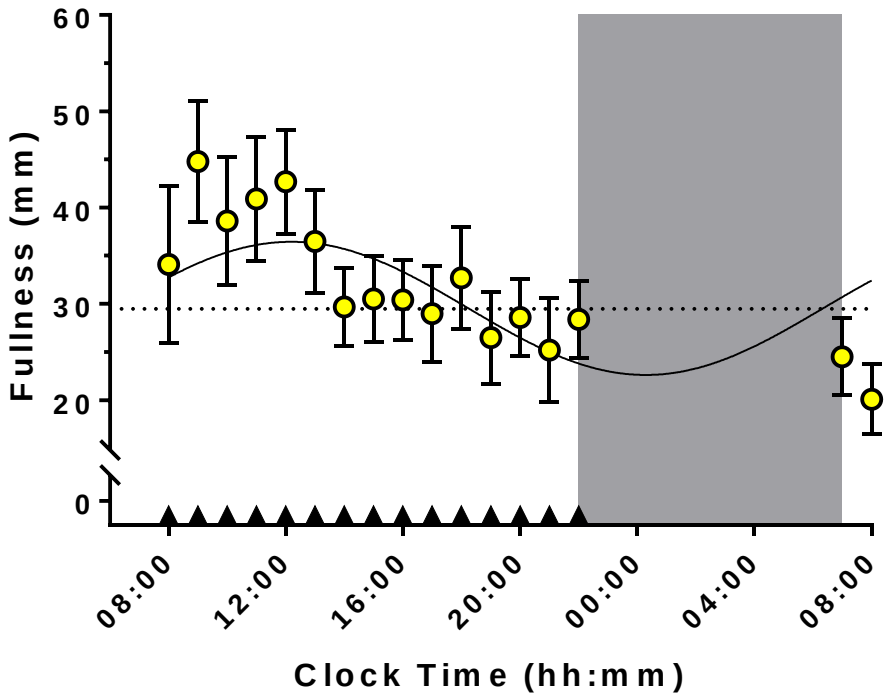

Figure 4.5: 24-hour profile for subjective ratings of fullness, as measured by VAS throughout waking periods. Values are presented as mean ± SEM. The solid line denotes the regression that best fits the data and the dotted horizontal line shows the 24-hour mean concentration used for the null comparison. The black triangles denote hourly feedings and the shaded area represents the 9-hour sleep window (0 lux).

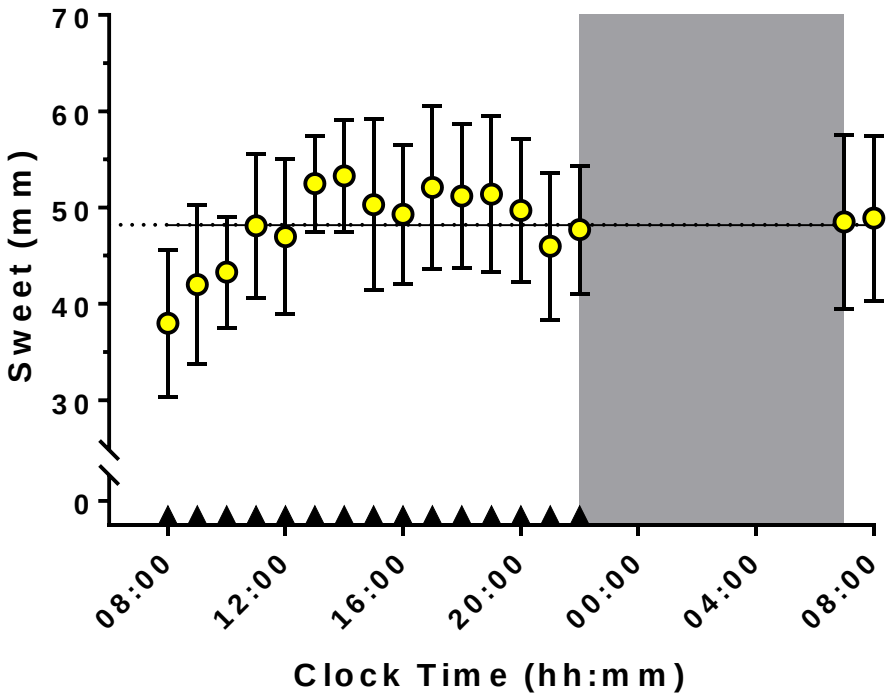

Figure 4.6: 24-hour profile for subjective ratings of desire to consume sweet foods, as measured by VAS throughout waking periods. Values are presented as mean ± SEM. The solid line denotes the regression that best fits the data and the dotted horizontal line shows the 24-hour mean concentration used for the null comparison. The black triangles denote hourly feedings and the shaded area represents the 9-hour sleep window (0 lux).

4.3.3 – Unacylated Ghrelin

A diurnal rhythm in unacylated ghrelin concentration emerged (p<0.01), as shown in **Figure 4.7**. The acrophase occurred shortly after waking at 08:26 ± 03:51 and fell to the nadir in the evening with a peak-to-trough amplitude of 12 ± 11%. This provided group-averaged maximum and minimum concentrations of 41.1 ± 17.8 pg·ml^{-1} and 28.8 ± 12.2 pg·ml^{-1}, respectively. Concentrations were also lower at the end of the measurement window when compared to the beginning (start = 41.1 ± 17.8 pg·ml^{-1}, end = 35.7 ± 13.2 pg·ml^{-1}; p=0.05).

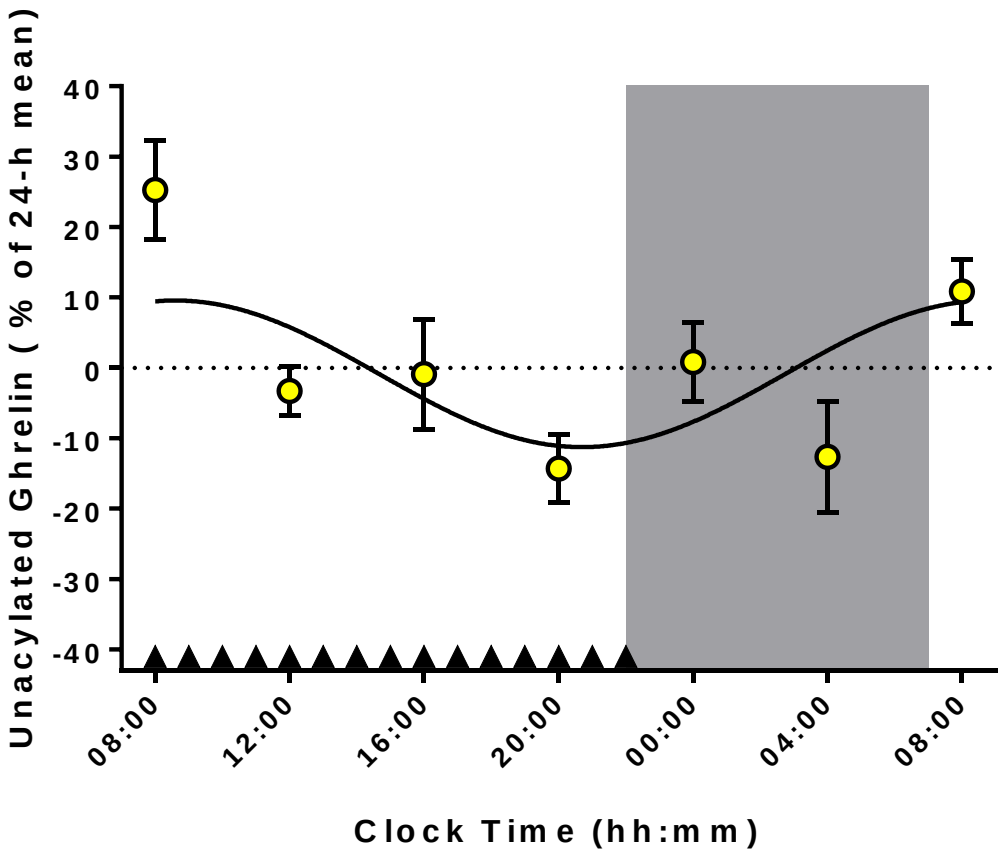

Figure 4.7: Normalised 24-hour unacylated ghrelin profile under semi-constant conditions. Values are presented as mean ± SEM. The solid line denotes the regression that best fits the data. The black triangles denote hourly feedings and the shaded area represents the 9-hour sleep window (0 lux).

4.3.4 – Leptin Profile

Plasma leptin concentration characterised a diurnal rhythm (p<0.01), as shown in **Figure 4.8**. The acrophase occurred shortly after lights out (00:35 ± 01:20) and concentrations were at their lowest at midday. The peak-to-trough amplitude for this rhythm was 25 ± 9%, with a peak concentration of 175.4 ± 277.1 pg·ml^{-1} and a minimum concentration of 127.2 ± 201.2 pg·ml^{-1} when averaged across the group. Concentrations were not different between the two 08:00 measurements (start = 163.2 ± 241.8 pg·ml^{-1}, end = 147.2 ± 215.9 pg·ml^{-1}; p=0.58).

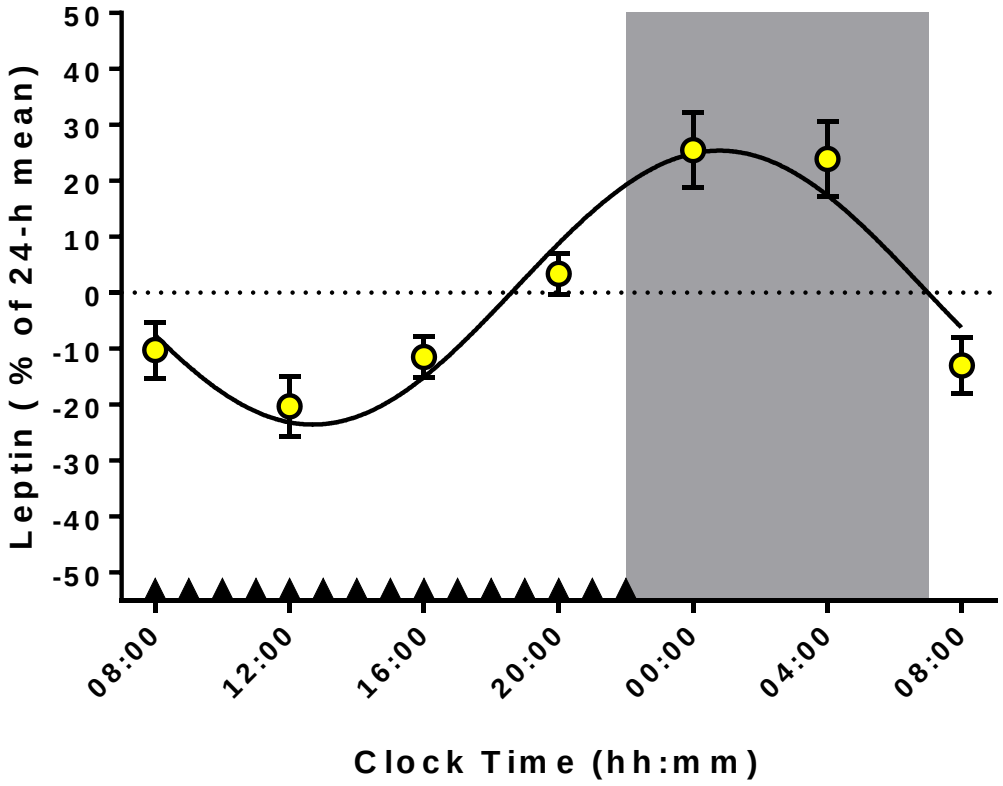

Figure 4.8: Normalised 24-hour plasma leptin profile under semi-constant conditions. Values are presented as mean ± SEM. The solid line denotes the regression that best fits the data. The black triangles denote hourly feedings and the shaded area represents the 9-hour sleep window (0 lux).

4.4 – Discussion

The aim of this study was to quantify diurnal rhythms in subjective appetite over 24-hours in response to a feeding protocol designed to suppress the associated rhythm in ghrelin concentration. With the exception of desire to consume sweet foods, all measured dimensions of subjective appetite characterised a diurnal rhythm. Hunger, prospective consumption and desire to eat savoury, salty and fatty foods all increased during waking hours to a peak in the evening, before declining overnight to a trough shortly after waking. However, there was also a tendency for these ratings to increase throughout the 24-hour measurement period, which was unexpected. Although a diurnal rhythm was also identified for unacylated ghrelin concentration, this ran in antiphase to subjective ratings of appetite, contrary to its proposed regulatory effects. Similarly, subjective ratings of fullness peaked at midday and fell to their lowest levels during sleep, despite leptin oscillating in an antiphasic manner.

This is the first study to show a 24-hour rhythm in subjective appetite in the context of diurnal behavioural influences. Ratings of hunger increased throughout the day to a peak at 20:44 before declining overnight. Despite the diurnal influences of feeding and sleep, this shows excellent agreement with the original study of Scheer, Morris and Shea (2013), wherein a circadian rhythm in hunger was established that peaked at 19:50 before falling to a trough in the biological morning. Comparable peaks in the biological evening were also apparent for prospective consumption and the desire to consume salty foods (19:10-20:30). Contrary to this, Scheer, Morris and Shea (2013) also identified an endogenous rhythm in the desire to consume sweet foods. This rhythm was not seen in the present study but this disparity is likely to reflect the sweet taste of the meal-replacement supplement used, which may have masked the underlying rhythm. A further contrast relates to subjective ratings of fullness, which Scheer, Morris and Shea (2013) suggest are not rhythmic under conditions of forced desynchrony. Although it could be argued that the diurnal rhythm in fullness in the present study is therefore a product of the behavioural cycle, Sargent *et al.* (2016) observed similarly phased rhythms in hunger, prospective consumption and fullness to the present study when using a 28-hour forced desynchrony protocol, which casts doubt over this suggestion.

Interestingly, these diurnal rhythms in subjective appetite were apparent despite an antiphasic rhythm in unacylated ghrelin concentration, in which the acrophase was identified at 08:26 before declining throughout waking hours. This is contrary to studies of continuous fasting, in which total ghrelin concentrations have been shown to increase at habitual meal times before decreasing spontaneously within 1-2 hours (Espelund *et al.*, 2005; Natalucci *et al.*, 2005). Similarly contrasting rhythms have also been observed under diurnal and forced desynchrony conditions, with the zenith and nadir reported to be in the region of 23:00-01:00 and 09:00-11:00, respectively (Cummings *et al.*, 2001; Dzaja *et al.*, 2004; McHill *et al.*, 2018). Although inadequate sample preparation meant accurate determination of acylated ghrelin concentration was not possible (Chandarana *et al.*, 2009), the 24-hour profiles of acylated and unacylated ghrelin do broadly align with one another (Spiegel *et al.*, 2011; Nass *et al.*, 2014), meaning that this is unlikely to fully explain the observed antiphasic rhythm. Consequently, this suggests that the feeding protocol was effective in modifying rhythmic ghrelin concentrations in accordance with the aims of the study.

To the knowledge of the authors, 24-hour ghrelin concentrations have not been measured under conditions of constant routine with hourly isocaloric feedings, making affirmation of this effect of feeding patterns difficult. In this sense, the study would have benefitted from the inclusion of a control trial, wherein feeding could have followed a conventional meal pattern as opposed to hourly feeds. This would have allowed a direct evaluation of how the present feeding strategy influenced key outcomes and should therefore be incorporated in ensuing studies. However, given that decreasing postprandial suppression of ghrelin is believed to drive diurnal rhythms (Cummings *et al.*, 2001), it is conceivable that a steadier influx of nutrients throughout the day could lead to continued suppression of ghrelin secretion by providing sustained increases in plasma metabolites known to inhibit its release (Möhlig *et al.*, 2002; Greenman *et al.*, 2004; Sato *et al.*, 2012). Whilst this is speculative, experimental data emerging from studies of meal frequency do lend credence to this notion. For instance, over an 11-hour period, Leidy *et al.* (2010) observed that when energy-matched diets were consumed as either six or three equally spaced meals, more frequent feeding eliminated the eating-related oscillations in acylated ghrelin. Similarly, Solomon *et al.* (2008) showed that consuming an isocaloric diet through two large meals resulted in

more profound peaks and troughs in ghrelin concentration when compared to consuming the same diet as 12 equally spaced boluses (Leidy and Campbell, 2011).

Leptin was also found to exhibit a diurnal oscillation in the present study, peaking at 00:35 and declining to its lowest levels at midday. Once again, this is seemingly misaligned with diurnal rhythms in hunger and fullness considering its proposed role in inducing satiety (Baicy *et al.*, 2007; Klok, Jakobsdottir and Drent, 2007), but the observed rhythm is well-supported by the prior literature (Simon *et al.*, 1998; Westerterp-Plantenga *et al.*, 2016). Under conditions of forced desynchrony, Scheer *et al.* (2009) established that leptin rhythms track the behavioural rather than the circadian phase, rising throughout waking hours from a trough prior to breakfast to a peak at the onset of sleep, several hours after the final meal. This was also affirmed by a constant routine protocol from the same group (Shea *et al.*, 2005), in which leptin concentrations rose systematically throughout 38 hours of constant wakefulness with hourly isocaloric feedings. The study of Schoeller *et al.* (1997) suggests that this rhythm is primarily driven by meal timing, with a 6-hour phase shift in the leptin rhythm occurring in response to a 6.5-hour delay in meal times. Collectively, the present study therefore affirms the conclusions of Poggiogalle, Jamshed and Peterson (2018), who argue that hormones implicated in energy balance track feeding behaviour, whilst subjective ratings of appetite are regulated by the SCN.

An intriguing contrast with these studies however, is the slight delay in the timing of the nadir in the leptin rhythm in the present study, which occurred at midday rather than breakfast. This is particularly surprising when comparing against the study of Mäntele *et al.* (2012), who employed in an essentially identical schedule of sleeping and feeding, as emphasised by the similar DLMO. However, this discrepancy may be explained by residual effects of the meal provided during the acclimatisation period. This is likely to have been larger than average for most participants (~1500 kcal) and came following a day of habitual intake, which may have led to an energy surplus at sleep onset given that physical activity was constrained. If the rhythm in leptin reflects energy balance, as suggested by Schoeller *et al.* (1997) and the abolition of the leptin rhythm during short-term fasting (Dallongeville *et al.*, 1998), then this surplus at sleep onset could have led to a phase delay in the nocturnal peak and the morning nadir. This may also explain why ratings of appetite appeared to increase during the 24-hour

measurement period, with the residual effects of this large meal lowering the initial ratings when compared to the 300-600 kcal consumed within the same timeframe the following night. Although this is an important consideration, such a carryover effect is not likely to have caused the observed rhythms in subjective appetite given the concordance with prior literature. Furthermore, such an effect was also equally apparent in ratings of sweet preference, which remained non-rhythmic despite this influence.

Taken together, these findings show that subjective appetite characterises a 24-hour rhythm in the context of diurnal behavioural influences, being lower in the morning and higher in the evening. Moreover, sensations of hunger do not appear to be modified by acute changes in feeding pattern, despite apparent inversion of the rhythm in key regulatory peptides. This reinforces the proposed rhythm in, and central regulation of, subjective hunger reported by Scheer, Morris and Shea (2013), and argues that these rhythms in perceived appetite provide a robust foundation upon which time-related dietary interventions can be structured. However, there remains a definite need to explore the interplay between subjective ratings of appetite and a broader range of proposed regulatory peptides under different feeding conditions, to better understand the regulatory processes.

Chapter 5: Impact of intermittent fasting on energy balance and associated health outcomes in lean adults.

5.1 – Introduction

Intermittent fasting is a dietary strategy in which typical patterns of food and drink consumption are punctuated by scheduled periods of energy restriction or abstinence from all energy-providing nutrients. The objective of this is often to create a net reduction in energy intake, and work from various laboratories consistently shows that such approaches are associated with reductions in body mass and improvements in a range of fasted health markers (Johnson *et al.*, 2007; Varady, Bhutani, *et al.*, 2009; Varady, Hudak and Hellerstein, 2009; Bhutani *et al.*, 2010). These responses are usually similar in magnitude to those observed when simply restricting calorie intake across all eating occasions, wherein a typical eating pattern is largely maintained (Larson-Meyer *et al.*, 2006; Pi-Sunyer *et al.*, 2007; Harvie *et al.*, 2011; Varady, 2011; Barnosky *et al.*, 2014; Ravussin *et al.*, 2015; Catenacci *et al.*, 2016; Headland *et al.*, 2016; Trepanowski, Kroeger, Barnosky, Klempel, Bhutani, Hoddy, Gabel, *et al.*, 2017). However, current understanding of precisely how intermittent fasting may affect human health and metabolism is far from complete.

While the reductions in body mass reported in prior studies of intermittent fasting reflect a state of negative energy balance, the changes that occur within the components of energy balance have not been well-characterised. This is of importance because prolonged periods of daily calorie restriction can elicit compensatory reductions in energy expenditure that undermine the resultant weight loss (Heilbronn *et al.*, 2006; Rosenbaum, Sy, *et al.*, 2008; Redman *et al.*, 2009; Martin *et al.*, 2011; Kissileff *et al.*, 2012). However, there is currently no evidence to suggest that this adaptive response is invoked by intermittent fasting strategies. Reductions in leptin concentration, a proposed hormonal regulator of energy balance (Mantzoros *et al.*, 2011), have been shown to be similar with intermittent fasting and daily calorie restriction (Johnson *et al.*, 2007; Bhutani *et al.*, 2010; Harvie *et al.*, 2011; Varady *et al.*, 2013). However, this does not seem to translate into measurable changes within

the specific components of energy expenditure, with studies failing to detect corresponding reductions in resting metabolic rate (Heilbronn, Smith, *et al.*, 2005) and physical activity (Klempel *et al.*, 2010, 2012; Varady *et al.*, 2016). Although promising, these studies have focused on isolated aspects of energy expenditure, which miss the additive effect of metabolic and behavioural adaptations to energy restriction (Spiegelman and Flier, 2001; Redman *et al.*, 2009; Hill, Wyatt and Peters, 2012; Muller *et al.*, 2015). Furthermore, physical activity has generally been quantified using pedometers and accelerometers, which may lack the sensitivity to detect the subtle modifications in activity that accompany changes in feeding patterns (Crouter *et al.*, 2003; Plasqui and Westerterp, 2007; Betts *et al.*, 2016; Jeran, Steinbrecher and Pischon, 2016).

Beyond this, reductions in fasted insulin concentration have been consistently observed in response to various intermittent fasting interventions, as reviewed by Barnosky *et al.* (2014). The authors also highlight that a subset of the studies reviewed report that fasted indices of insulin sensitivity generally increase following a period of intermittent fasting. However, it is important to note that these fasted measurements can overlook changes in glucose metabolism under dynamic conditions (Borai *et al.*, 2011). To date, few studies have examined postprandial effects, and those that have reveal equivocal findings: with some showing favourable effects (Halberg *et al.*, 2005; Antoni, Johnston, *et al.*, 2018), others no effect (Soeters *et al.*, 2009), and others a sexual dimorphism (Heilbronn, Civitarese, *et al.*, 2005). Consequently, the effects of intermittent fasting on postprandial metabolism remain unclear, which is important given that individuals in Western cultures typically spend the majority of their waking time in a postprandial state (Travers *et al.*, 2017). Further to this, there has been little exploration of how such diets interact with the second-meal effect, which compounds the issue (Silva *et al.*, 2005; Gonzalez, 2014; Antoni, Johnston, *et al.*, 2018).

In addition, isolating the distinct effects of fasting relative to negative energy balance or weight-loss *per se* is a key question to address. It has been suggested that temporal modifications in energy intake exert direct influences on metabolic health, which are independent of any net effect on energy balance (Johnston, 2014; Mattson *et al.*, 2014; Bandín *et al.*, 2015; St-Onge *et al.*, 2017; Anton *et al.*, 2018). This means that feeding times could constitute a novel dimension of what is considered a healthy diet.

Therefore, although the limited body of prior literature offers promising insights as to the efficacy of intermittent fasting in improving metabolic health, numerous uncertainties remain. For this reason, the present study will employ a complete alternate-day fasting intervention, as defined by Patterson and Sears (2017), to explore the therapeutic potential of intermittent fasting by addressing three core objectives:

1. To establish whether hypocaloric intermittent fasting elicits compensatory changes in the components of energy balance, and to compare these against the changes arising from an energy-matched daily calorie restriction intervention.

2. To examine the effect of hypocaloric intermittent fasting on postprandial metabolism relative to an energy-matched daily calorie restriction intervention.

3. To explore whether hypocaloric intermittent fasting affects the components of energy balance or postprandial metabolism independently from chronic energy imbalance, by contrasting against a eucaloric intermittent fasting intervention.

Focusing initially upon energy expenditure, it was hypothesised that resting metabolic rate and postprandial thermogenesis would be unaffected by the two intermittent fasting conditions (Heilbronn, Smith, *et al.*, 2005; Byrne *et al.*, 2018) but reduced by the daily calorie restriction diet (Rosenbaum, Hirsch, *et al.*, 2008; Muller *et al.*, 2015). Conversely, based on the observations of Betts and colleagues, it was anticipated that the intermittent fasting conditions would reduce physical activity thermogenesis to a similar extent as the daily calorie restriction treatment (Martin *et al.*, 2011; Betts *et al.*, 2016). In terms of the effects upon postprandial metabolism, with the enhanced opportunity for metabolism of lipid-derived substrates offered by intermittent fasting (Soeters *et al.*, 2012; Anton *et al.*, 2018), it was expected that this would result in greater reductions in insulinaemia and glycaemia in the hypocaloric intermittent fasting group when compared to the other two groups (Halberg *et al.*, 2005; Heilbronn, Civitarese, *et al.*, 2005).

5.2 – Methods

This randomised–controlled trial sought to establish the effects of intermittent fasting on energy expenditure and postprandial metabolism in a cohort of lean adults, utilising a complete alternate-day approach. Briefly, participants completed a four-week control phase to provide data on habitual dietary intake and activity patterns. Once completed, they were then randomised to one of three conditions for 20 days: daily calorie restriction (75% of habitual intake daily), intermittent fasting with calorie restriction (alternating 24-hour periods of fasting and feeding to 150% of habitual intake) or intermittent fasting without calorie restriction (alternating 24-hour periods of fasting and feeding to 200% of habitual intake). Monitoring of dietary intake and physical activity continued throughout the intervention, whilst laboratory visits before and after evaluated body composition, metabolic rate and postprandial responses to sequential meals. The protocol for this study (detailed below) was approved by the NHS Research Ethics Committee (reference: 15/SW/0007).

5.2.1 – Participants

To address the objectives if this study, 36 lean adults were recruited including both men and women. Participants were initially classified as lean based on body mass index (BMI; 20.5-25.0 $kg \cdot m^{-2}$), which was subsequently confirmed using fat mass index (FMI) obtained from a dual-energy x-ray absorptiometry (DEXA) scan. Values of $\leq$7.5 $kg \cdot m^{-2}$ and $\leq$11.0 $kg \cdot m^{-2}$ were classified as lean for males and females, respectively. Further eligibility criteria for this study are listed below.

Inclusion: 1. Aged 18-65 years; 2. Stable body mass (±3 kg) for at least 6 months; 3. Able and willing to comply with study procedures; 4. Willing to undertake required fasting durations; 5. Have the capacity to provide informed consent.

Exclusion: 1. Body mass >120 kg; 2. Recent or planned engagement in fasting practices (within 3 months of start date); 3. Recent or planned change in diet/physical activity habits; 4. Suffering from an eating disorder as assessed using the EDE-Q 6.0 (Fairburn, 2008); 5. Have been diagnosed with diabetes or other metabolic health disturbances; 6. Ongoing medical condition or treatment which may interfere with

study variables; 7. Menopausal; 8. Pregnant, recently pregnant, planning to become pregnant (within 3 months) or currently breastfeeding; 9. Having donated blood within the last 3 months; 10. Lack of capacity/language skills to independently follow the protocol; 11. Unable to consume test meals due to intolerances/dietary preferences (i.e. vegan, gluten, milk proteins); 12. Any other behaviour or condition which introduces bias to the experiment or poses undue personal risk.

Power Calculation – The sample size for this study was estimated using *a priori* power analysis of studies employing similar durations of daily calorie restriction and considered a range of the outcome measures in question. Specifically, Friedlander *et al.* (2005) observed a decline in resting metabolic rate following 21 days of daily calorie restriction (pre = 1898 ± 262 $kcal \cdot day^{-1}$, post = 1670 ± 203 $kcal \cdot day^{-1}$). Applying a two-tailed *t*-test to these data suggested that 11 participants would be required to achieve 80% power when detecting such an effect at an alpha level of 0.05. Focusing instead on changes in postprandial glucose metabolism, Molfino *et al.* (2010) reported a reduction in 2-hour post-bolus plasma glucose concentrations during an oral glucose tolerance test following 10 days of daily calorie restriction (pre = 10.72 ± 3.56 $mmol \cdot l^{-1}$, post = 7.10 ± 2.96 $mmol \cdot l^{-1}$). In this instance 9 participants were deemed necessary to detect a comparable within-treatment effect with 80% power at an alpha level of 0.05. Collectively, this suggested that 9-11 participants were required per group to establish within–treatment effects in the primary outcomes. However, given the potential for variability in the outcomes of interest, a more conservative requirement of 12 per group was decided upon. Recruitment proceeded on a rolling basis until the desired sample size was attained. To minimise loss to follow-up, emphasis was placed on considering the demands of the study before enrolling. In cases of withdrawal, additional participants were sought to maintain statistical power.

5.2.2 – Experimental Protocol

Overview – Following the provision of written informed consent, eligibility was assessed using a series of self–report questionnaires together with a BMI calculation. Eligible participants then undertook the 8-week protocol shown in **Figure 5.1a**. For all laboratory sessions, participants were required to avoid caffeine, alcohol, smoking and strenuous exercise throughout the preceding 24 hours, whilst also standardising

their dietary intake on a within-participant basis. Following an overnight fast (minimum 10 hours), participants reported to the laboratory at 07:30 ± 01:00 having consumed 500 ml of water upon waking. For female participants, laboratory sessions were scheduled to coincide with the follicular phase of their menstrual cycle (i.e. 3-10 days after onset of menses) to account for the associated changes.

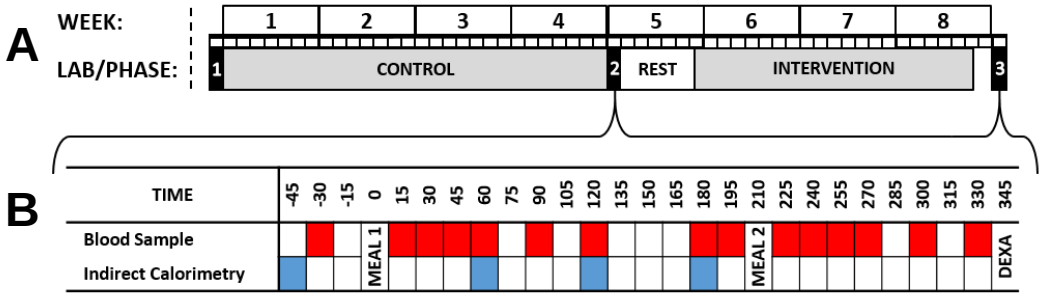

Figure 5.1: Schematic of the 8-week study design (A) and the sampling intervals for laboratory sessions 2 and 3 (B). *Abbreviations: DEXA = Dual Energy X-ray Absorptiometry.*

LAB 1 (Baseline) – The initial lab session provided a reference point for examining the stability of body mass, as an indicator of overall energy balance, throughout the ensuing 4-week control phase in which habitual dietary intake and physical activity were quantified. In addition, this visit served to familiarise participants with key procedures to improve reliability over subsequent laboratory sessions, particularly measurements of resting metabolic rate (Soares *et al.*, 1989; Alam *et al.*, 2005). A urine sample was collected when voiding prior to measurements of height and body mass to ensure adequate hydration for these measurements (i.e. urine specific gravity <1.020). Following a 20-minute rest in a semi-recumbent position, resting metabolic rate and substrate oxidation were then measured via indirect calorimetry of expired air samples, after which a fasted blood sample was drawn. To conclude this session, a submaximal treadmill protocol was undertaken to individually calibrate the physical activity monitors being used throughout the study (Actiheart^{TM}, Cambridge Neurotechnology; Cambridge, UK). Before departing, participants were given the materials to capture free-living measurements of dietary intake and physical activity.

Control Phase – During this phase, both energy intake and physical activity energy expenditure were measured concurrently in four designated monitoring windows of three consecutive days each. Each of these windows was separated by at least two days from any other and the final window covered the three days leading up to LAB 2, to ensure compliance with standardisation procedures. Physical activity energy expenditure and intensity were measured using individually–calibrated Actiheart^{TM} monitors (Brage *et al.*, 2005, 2007), whilst energy intake was measured by means of a weighed record of food and fluid intake. To proceed into the intervention phase, participants were required to maintain energy balance throughout the control period and provide accompanying measurements of energy intake and expenditure. Energy balance was ascertained by maintaining a stable body weight (≤1.0 kg increase or decrease) between LAB 1 and LAB 2 (Betts *et al.*, 2014; Chow and Hall, 2014).

LAB 2 (Pre-Intervention) – Having completed the control phase, participants returned to the laboratory for measurement of a series of fasted and postprandial outcomes. Once again, adequate hydration status was ascertained prior to measuring body mass. Fasting measurements of resting metabolic rate and substrate oxidation were then obtained before an intravenous cannula was fitted. At this stage, a venous blood sample was also drawn to provide fasted concentrations of relevant metabolites and hormones. This was then followed by two sequential mixed-macronutrient meal tests: a homogenous porridge meal (meal 1) and a meal-replacement shake (meal 2). The first featured indirect calorimetry measurements from expired gases and venous blood samples, whereas the second only involved sampling of arterialised–venous blood. **Figure 5.1b** shows the sampling intervals for these measures. To conclude, a DEXA scan was used to quantify fat mass, lean mass and visceral adipose tissue mass.

Diet Allocation – During a 6-day rest period following LAB 2, which was included to avoid prolonged periods of diet monitoring (Tucker, 2007) and maintain the 4-week testing interval, participants were randomised to one of three parallel intervention arms (**Table 5.1**) in a 1:1:1 allocation ratio using a stratified randomisation scheme. This featured a factor for objectively-measured physical activity level (PAL) to ensure an even distribution of lower activity (PAL: <1.75) and higher activity (PAL: ≥1.75) participants in each condition. In accordance with best practice recommendations, allocation concealment was employed to minimise bias (Clark, Fairhurst and

Torgerson, 2016). The randomisation scheme, including block sizes and sequences, was generated by a senior author who was not involved in participant management and was concealed from those that were (Schulz and Grimes, 2002; Schulz *et al.*, 2002). Participant assignment was requested via email by providing the participant code, BMI/FMI and PAL. As only the delivery team knew which code corresponded to which participant, and only the senior author knew the block sizes and sequence, it was not possible for the allocation to be reliably predicted.

Table 5.1: Intervention arms employed in the study protocol

Intervention	Description
Daily calorie restriction (75:75)	Reduce normal intake by 25% every day
Intermittent fasting with calorie restriction (0:150)	Alternate between 24-hour periods of fasting and feeding (transitions at 15:00), with 150% of normal intake on fed days.
Intermittent fasting without calorie restriction (0:200)	Alternate between 24-hour periods of fasting and feeding, (transitions at 15:00), with 200% of normal intake on fed days.

Intervention Phase – The three diets listed in **Table 5.1** each lasted for 20 days, with transitions between 24-hour cycles occurring at 15:00 each day. This transition point was identified as being optimal for the two intermittent fasting conditions based on the observed diurnal rhythms in subjective appetite established in Chapter 4. Specifically, it emerged that both hunger and prospective consumption were at their lowest in the morning and increased throughout the day to peak in the evening before falling overnight. Consequently, in bisecting the upward slope from nadir to zenith, a transition from fasting to feeding and *vice versa* at 15:00 ensured that food provision was proximal to periods of rhythmically increased hunger, whilst aligning the latter elements of the fast with periods of rhythmically reduced hunger. Furthermore, this transition allowed the consumption of at least one main meal per day to aid motivation, as advocated by Varady, Bhutani, *et al.* (2009), whilst also retaining a 24-hour fasting period. This is around double that achieved in most prior studies of intermittent fasting (Barnosky *et al.*, 2014) and matches the longest reported in studies of complete alternate-day fasting (Heilbronn, Smith, *et al.*, 2005). Lastly, this point also broadly aligns with the middle of the waking day, based on respective wake and sleep times of 06:00-08:00 and 22:00-00:00 (Betts *et al.*, 2014; Chowdhury *et al.*, 2016).

For the intermittent fasting groups, during fasted cycles participants were only permitted water, herbal teas and black tea/coffee with no sugar (i.e. unsweetened energy-free drinks). During feeding cycles, and throughout the daily calorie restriction intervention, participants were asked to appropriately modify their normal diet to give 75%, 150% or 200% of their control phase energy intake in accordance with their group assignment. Physical activity and energy intake were also monitored over the first and last 6 days of the intervention period. In the context of physical activity this was to examine behavioural adaptations to the different diets, whilst measurements of energy intake were used to monitor compliance with intake targets and provide feedback to maximise accuracy. It was anticipated that simply doubling their habitual diet to achieve the calorie target whilst maintaining macronutrient balance would not be feasible for some participants. In these circumstances, achieving the required caloric intake took precedence over maintaining macronutrient balance. The data reported in Section 5.3.3 show the minor variance that occurred due to these instances.

LAB 3 (Post-Intervention) – Following the completion of 20 consecutive 24-hour dietary cycles and a wash-out day of diet and activity standardisation, as described previously, participants returned to the laboratory and repeated the protocol outlined earlier for LAB 2, thereby providing a pre-post comparison.

5.2.3 – Outcome Measures

Body Composition – Post-void body mass was measured to the nearest 0.1 kg (Weylux 424, UK) and height was measured to the nearest 0.1 cm (Seca Stadiometer, Germany). Body composition was assessed using a DEXA scan (QDR Discovery W, Hologic; MA, USA) conducted in accordance with the manufacturer's instructions.

Dietary Intake – Participants were provided with a set of compact kitchen scales (Pocket Pro 2000, Smart Weigh; NY, USA) and a log-book with which all food and drink items were to be recorded. A member of the research team discussed best practice with them and emphasised the level of detail required. Weighed records were analysed (Nutritics^{TM} version 5.031; Ireland) to determine energy and macronutrient intake.

Physical Activity – Physical activity was measured using Actiheart^{TM} monitors (Cambridge Neurotechnology; Cambridge, UK) (Brage *et al.*, 2005). These monitors were individually calibrated using an adapted version of the treadmill protocol described by Brage *et al.* (2007). This involved completing four 3-minute stages of incremental treadmill locomotion with concurrent measurements of heart rate and energy expenditure (indirect calorimetry of expired air samples) to yield a heart rate–physical activity intensity regression equation on which estimates were based.

Meal Tests – Two successive meal tests were completed at pre- and post-intervention. Both meals were prescribed to provide one third of resting metabolic rate, as measured in the fasted state at pre-intervention. Meal 1 was a homogenous porridge meal (1.31 kcal·g^{-1}; 59% carbohydrate, 29% fat, 12% protein) composed of golden syrup flavour instant oats (Sainsbury's, UK), whole milk (Tesco, UK) and white granulated sugar (Silver Spoon, UK). This was cooked in a microwave and cooled for 10 minutes before being consumed in its entirety within a 10-minute eating opportunity following a pre-meal blood draw. Meal 2 took the form of a liquid meal-replacement supplement (1.50 kcal·ml^{-1}; 54% carbohydrate, 30% fat, 16% protein) (Ensure Plus; Abbott Nutrition, OH). This was consumed following a pre-meal arterialised-venous blood draw within a 5-minute feeding window commencing 3.5 hours after the consumption of meal 1.

Indirect Calorimetry – Resting metabolic rate and substrate oxidation were measured using indirect calorimetry of expired air samples (Frayn, 1983). In each instance, three consecutive 5-minute samples were taken in accordance with best practice guidelines (Compher *et al.*, 2006), with the values from two or more samples that agree to within 100 kcal·day^{-1} averaged and used in further analysis (Nightingale *et al.*, 2016).

Blood Sampling and Analysis – At the pre-intervention and post-intervention visits all blood samples were procured by means of an intravenous cannula. Samples were drawn and dispensed into an EDTA-coated tube for processing before the cannula was flushed to keep it patent. Analysis of plasma samples for concentrations of metabolites was performed using an automated analyser (RX Daytona; Randox Laboratories, Northern Ireland) and commercially available reagents (Randox Laboratories, Northern Ireland). Plasma insulin and leptin concentrations were determined using commercially available ELISAs (Mercodia, Sweden). Where concentrations fell

below the limit of detection for the assay, values were supplanted by the limit of detection specified by the assay manufacturer.

Urine Collection and Analysis – Urine samples collected at the outset of each lab session were analysed for specific gravity via refractometry (SUR-NE Clinical, Japan) and osmolality via the freeze-point depression method (Micro Osmometer 3300; Advanced Instruments, USA). Throughout the 3-hour postprandial period following meal 1, total urine output was also collected in order to correct rates of energy and substrate metabolism for protein oxidation. Urinary urea concentration was determined using an automated analyser, as described previously for plasma samples.

5.2.4 – Statistical Analysis

Analysis centred upon a two-way mixed model analysis of variance (ANOVA), featuring a between-subjects factor for diet allocation and a within-subjects factor for time. This examined the effect of each diet on the outcomes of interest (i.e. pre *versus* post, control phase *versus* intervention phase) and whether this effect varied between the three dietary conditions (i.e. group*time interaction). Where the time course of postprandial biochemical outcomes was examined, a three-way ANOVA was utilised due to the inclusion of timepoint (i.e. sampling interval) as a factor. Significant differences were followed up by appropriate *post-hoc* tests to isolate the source(s) of variance, and a Ryan-Holm-Bonferroni stepwise correction was used to adjust the resulting *p*-values for multiple comparisons (Ludbrook, 2000; Atkinson, 2002). All analysis was performed using SPSS 23.0 (IBM, USA). Statistical significance was accepted at $p \leq 0.05$ and all data are presented as mean ± SD unless otherwise stated. For clarity, *p*-values reflecting the interaction effect of an ANOVA are preceded by the notation 'group*time' or 'group*time*timepoint' to reflect the factors included (e.g. group*time, $p=0.01$), whilst time effects emerging from any ANOVA are noted accordingly (e.g. time, $p=0.01$). All post-hoc tests are noted in a similar fashion to highlight the comparison being made, for instance, 'Δ-between, $p=0.01$' denotes the *p*-value for a comparison of change scores between specific groups. Where the comparison being referred to is not clear from the preceding text, the contrasting group will also be provided in the notation (e.g. Δ-between, $p=0.01$ vs 75:75).

5.3 – Results

5.3.1 – Participants

The baseline characteristics of the participants in each intervention arm are shown in **Table 5.2**. In total, 42 participants provided informed consent for their participation and completed the baseline laboratory session. Of those completing this initial lab session, three withdrew during the control phase, with illness, work commitments and relocation cited as the reasons. Two participants were also excluded during the control phase, one due to changes in body mass that exceeded the permissible range and another for reporting implausibly low energy intake values. Post-randomisation, only one participant withdrew from the study; this was from the 0:150 condition and was attributed to difficulty fasting for the required durations. No baseline differences were apparent between the three groups prior to the intervention.

Table 5.2: Participant characteristics for the three intervention arms. Data are presented as mean ± SD.

Variable	Diet Allocation		
	75:75	**0:150**	**0:200**
n	12	12	12
Age (years)	45 ± 6	42 ± 11	41 ± 14
Female (n)	7	5	9
Height (m)	1.728 ± 0.075	1.737 ± 0.083	1.688 ± 0.055
Body Mass (kg)	72.0 ± 10.2	72.1 ± 8.3	67.4 ± 7.8
BMI ($kg \cdot m^{-2}$)	24.0 ± 1.9	23.9 ± 2.4	23.6 ± 2.1
FMI ($kg \cdot m^{-2}$)	6.35 ± 1.54	5.62 ± 2.15	6.37 ± 2.54
PAL [1]	1.68 ± 0.17	1.74 ± 0.23	1.68 ± 0.23
RMR ($kcal \cdot day^{-1}$)	1570 ± 305	1641 ± 301	1502 ± 251
Fasting Plasma Glucose ($mmol \cdot l^{-1}$)	5.25 ± 0.39	5.54 ± 0.58	5.17 ± 0.54
Fasting Plasma Insulin ($pmol \cdot l^{-1}$)	19.16 ± 10.48	18.16 ± 5.46	21.72 ± 8.93
HOMA-IR [2]	0.76 ± 0.45	0.74 ± 0.23	0.83 ± 0.33
Plasma Total Cholesterol ($mmol \cdot l^{-1}$)	4.53 ± 1.10	4.84 ± 0.72	5.00 ± 1.18
Plasma LDL Cholesterol ($mmol \cdot l^{-1}$)	2.77 ± 0.75	3.11 ± 0.90	2.95 ± 1.30
Plasma HDL Cholesterol ($mmol \cdot l^{-1}$)	1.58 ± 0.48	1.50 ± 0.45	1.70 ± 0.46
Fasting Plasma Triacylglycerol ($mmol \cdot l^{-1}$)	0.82 ± 0.31	0.95 ± 0.42	0.86 ± 0.32

[1] PAL = Total Daily Energy Expenditure/RMR

[2] HOMA-IR = (fasted insulin $\mu IU \cdot ml^{-1}$ * fasted glucose $mmol \cdot l^{-1}$)/22.5

Abbreviations: BMI = Body Mass Index; FMI = Fat Mass Index; PAL = Physical Activity Level; RMR = Resting Metabolic Rate; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; LDL= Low-Density Lipoprotein; HDL = High-Density Lipoprotein

5.3.2 – Body Composition

Changes in body mass and composition are shown in **Table 5.3**. Body mass was stable throughout the control phase in all three groups in accordance with the requirements of the study (75:75 = +0.1±0.6 kg, 0:150 = +0.1±0.8 kg, 0:200 = +0.2±0.5 kg). However, the change in response to the intervention differed across groups (group*time, p=0.01); body mass in the 75:75 and 0:150 conditions decreased to a similar degree, which differed from the stability of body mass in the 0:200 group (Δ-between, p=0.01 vs 75:75, p=0.04 vs. 0:150). No differences were apparent in the magnitude of this decrease between the 75:75 and 0:150 groups (Δ-between, p=0.46). This pattern was also mirrored by measures of BMI and waist circumference, although the latter did not reach significance for the interaction (group*time, p=0.08).

The change in fat mass also differed between groups (group*time, p<0.01). The decrease seen in response to the 75:75 diet was greater than the decrease accompanying the 0:150 diet (Δ-between, p=0.01 vs 75:75), both of which differed from the stability seen in response to the 0:200 diet (Δ-between, p<0.01 vs 75:75, p=0.05 vs 0:150). The differences in fat mass in the 0:150 group relative to the 75:75 group despite comparable reductions in body mass, appear to be driven by accompanying losses of lean mass from pre- to post-intervention in the 0:150 group. However, no interaction effect emerged for changes in lean mass (group*time, p=0.24), with only the time effect achieving significance (time, p=0.01).

Consequently, FMI decreased in the 75:75 group to a greater extent than in the 0:150 group (Δ-between, p=0.03 vs 75:75). This decrease also contrasted with the 0:200 group (Δ-between, p<0.01 vs 75:75), in which FMI was unchanged. However, the difference in the change seen with the 0:150 and 0:200 conditions did not achieve significance (Δ-between, p=0.07). Lastly, the change in visceral fat mass from pre- to post-intervention was not different between groups (group*time, p=0.30) despite decreases in both the 75:75 and 0:150 conditions, which is in accordance with the findings for waist circumference.

Table 5.3: Body composition outcomes. Data are mean (SD) at pre- and post-intervention, with delta change and 95% confidence intervals (CI) for the response within each group.

Variable	75:75 (n=12)			0:150 (n=12)			0:200 (n=12)			Two-way ANOVA		
	PRE	**POST**	**Δ from PRE**	**PRE**	**POST**	**Δ from PRE**	**PRE**	**POST**	**Δ from PRE**	**Interaction**	**Time**	**Group**
Body Mass (kg)	72.1 (10.2)	70.2 (9.9)	**-1.9 *[C]** **(-2.5, -1.4)**	72.3 (8.2)	70.7 (8.2)	**-1.6 *[C]** **(-2.2, -1.0)**	67.7 (7.8)	67.2 (7.4)	-0.5 [A,B] (-1.1, 0.1)	**0.01**	**<0.01**	0.46
Body Mass Index (kg·m^{-2})	24.0 (1.9)	23.4 (1.8)	**-0.6 *[C]** **(-0.8, -0.5)**	24.0 (2.3)	23.4 (2.3)	**-0.5 *[C]** **(-0.7, -0.3)**	23.7 (2.1)	23.5 (1.9)	-0.2 [A,B] (-0.4, 0.0)	**0.01**	**<0.01**	0.99
Waist Circumference (cm)	84.0 (7.1)	81.5 (6.8)	-2.4 (-3.5, -1.4)	83.2 (4.6)	81.5 (4.0)	-1.7 (-2.7, -0.6)	80.0 (7.9)	79.4 (7.6)	-0.7 (-1.8, 0.4)	0.08	**<0.01**	0.46
Fat Mass (kg)	18.5 (4.1)	16.7 (3.8)	**-1.8 *[B,C]** **(-2.2, -1.3)**	16.1 (5.1)	15.4 (5.3)	**-0.8 *[A,C]** **(-1.3, -0.3)**	17.4 (6.8)	17.3 (6.4)	-0.1 [A,B] (-0.5, 0.3)	**<0.01**	**<0.01**	0.66
Fat Mass Index (kg·m^{-2})	6.35 (1.54)	5.73 (1.43)	**-0.62 *[B,C]** **(-0.78, -0.46)**	5.62 (2.15)	5.36 (2.23)	**-0.26 *[A]** **(-0.47, -0.06)**	6.37 (2.54)	6.36 (2.39)	-0.01 [A] (-0.17, 0.15)	**<0.01**	**<0.01**	0.61
Lean Mass (kg)	53.6 (10.5)	53.5 (10.2)	-0.1 (-0.6, 0.3)	56.1 (10.1)	55.3 (10.0)	-0.8 (-1.5, -0.2)	50.3 (8.6)	49.8 (8.6)	-0.4 (-0.9, 0.0)	0.24	**0.01**	0.39
Visceral Fat Mass (g)	382.6 (163.5)	351.3 (159.7)	-31.3 (-48.6, -13.9)	355.7 (94.6)	321.4 (81.9)	-34.3 (-43.4, -15.2)	311.4 (161.5)	304.0 (145.8)	-7.5 (-43.7, 28.8)	0.30	**<0.01**	0.57

* denotes $p \leq 0.05$ for within-group effect (paired *t*-test)

[A] denotes $p \leq 0.05$ for between-group change score vs. 75:75 (independent samples *t*-test)

[B] denotes $p \leq 0.05$ for between-group change score vs. 0:150 (independent samples *t*-test)

[C] denotes $p \leq 0.05$ for between-group change score vs. 0:200 (independent samples *t*-test)

5.3.3 – Energy Intake

Energy intake, and the fraction of energy intake derived from each of the four macronutrients, is shown in **Figure 5.2**. During the intervention, the interaction effect (group*time, $p<0.01$) highlighted that the 75:75 and 0:150 groups reduced their energy intake to a similar degree (75:75 = -622±216 kcal·day^{-1}, 0:150 = -503±264 kcal·day^{-1}; Δ-between, $p=0.24$), reductions which differed to the 0:200 group in which energy intake was stable to within 15 kcal·day^{-1} (Δ-between, $p<0.01$ vs 75:75, $p<0.01$ vs 0:150). This was accompanied by interaction effects for both carbohydrate (group*time, $p=0.02$) and fat (group*time, $p<0.01$) intake, which appear to drive these changes. In the 75:75 group, both carbohydrate (-54±26 g·day^{-1}) and fat (-35±19 g·day^{-1}) intake decreased in response to the intervention, a pattern which was largely mirrored by the 0:150 group, with carbohydrate and fat intake decreasing by -45±40 g·day^{-1} and -21±10 g·day^{-1}, respectively. Comparatively, there were no meaningful changes in the consumption of any one macronutrient in the 0:200 group.

Upon comparing these changes across groups, the change in carbohydrate intake was not different between 75:75 and 0:150 (Δ-between, $p=0.52$), nor 0:150 and 0:200 (Δ-between, $p=0.12$), only achieving significance when comparing 75:75 against 0:200 (Δ-between, $p=0.04$). Conversely, the disparate changes in fat intake highlighted a difference between the two energy-restricted diets, decreasing to a greater extent in the 75:75 group when compared to the 0:150 group (Δ-between, $p=0.03$ vs 75:75), both of which contrasted with the 0:200 group (Δ-between, $p<0.01$ vs 75:75, $p<0.01$ vs 0:150). The changes in protein (group*time, $p=0.12$) and alcohol intake (group*time, $p=0.13$) were not meaningful.

The net effect of this in terms of macronutrient balance was a disproportionate change in the fraction of energy intake derived from fat between groups (group*time, $p<0.01$). A 4±4% decrease in fat-derived energy intake was observed in the 75:75 group, which diverged from the stability of the 0:150 group (+0±3%; Δ-between, $p=0.04$ vs 75:75) and an increase in the 0:200 group (+4±7%; Δ-between, $p=0.01$ vs 75:75), which tended to differ themselves (Δ-between, $p=0.06$, 0:150 vs 0:200). These changes were offset by between-group differences in the proportion of energy intake obtained from both protein (group*time, $p=0.03$) and alcohol (group*time, $p=0.05$). Specifically, the

75:75 group tended to increase the fraction of dietary energy derived from protein (+1±2%) and alcohol (+2±3%), whilst in the 0:200 group no meaningful changes in the contribution of any one macronutrient were apparent.

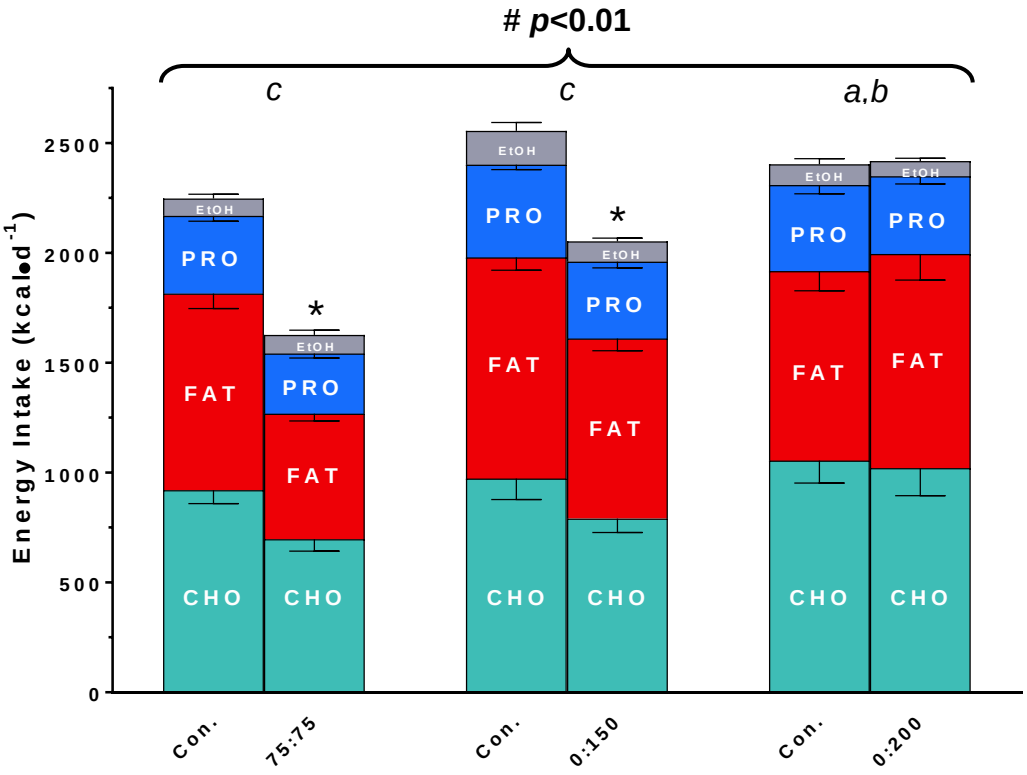

Figure 5.2: Energy and macronutrient intake during the control and intervention phases in the daily calorie restriction (75:75), intermittent fasting with restriction (0:150) and intermittent fasting without restriction groups (0:200). Energy derived from the respective macronutrients was estimated by multiplying the reported intake in grams by the accompanying Atwater general factor. # denotes $p \leq 0.05$ for group*time interaction (two-way ANOVA). * denotes $p \leq 0.05$ for within-group time effect (paired *t*-test). a, b and c denote $p \leq 0.05$ for between-group change score vs. 75:75, 0:150 and 0:200, respectively (independent *t*-test). Data are presented as mean and SEM. Abbreviations: Con = control; CHO = carbohydrate; FAT = lipids; PRO = protein; EtOH = alcohol.

5.3.4 – Energy Expenditure

Total daily energy expenditure in the control and intervention phases in each of the three groups is shown in **Figure 5.3** as a sum of resting metabolic rate, diet-induced thermogenesis and physical activity thermogenesis. The interaction effect (group*time, $p<0.01$) highlighted that the reduction in energy expenditure in the 0:150 group (-230±166 kcal·day^{-1}) differed to the stability of the 0:200 condition (+87±236 kcal·day^{-1}; Δ-between, $p<0.01$ vs 0:150). Although, energy expenditure was not meaningfully altered by the 75:75 diet either (-112±244 kcal·day^{-1}), this change was not different from either the 0:150 or 0:200 diets (Δ-between, $p=0.18$ vs 0:150, $p=0.11$ vs 0:200).

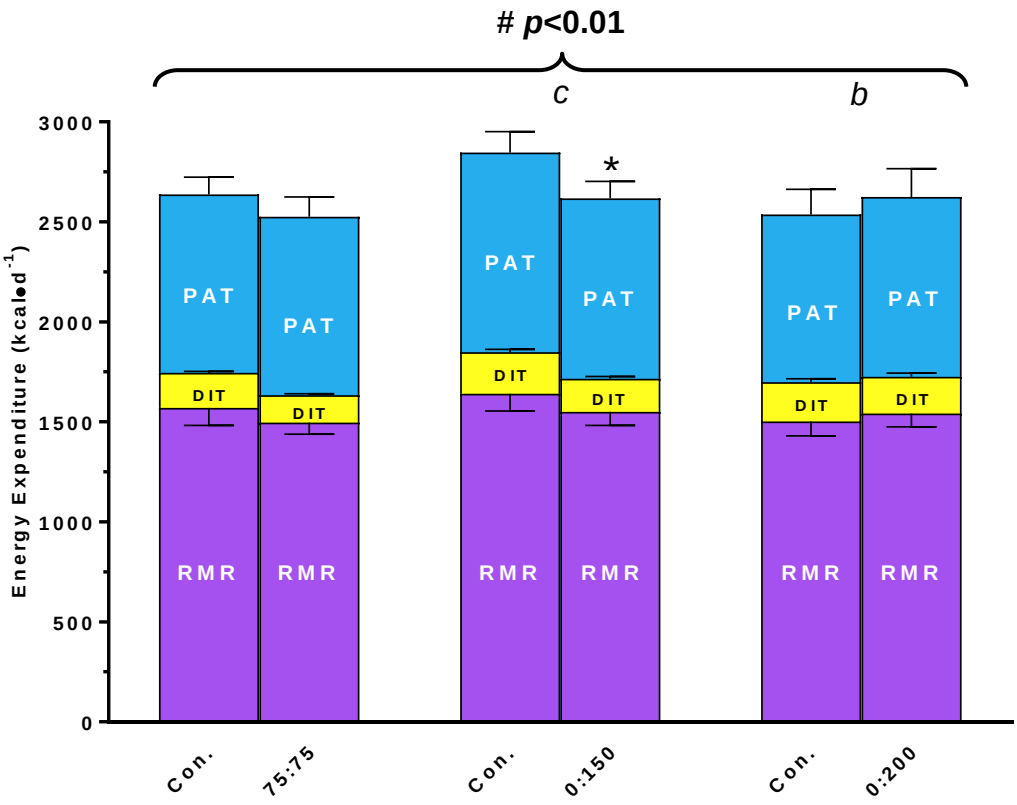

Figure 5.3: Total daily energy expenditure as a product of the three main components during the control and intervention phases in the daily calorie restriction (75:75), intermittent fasting with restriction (0:150) and intermittent fasting without restriction (0:200) groups. RMR was measured via indirect calorimetry during the pre- and post-intervention laboratory sessions, DIT was estimated by multiplying reported intakes by the equivalents proposed by Westerterp (2004), and PAT was measured using Actiheart™ monitors. # denotes $p \leq 0.05$ for group*time interaction (two-way ANOVA). * denotes $p \leq 0.05$ for within-group time effect (paired t-test). a, b and c denote $p \leq 0.05$ for between-group change score vs. 75:75, 0:150 and 0:200, respectively (independent t-test). Data are presented as mean and SEM. Abbreviations: Con = control; RMR = resting metabolic rate; DIT = diet-induced thermogenesis; PAT = physical activity thermogenesis.

5.3.5 – Energy Balance

For clarity, the energy balance data discussed thus far are summarised in **Figure 5.4**. This serves to better visualise the effect of the three interventions on energy intake and the resultant impacts upon the components of energy expenditure, which was a primary outcome in this study. However, direct comparisons between intake and expenditure data were deemed inappropriate in light of the different measurement techniques employed and the associated limitations (Hall *et al.*, 2012).

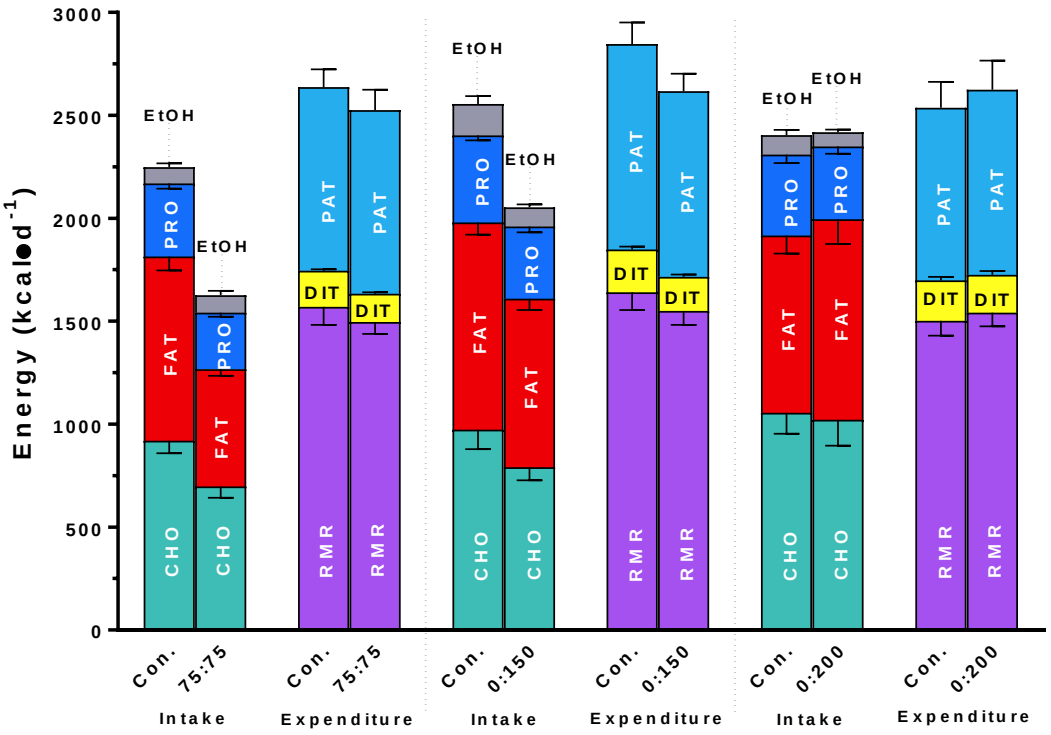

Figure 5.4: Components of energy intake and energy expenditure measured during the control and intervention phases in the daily calorie restriction (75:75), intermittent fasting with restriction (0:150) and intermittent fasting without restriction groups (0:200). Data are presented as mean and SEM for each stacked-bar component. Abbreviations: Con = control; CHO = carbohydrate; FAT = lipids; PRO = protein; EtOH = alcohol; RMR = resting metabolic rate; DIT = diet-induced thermogenesis; PAT = physical activity thermogenesis.

5.3.6 – Metabolic Rate

Following adjustment for changes in body mass, resting metabolic rate was unchanged by the three interventions (75:75 = -0.35±2.43 kcal·kg^{-1}·day^{-1}, 0:150 = -0.77±2.65 kcal·kg^{-1}·day^{-1}, 0:200 = +0.80±2.38 kcal·kg^{-1}·day^{-1}), showing neither an interaction (group*time, p=0.30) nor a time effect (time, p=0.80). Furthermore, despite the seemingly large changes in carbohydrate and lipid oxidation shown in **Figure 5.5**, only time effects were apparent with no meaningful interactions (group*time, all $p \geq 0.51$). This pattern was also mirrored by the changes in respiratory exchange ratio (75:75 = -0.03±0.07, 0:150 = -0.06±0.06, 0:200 = -0.02±0.06; group*time, p=0.42), with the time effect (time, $p<0.01$) being driven by the response to the 0:150 diet.

In the postprandial state (i.e. the metabolic responses monitored over 3-hours following meal 1 at pre- and post-intervention), once again metabolic rate (group*time, p=0.55) and substrate oxidation (group*time, all $p \geq 0.10$) were not affected by the three diets (**Figure 5.6**). Only lipid oxidation achieved significance for a time effect (time, p=0.05), which was driven by a tendency to increase in the two intermittent fasting groups. This was mirrored by measurements of respiratory exchange ratio, which also characterised a time effect (75:75 = +0.00±0.05, 0:150 = -0.04±0.05, 0:200 = -0.02±0.03; time, p=0.01) but no interaction (group*time, p=0.10).

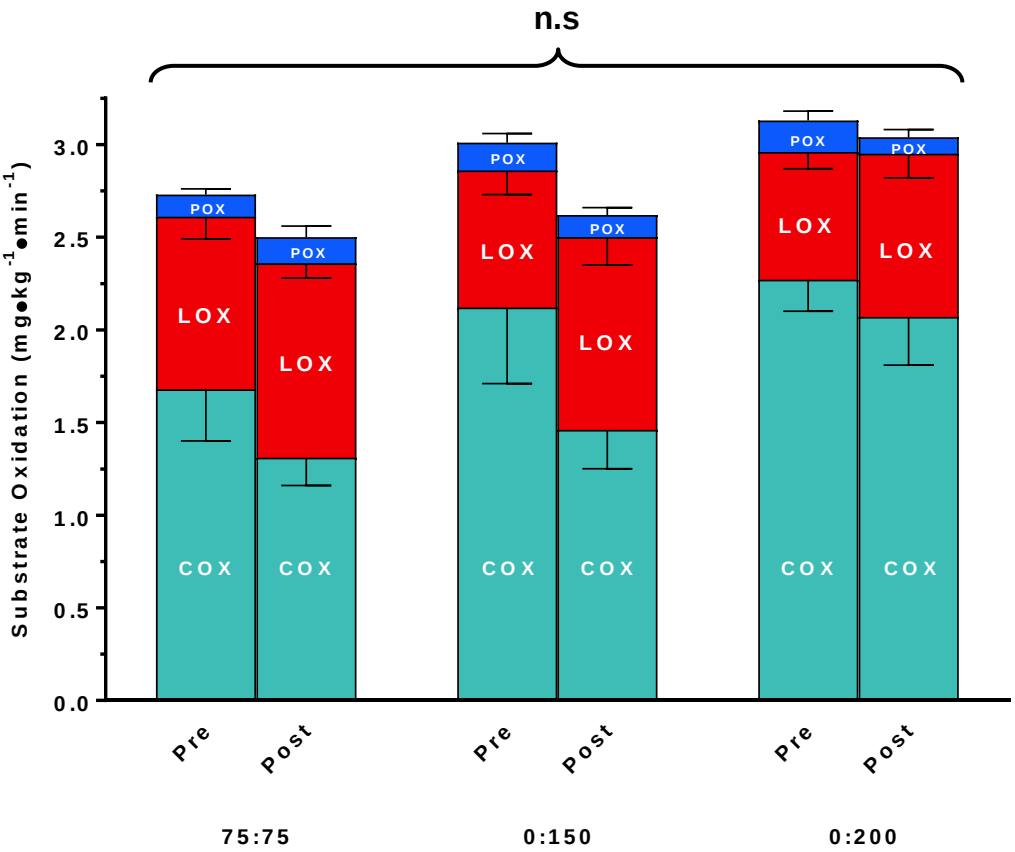

Figure 5.5: Total fasting substrate oxidation as accounted for by each macronutrient at pre- and post-intervention in the daily calorie restriction (75:75), intermittent fasting with restriction (0:150) and intermittent fasting without restriction groups (0:200). # denotes $p \leq 0.05$ for group*time interaction (two-way ANOVA). * denotes $p \leq 0.05$ for within-group time effect (paired t-test). a, b and c denote $p \leq 0.05$ for between-group change score vs. 75:75, 0:150 and 0:200, respectively (independent t-test). Data are presented as mean and SEM. Abbreviations: COX = carbohydrate oxidation; LOX = lipid oxidation; POX = protein oxidation.

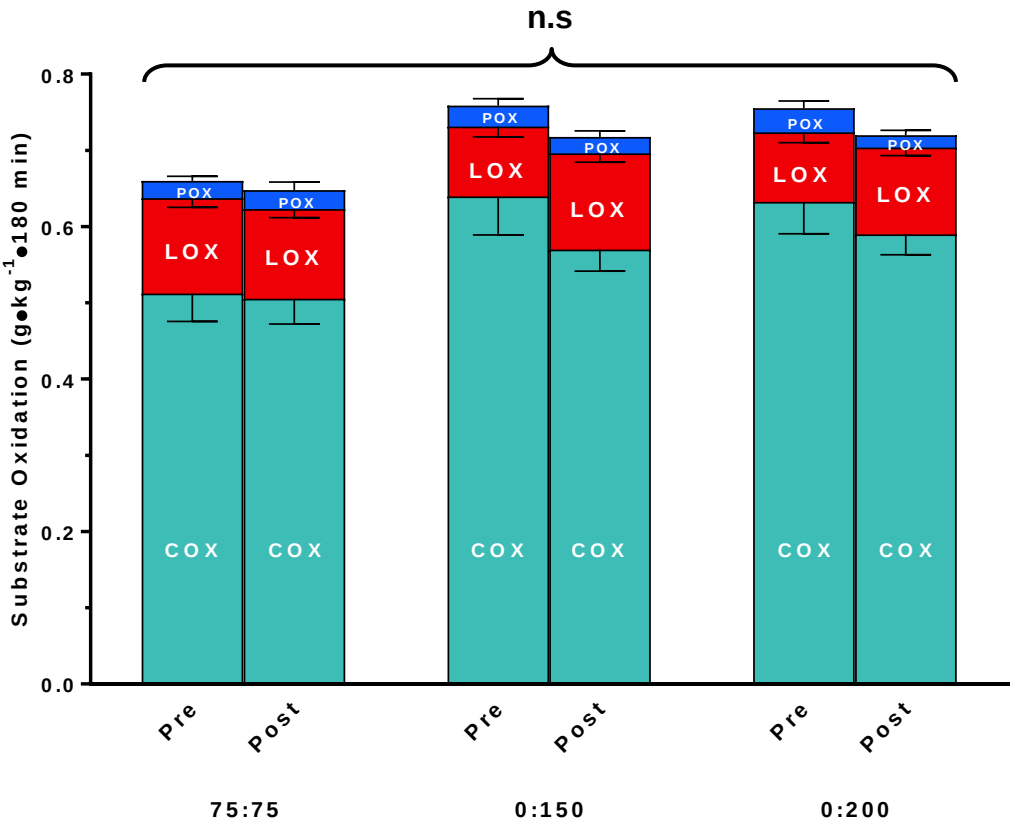

Figure 5.6: Average substrate oxidation throughout the 3-hour postprandial period presented as a sum of measured oxidation of specific macronutrients at pre- and post-intervention in the daily calorie restriction (75:75), intermittent fasting with restriction (0:150) and intermittent fasting without restriction groups (0:200). # denotes $p \leq 0.05$ for group*time interaction (two-way ANOVA). * denotes $p \leq 0.05$ for within-group time effect (paired t-test). a, b and c denote $p \leq 0.05$ for between-group change score vs. 75:75, 0:150 and 0:200, respectively (independent t-test). Data are presented as mean and SEM. Abbreviations: COX = carbohydrate oxidation; LOX = lipid oxidation; POX = protein oxidation.

5.3.7 – Diet-Induced Thermogenesis

Given that diet-induced thermogenesis (**Figure 5.7**) was estimated from reported intakes of the four main macronutrients, the effects of the interventions on this outcome broadly align with those discussed previously for energy intake, with slight amplification in some cases due to the differing thermogenic effects of each macronutrient. Briefly, diet-induced thermogenesis (group*time, $p<0.01$) was similarly decreased in the 75:75 and 0:150 groups during the intervention (75:75 = -39±12 kcal·day^{-1}, 0:150 = -42±25 kcal·day^{-1}; Δ-between, $p=0.68$). These reductions differed from the stability of the 0:200 group (-11±19 kcal·day^{-1}; Δ-between, $p<0.01$ vs 75:75, $p=0.01$ vs 0:150), capturing the energy-restricted nature of the two diets. Accordingly, the change in the thermogenic fraction accounted for by fat (group*time, $p<0.01$) and carbohydrate (group*time, $p=0.02$) was also different between groups, mirroring the divergent changes in macronutrient intake described previously.

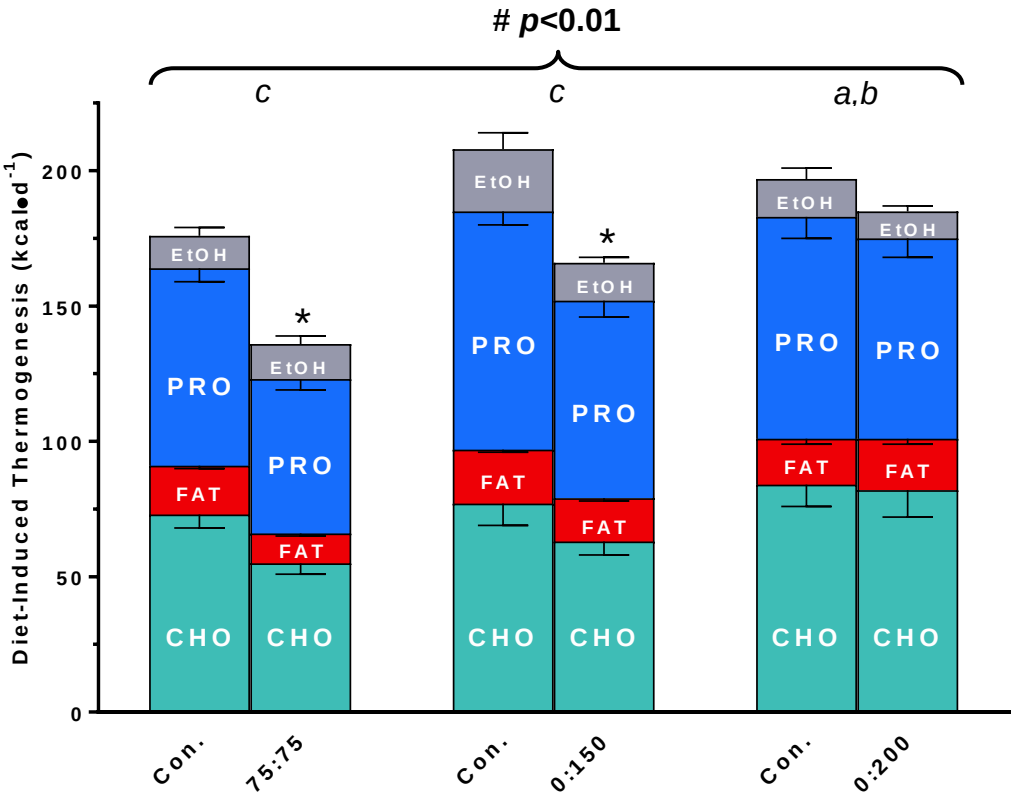

Figure 5.7: Diet-induced thermogenesis as a product of the thermogenic fractions of the four main macronutrients during the monitoring and intervention phases for the daily calorie restriction (75:75), intermittent fasting with restriction (0:150) and intermittent fasting without restriction groups (0:200). Estimates were made by multiplying reported intakes in the respective phases by the equivalents proposed by Westerterp (2004). # denotes $p \leq 0.05$ for group*time interaction (two-way ANOVA). * denotes $p \leq 0.05$ for within-group time effect (paired t-test). a, b and c denote $p \leq 0.05$ for between-group change score vs. 75:75, 0:150 and 0:200, respectively (independent t-test). Data are presented as mean and SEM. Abbreviations: Con = control; CHO = carbohydrate; FAT = lipid; PRO = protein; EtOH = alcohol.

5.3.8 – Physical Activity Thermogenesis

Physical activity thermogenesis, as measured by individually-calibrated Actiheart™ monitors, is shown in **Figure 5.8**. The interaction effect (group*time, p=0.04) highlighted a trend for reduced physical activity thermogenesis in response to the 0:150 diet when contrasted against the tendency to increase in response to the 0:200 diet (0:150 = -98±169 kcal·day^{-1}, 0:200 = +59±142 kcal·day^{-1}; Δ-between, p=0.07); whereas physical activity thermogenesis in the 75:75 group was stable to within 1 kcal·day^{-1}, which did not differ meaningfully from the change in either the 0:150 or 0:200 groups (Δ-between, p=0.24 vs 0:150, p=0.31 vs 0:200). Upon stratifying this by calories expended at differing intensity thresholds, as defined by metabolic equivalents, the only effect worthy of mention was a trend for between-group differences in the change in energy expended performing light activities (group*time, p=0.08). This seems to have been driven by the slight decrease in the 0:150 group relative to the other two conditions, as illustrated in **Figure 5.8** (75:75 = +12±52 kcal·day^{-1}, 0:150 = -33±63 kcal·day^{-1}, 0:200 = +21±68 kcal·day^{-1}).

To explore these changes further, the modifications in physical activity thermogenesis and intensity observed during the intervention (**Figure 5.9a**) were sub-divided according to cycles of fasting and feeding for the 0:150 and 0:200 groups (**Figure 5.9b**). When expressed in this manner, the change in physical activity thermogenesis from fasted to fed days was not different between groups (group*time, p=0.37), and nor was this change different between groups when specific intensity thresholds were analysed. Only the change in vigorous activity approached significance for the interaction (group*time, p=0.07), which most likely reflects the decrease from fasted to fed days in the 0:150 group relative to the slight increase in the 0:200 group (0:150 = -27±68 kcal·day^{-1}, 0:200 = +21±58 kcal·day^{-1}). It is interesting to note however, that time effects were established for calories expended in the sedentary (time, p<0.01) and light (time, p<0.01) intensity ranges, suggesting divergent activity patterns on fasted and fed days that were similar across the two intermittent fasting groups. Specifically, participants in both groups tended to reduce the calories expended through sedentary (0:150 = -34±31 kcal·day^{-1}, 0:200 = -41±26 kcal·day^{-1}) and light (0:150 = -38±43 kcal·day^{-1}, 0:200 = -45±53 kcal·day^{-1}) intensity physical activities to a greater degree

on fasting days when compared to fed days. This resulted in a trend for lower physical activity thermogenesis on fasting days when compared to fed days (time, p=0.07).

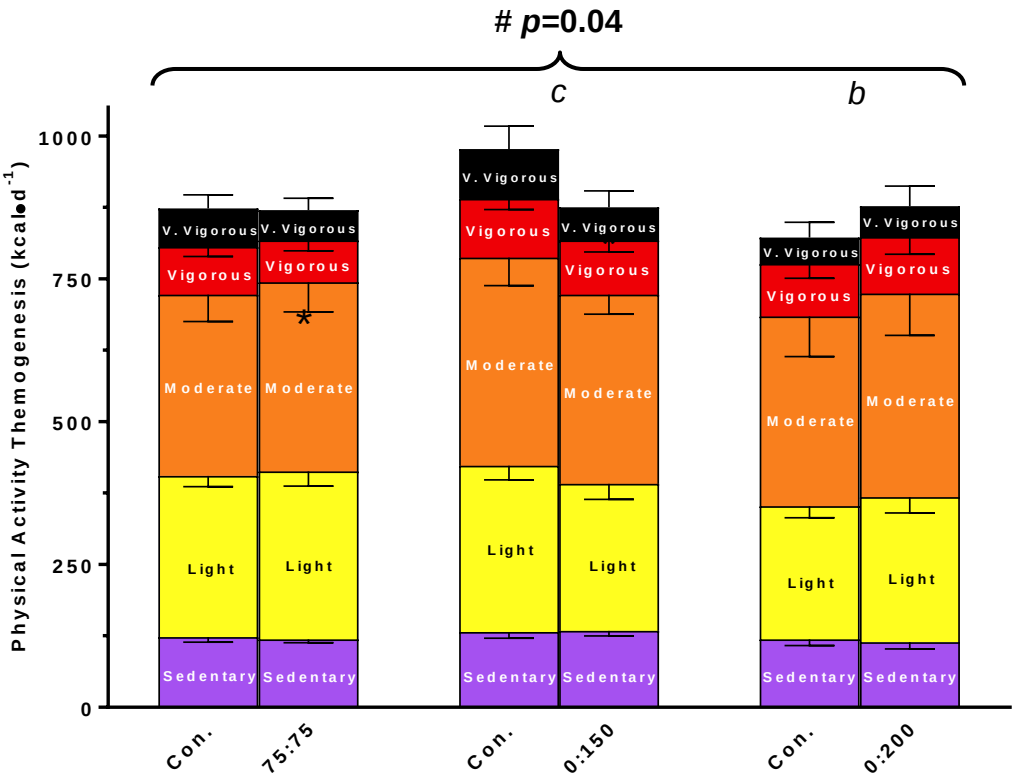

Figure 5.8: Physical activity thermogenesis presented as a sum of the kilocalories expended at differing intensities of activity during the monitoring and intervention phases for the daily calorie restriction (75:75), intermittent fasting with restriction (0:150) and intermittent fasting without restriction groups (0:200). Intensity thresholds were defined as multiples of resting metabolic rate (i.e. METs), as measured prior to the intervention, using the following ranges: sedentary = ≤1.5 METs, light = 1.5-2.9 METs, moderate = 3.0-5.9 METs, vigorous = 6.0-10.1 METs, v.vigorous = ≥10.2 METs. # denotes p≤0.05 for group*time interaction (two-way ANOVA). * denotes p≤0.05 for within-group time effect (paired t-test). a, b and c denote p≤0.05 for between-group change score vs. 75:75, 0:150 and 0:200, respectively (independent t-test). Data are presented as mean and SEM. Abbreviations: Con = control; v. vigorous = very vigorous.

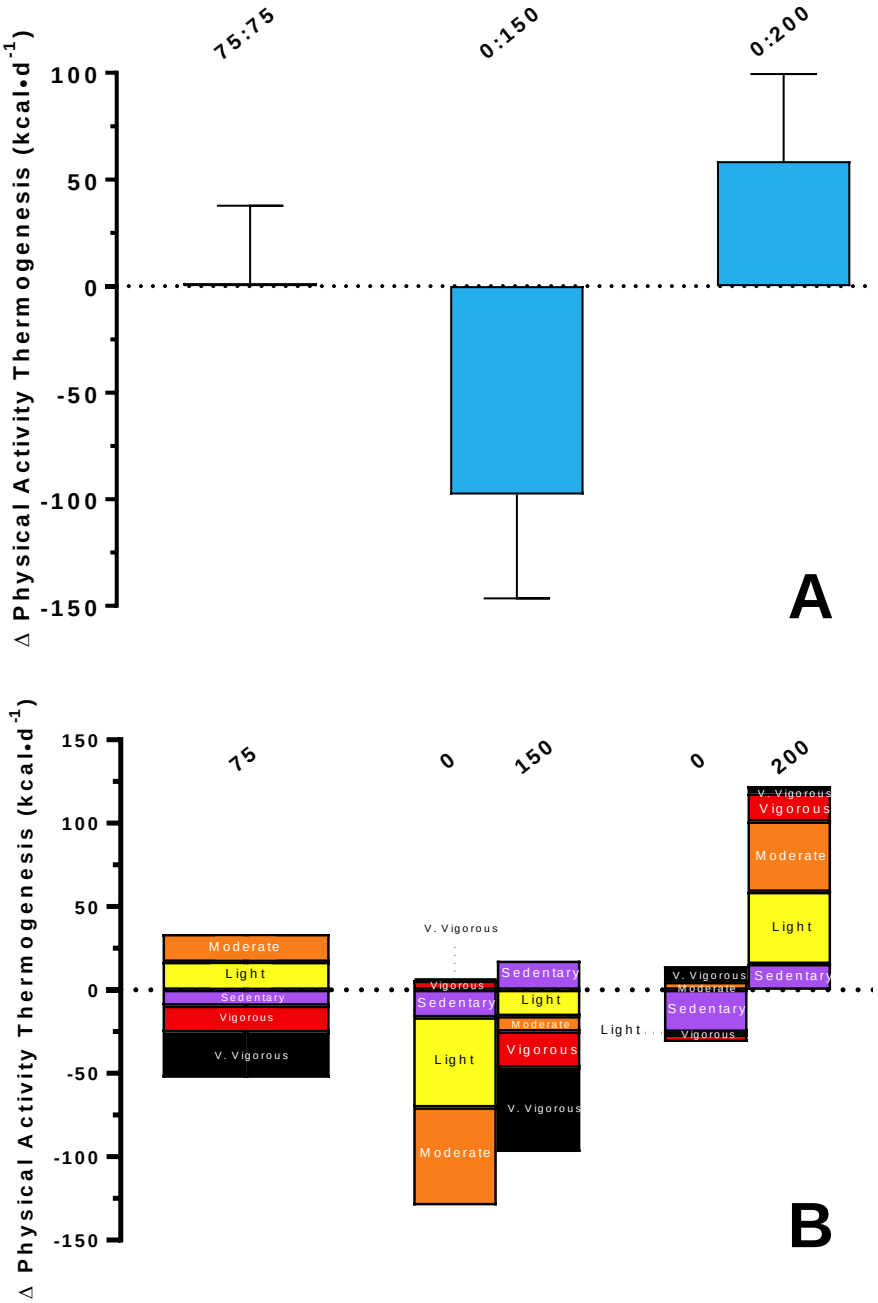

Figure 5.9: Change in physical activity thermogenesis during the intervention (A) as a sum of the kilocalories expended at differing intensities of activity during fasted and fed periods (B) for intermittent fasting with restriction (0:150) and intermittent fasting without restriction groups (0:200). Intensity thresholds were defined as multiples of resting metabolic rate (i.e. METs), as measured prior to the intervention, using the following ranges: sedentary = ≤1.5 METs, light = 1.5-2.9 METs, moderate = 3.0-5.9 METs, vigorous = 6.0-10.1 METs, v.vigorous = ≥10.2 METs. # denotes $p \leq 0.05$ for

group*time interaction (two-way ANOVA). * denotes $p \leq 0.05$ for within-group time effect (paired t-test). a, b and c denote $p \leq 0.05$ for between-group change score vs. 75:75, 0:150 and 0:200, respectively (independent t-test). Data are presented as mean and SEM. Abbreviations: Con = control; v.vigorous = very vigorous.

5.3.9 – Fasting Biochemistry

All fasting biochemistry outcomes are shown in **Table 5.4**. The only meaningful interaction effect that emerged was for plasma leptin concentration (group*time, p=0.04), which decreased following the 75:75 diet but not the 0:200 diet (Δ-between, p=0.01). Although there was also a tendency for leptin to decrease following the 0:150 intervention, this did not differ from the changes seen in response to the other two conditions (Δ-between, p=0.58 vs 75:75, p=0.16 vs 0:200). Aside from this, there were minimal impacts of the three interventions on these outcomes, as emphasised by the accompanying dearth of time effects. The only time effect that even approached significance was the pre- to post-intervention change in LDL cholesterol, which was driven by a decrease in response to the 75:75 diet.

Table 5.4: Fasting and postprandial biochemistry outcomes. Data are mean (SD) at pre- and post-intervention, with delta change and 95% confidence intervals (CI) for the response within each group.

Outcome	75:75 (n=12)			0:150 (n=12)			0:200 (n=12)			Two-way ANOVA		
	PRE	POST	Δ from PRE	PRE	POST	Δ from PRE	PRE	POST	Δ from PRE	Interaction	Time	Group
Plasma Glucose												
Fasted (mmol·l^{-1})	5.27 (0.38)	5.28 (0.31)	0.01 (-0.13, 0.15)	5.62 (0.37)	5.66 (0.39)	0.04 (-0.29, 0.38)	5.14 (0.67)	5.24 (0.40)	0.10 (-0.20, 0.40)	0.88	0.48	**0.02**
iAUC (mmol·l^{-1}·330 min)	332 (146)	360 (228)	28 (-55, 111)	267 (107)	268 (140)	1 (-97,99)	268 (88)	243 (73)	-24 (-77, 28)	0.60	0.94	0.17
Meal 1 Peak (mmol·l^{-1})	7.81 (1.26)	8.26 (1.15)	0.45 (-0.15, 1.05)	8.22 (1.09)	8.24 (1.05)	0.01 (-0.79, 0.82)	7.72 (1.00)	7.51 (1.20)	-0.21 (-0.81, 0.38)	0.31	0.64	0.31
Meal 2 Peak (mmol·l^{-1})	8.10 (0.42)	8.13 (0.54)	0.04 (-0.25, 0.32)	8.21 (0.95)	8.27 (0.81)	0.06 (-0.41, 0.53)	8.02 (0.90)	8.03 (0.82)	0.01 (-0.43, 0.44)	0.98	0.74	0.75
Meal 1 Time to Peak (min)	35 (17)	34 (9)	-1 (-14, 12)	33 (13)	31 (8)	-1 (-10, 7)	33 (9)	34 (11)	1 (-8, 11)	0.91	0.88	0.75
Meal 2 Time to Peak (min)	44 (23)	39 (14)	-5 (-14, 4)	41 (27)	39 (19)	-3 (-24, 19)	44 (25)	38 (14)	-6 (-22, 10)	0.94	0.30	0.98
Plasma Insulin												
Fasted (pmol·l^{-1})	20.63 (8.19)	17.18 (12.13)	-3.45 (-7.94, 1.04)	19.02 (5.04)	19.01 (7.91)	-0.01 (-4.73, 4.71)	18.50 (5.78)	21.21 (8.33)	2.70 (-2.13, 7.54)	0.14	0.84	0.94
iAUC (nmol·l^{-1}·330 min)	28.45 (9.92)	30.01 (13.21)	1.56 (-5.32, 8.44)	35.59 (12.80)	38.65 (17.92)	3.06 (-4.28, 10.41)	39.79 (17.39)	39.36 (15.16)	-0.43 (-8.65, 7.79)	0.77	0.48	0.16
Meal 1 Peak (pmol·l^{-1})	216.15 (70.95)	224.47 (77.41)	8.32 (-45.61, 62.24)	280.69 (111.52)	311.52 (129.74)	30.83 (-27.58, 89.24)	302.83 (106.74)	303.40 (140.67)	0.58 (-75.11, 76.27)	0.74	0.43	0.08
Meal 2 Peak (pmol·l^{-1})	271.73 (118.89)	288.50 (155.84)	16.78 (-43.33, 76.89)	281.26 (78.13)	289.73 (99.56)	8.47 (-44.34, 61.27)	339.10 (185.98)	382.93 (212.22)	43.83 (-88.95, 176.61)	0.81	0.33	0.26
Meal 1 Time to Peak (min)	34 (9)	35 (10)	1 (-6,9)	41 (16)	50 (12)	9 (1, 16)	34 (11)	41 (19)	8 (-7, 22)	0.51	**0.04**	**0.03**
Meal 2 Time to Peak (min)	35 (10)	35 (13)	0 (-10, 10)	38 (15)	43 (20)	5 (-8, 18)	38 (21)	41 (13)	4 (-9, 16)	0.79	0.35	0.58
HOMA-IR[1]	0.809 (0.346)	0.685 (0.517)	-0.124 (-0.305, 0.057)	0.788 (0.210)	0.795 (0.320)	0.007 (-0.196, 0.211)	0.683 (0.154)	0.815 (0.302)	0.132 (-0.057, 0.321)	0.13	0.92	0.92
Plasma Total Cholesterol												
Fasted (mmol·l^{-1})	4.75 (0.97)	4.46 (0.92)	-0.28 (-0.48, -0.09)	4.74 (0.77)	4.74 (0.78)	0.00 (-0.23 ,0.23)	4.68 (0.96)	4.69 (0.80)	0.01 (-0.24, 0.27)	0.09	0.15	0.93

Table 5.4 cont. Fasting and postprandial biochemistry outcomes. Data are mean (SD) at pre- and post-intervention, with delta change and 95% confidence intervals (CI) for the response within groups.

Outcome	75:75 (n=12)			0:150 (n=12)			0:200 (n=12)			Two-way ANOVA		
	PRE	**POST**	**Δ from PRE**	**PRE**	**POST**	**Δ from PRE**	**PRE**	**POST**	**Δ from PRE**	**Interaction**	**Time**	**Group**
Plasma HDL Cholesterol												
Fasted (mmol·l^{-1})	1.64 (0.43)	1.57 (0.38)	-0.07 (-0.18, 0.03)	1.53 (0.43)	1.58 (0.39)	0.05 (-0.04, 0.14)	1.60 (0.41)	1.65 (0.38)	0.04 (-0.05, 0.13)	0.10	0.82	0.90
Plasma LDL Cholesterol												
Fasted (mmol·l^{-1})	2.96 (0.93)	2.72 (0.79)	-0.24 (-0.41, -0.07)	3.06 (0.85)	2.96 (0.79)	-0.10 (-0.31, 0.12)	2.82 (1.18)	2.80 (1.07)	-0.02 (-0.31, 0.27)	0.35	0.06	0.85
Plasma Triacylglycerol												
Fasted (mmol·l^{-1})	0.87 (0.32)	0.83 (0.20)	-0.04 (-0.21 ,0.13)	1.02 (0.33)	0.89 (0.22)	-0.12 (-0.27, 0.02)	0.96 (0.39)	0.93 (0.39)	-0.03 (-0.18, 0.13)	0.59	0.13	0.64
tAUC (mmol·l^{-1}·330 min)	291 (129)	280 (71)	-11 (-59, 36)	394 (126)	349 (117)	-45 (-93,2)	357 (164)	351 (144)	-6 (-51, 40)	0.37	0.10	0.21
Plasma NEFA												
Fasted (mmol·l^{-1})	0.48 (0.34)	0.46 (0.24)	-0.02 (-0.13, 0.08)	0.33 (0.09)	0.34 (0.14)	0.01 (-0.08, 0.09)	0.34 (0.18)	0.31 (0.13)	-0.04 (-0.16, 0.09)	0.83	0.54	0.12
tAUC (mmol·l^{-1}·330 min)	50 (21)	46 (14)	-4 (-13,5)	40 (11)	40 (11)	0 (-7,7)	41 (18)	39 (18)	-2 (-13, 9)	0.81	0.40	0.31
Plasma Glycerol												
Fasted (mmol·l^{-1})	0.06 (0.03)	0.06 (0.02)	0.00 (-0.02, 0.02)	0.04 (0.03)	0.04 (0.02)	0.00 (-0.02, 0.02)	0.04 (0.02)	0.03 (0.02)	0.01 (-0.02, 0.02)	0.98	0.90	**0.04**
tAUC (mmol·l^{-1}·330 min)	12.31 (4.98)	14.00 (3.63)	1.69 (-0.80, 4.19)	10.71 (4.08)	11.02 (3.87)	0.32 (-1.07, 1.70)	10.71 (4.10)	10.58 (4.90)	-0.13 (-1.57, 1.31)	0.29	0.20	0.26
Plasma Leptin												
Fasted (ng·ml^{-1})	9.30 (5.97)	5.50 (3.96)	**-3.60 *[C] (-6.07, -1.53)**	10.49 (13.92)	7.68 (10.10)	-2.81 (-6.01, 0.39)	16.43 (15.89)	16.61 (16.60)	0.18 [A] (-1.33, 1.69)	**0.04**	**<0.01**	0.15

[1] HOMA-IR = (fasted insulin μIU·ml^{-1} * fasted glucose mmol·l^{-1})/22.5

[A] denotes significantly different from 75:75 according to post-hoc independent samples t-test ($p \leq 0.05$)

[C] denotes significantly different from 0:200 according to post-hoc independent samples t-test ($p \leq 0.05$)

Abbreviations: AUC = Area Under Curve; iAUC = Incremental Area Under Curve; tAUC = Total Area Under Curve; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; LDL= Low-Density Lipoprotein; HDL = High-Density Lipoprotein; NEFA = Non-Esterified Fatty Acids

5.3.10 – Postprandial Glucose

The postprandial glucose response to the sequential meal tests ingested before and after the 20-day interventions is shown in **Figure 5.10**. No interaction effects emerged for any aspect of this profile, including incremental area under curve, peak concentration and time to peak concentration (**Table 5.4**). Furthermore, this was unaffected when these outcomes were determined separately for each meal.

5.3.11 – Postprandial Insulin

The postprandial insulin response to the sequential meal tests ingested before and after the 20-day interventions is shown in **Figure 5.11**. Once again, no interaction effects emerged for any aspect of this profile, including incremental area under curve, peak concentration and time to peak concentration (**Table 5.4**). Furthermore, this was unaffected when these outcomes were determined separately for each meal.

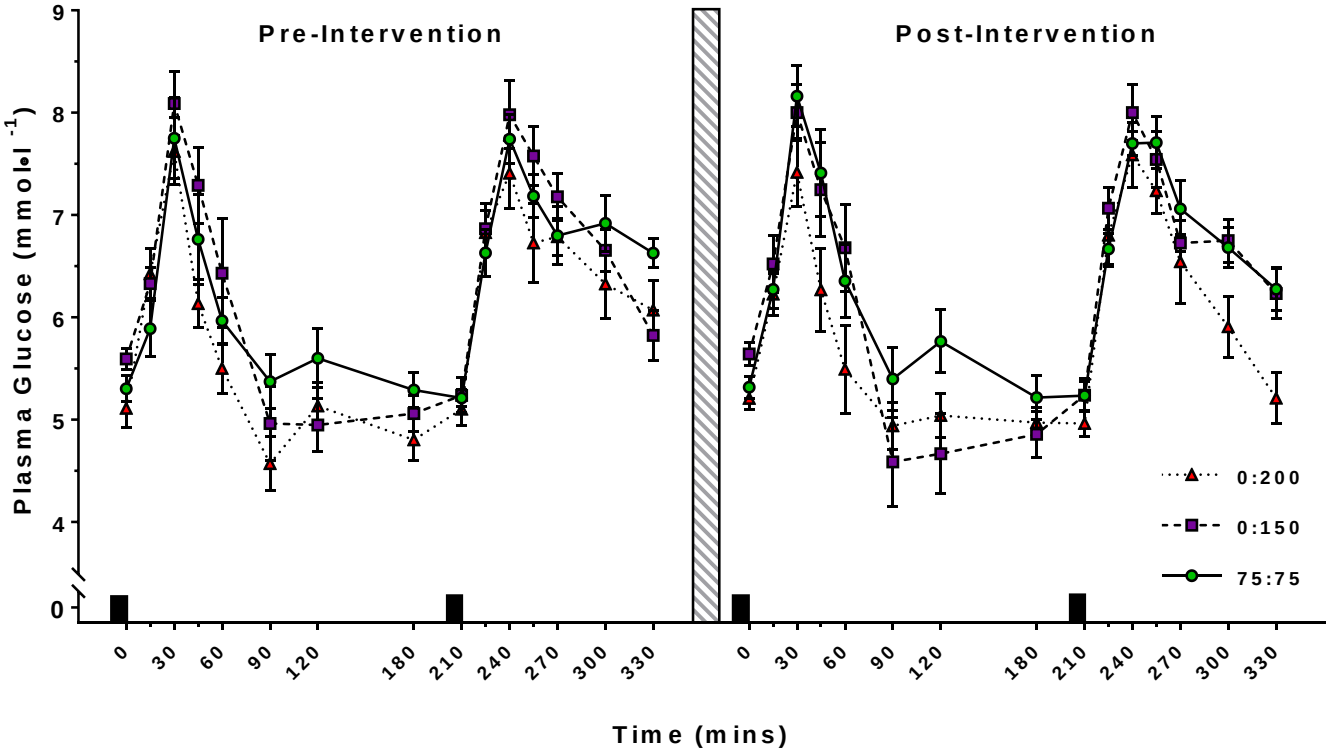

Figure 5.10: Postprandial plasma glucose profiles in response to two sequential meal tests conducted at pre- and post-intervention in the daily calorie restriction (75:75), intermittent fasting with restriction (0:150) and intermittent fasting without restriction (0:200) groups. The white box on the x-axis represents the 20-day intervention and the black boxes on the x-axis denote the timing of prescribed meals. No interaction effects were identified by a three-way ANOVA (group*time*timepoint; p=0.35). Data are presented as mean ± SEM.

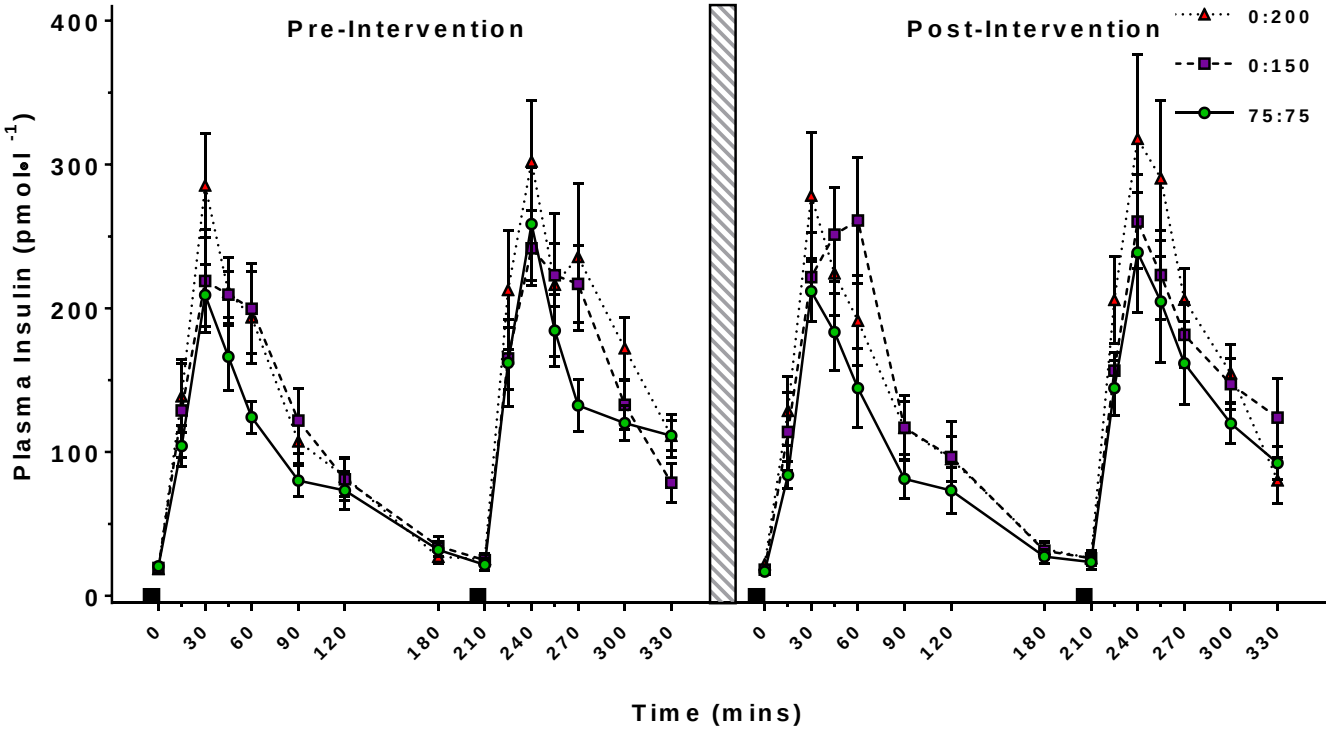

Figure 5.11: Postprandial plasma insulin profiles in response to two sequential meal tests conducted at pre- and post-intervention in the daily calorie restriction (75:75), intermittent fasting with restriction (0:150) and intermittent fasting without restriction (0:200) groups. The white box on the x-axis represents the 20-day intervention. The black boxes on the x-axis denote the timing of prescribed meals. No interaction effects were identified by a three-way ANOVA (group*time*timepoint; p=0.61). Data are presented as mean ± SEM.

5.3.12 – Postprandial Triacylglycerol

The postprandial triacylglycerol response to the sequential meal tests ingested before and after the 20-day interventions is shown in **Figure 5.12**. Once again, no interaction effects emerged, although the total area under curve for triglyceride concentrations approached significance for a time effect when meal 2 was analysed separately (time, p=0.08). As shown in the figure, this seemed to be driven by reductions in postprandial triacylglycerol concentration in response to the 0:150 diet.

5.3.13 – Postprandial NEFA

The postprandial NEFA response to the sequential meal tests ingested before and after the 20-day interventions is shown in **Figure 5.13**. Once again, no interaction effects emerged for any aspect of this profile (**Table 5.4**) and this was unaffected when each meal was analysed separately.

5.3.14 – Postprandial Glycerol

No interaction effects emerged for any aspect of the postprandial glycerol response (**Table 5.4**) and this was unaffected when each meal was analysed separately. However, due to the variable performance of the glycerol assay the postprandial profile is not shown and any results should be treated with caution.

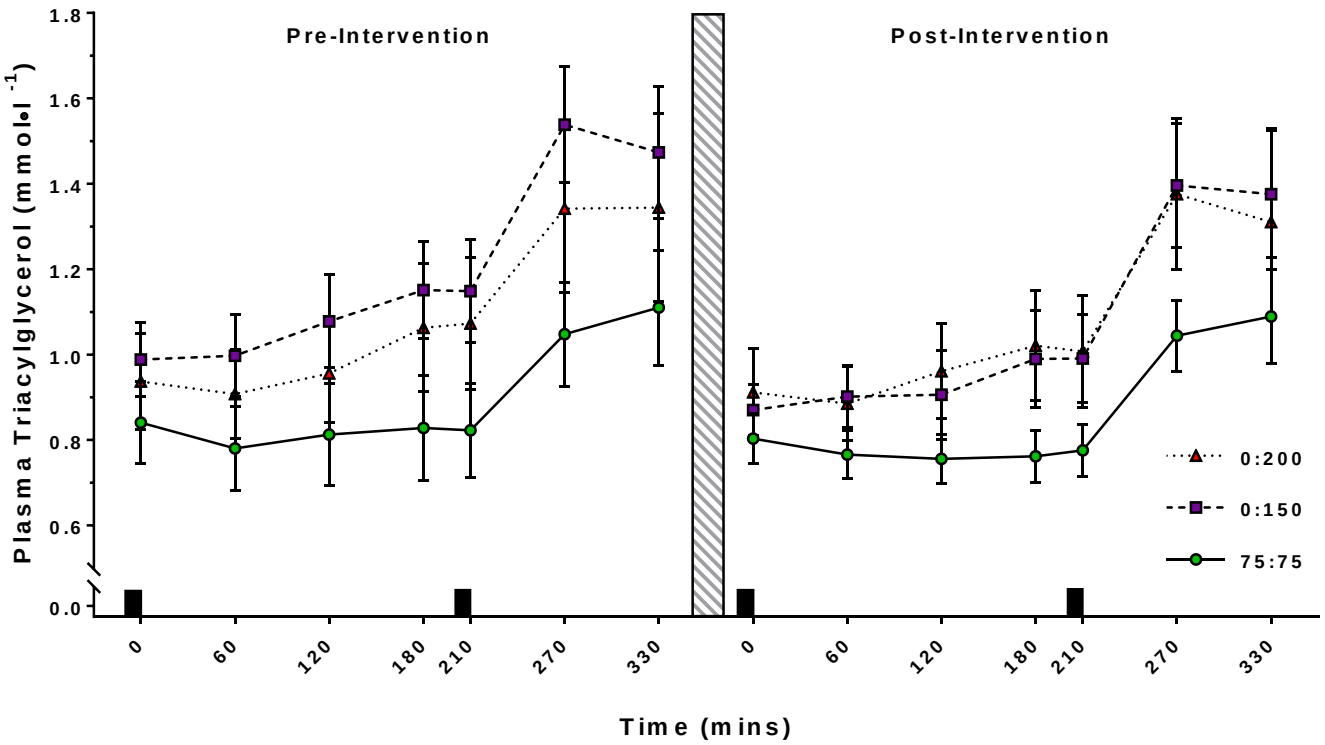

Figure 5.12: Postprandial plasma triacylglycerol profiles in response to two sequential meal tests conducted at pre- and post-intervention in the daily calorie restriction (75:75), intermittent fasting with restriction (0:150) and intermittent fasting without restriction (0:200) groups. The white box on the x-axis represents the 20-day intervention. The black boxes on the x-axis denote the timing of prescribed meals. No interaction effects were identified by a three-way ANOVA (group*time*timepoint; p=0.85). Data are presented as mean ± SEM.

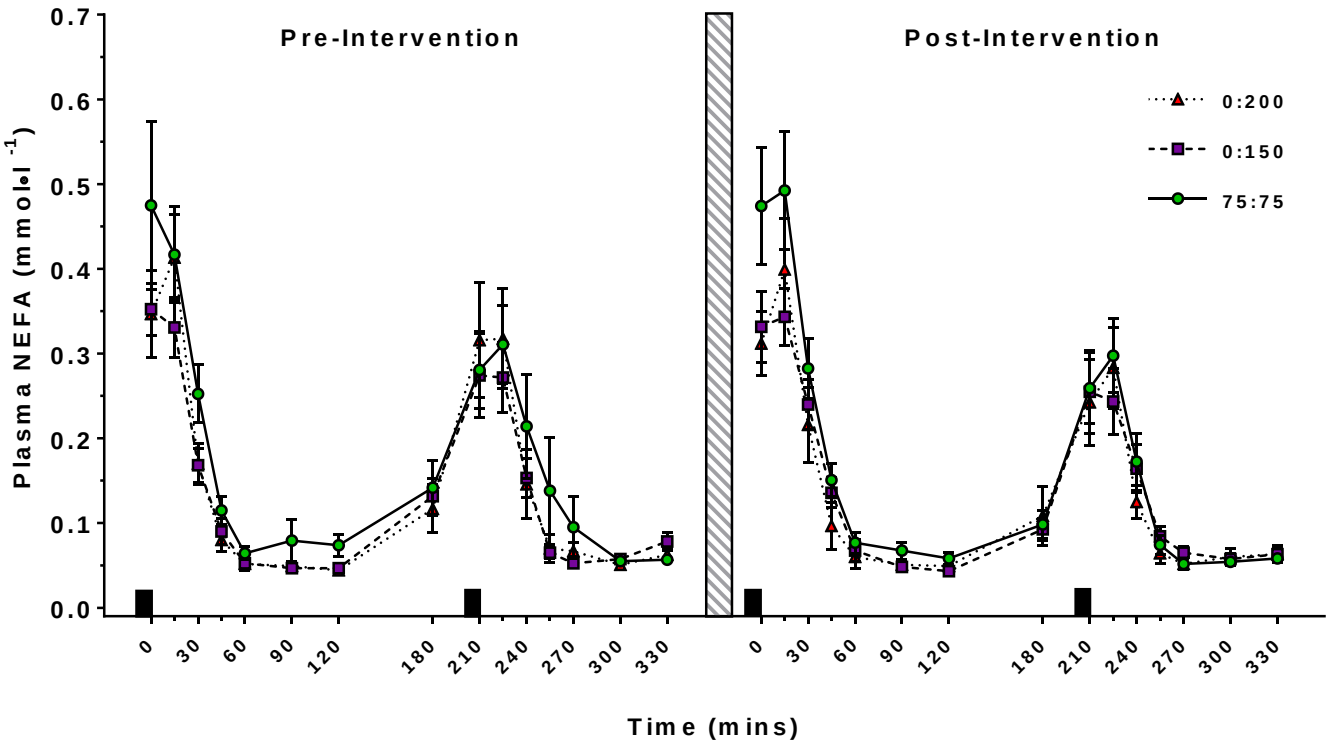

Figure 5.13: Postprandial plasma NEFA profiles in response to two sequential meal tests conducted at pre- and post-intervention in the daily calorie restriction (75:75), intermittent fasting with restriction (0:150) and intermittent fasting without restriction (0:200) groups. The white box on the x-axis represents the 20-day intervention. The black boxes on the x-axis denote the timing of prescribed meals. No interaction effects were identified by a three-way ANOVA (group*time*timepoint; p=0.68). Data are presented as mean ± SEM.

5.4 – Discussion

The present study established the effects of a diet combining intermittent fasting with calorie restriction on energy expenditure and metabolic health, compared to energy-matched (i.e. hypocaloric) daily calorie restriction and intermittent fasting *without* calorie restriction. In accordance with these prescriptions, energy intake was necessarily reduced to a similar degree in the two energy-restricted diets but maintained in the intermittent fasting without calorie restriction group. This pattern was also mirrored by expected changes in body mass, although fat mass was reduced to a greater degree by daily calorie restriction when compared to intermittent fasting. Most interestingly, the primary outcome revealed a decrease in physical activity energy expenditure upon combining intermittent fasting with calorie restriction when compared to intermittent fasting without calorie restriction, whilst physical activity was remarkably stable in response to daily calorie restriction. However, despite these changes in body mass/composition and physical activity levels, health outcomes ware largely unaltered by the three interventions.

The reduction in physical activity energy expenditure when combining intermittent fasting with calorie restriction is a key observation in this study. This differed from the slight increase seen when undertaking intermittent fasting without calorie restriction, whilst physical activity in the daily calorie restriction group was unperturbed. This is the first study to quantify changes in physical activity energy expenditure during complete alternate-day fasting, both alone and in combination with calorie restriction. Coupling this fasting model with the use of objective methods to quantify physical activity (i.e. combined heart rate/accelerometry), which show good agreement with free-living reference standards (Brage *et al.*, 2015), most likely explains the contradiction of prior studies on this topic. Both Klempel *et al.* (2010) and Hoddy *et al.* (2016) suggest that physical activity is maintained during 8-weeks of intermittent fasting despite weight loss. However, both of these studies employed daily step counts as a proxy for physical activity, the limitations of which are well-known (Crouter *et al.*, 2003; Corder, Brage and Ekelund, 2007). Although a similar conclusion was drawn by Klempel *et al.* (2012) based on accelerometry data from another 8-week intermittent fasting protocol, the intervention only required fasting on one day per week and recruited those reporting low levels of habitual activity at baseline. Instead,

the present results show good agreement with the findings of Betts *et al.* (2014), who observed reductions in physical activity energy expenditure in response to extended morning fasting in lean adults.

Collectively, these findings therefore suggest that neither prolonged fasting intervals nor the imposed energy restriction in isolation were sufficient to meaningfully alter physical activity thermogenesis. Instead, their combined influence in the intermittent fasting with calorie restriction condition seems to be exerting an interactive effect to elicit a change despite relatively small decreases in body mass. The observed tendency for reduced physical activity thermogenesis on fast days compared to fed days in response to intermittent fasting would certainly be consistent with an adaptive/compensatory response to prolonged fasting, which is perhaps exacerbated by behavioural adaptations to the energy deficit, as is often seen with longer periods of daily calorie restriction (Martin *et al.*, 2011). This would also be in keeping with the reduction being driven by decreases in sedentary and light activities, which is characteristic of a reduction in spontaneous physical activity rather than a conscious decision (Betts *et al.*, 2016). However, there are two potential confounding influences which could also explain the apparent decrease in physical activity on fasted days when compared to fed.

Firstly, instead of an adaptive response, these changes in activity between fasted and fed days may be capturing an effect of the intermittent fasting protocol. For instance, the reduction in light activity during fasting may reflect the reduction in food preparation time (Ainsworth *et al.*, 2011), or perhaps an ability to get up later in the morning as no time needs to be allowed for breakfast consumption. However, such a decrease in response to fasting would be apparent in both the 0:150 and 0:200 groups, when light activity was largely stable during fasted periods in the 0:200 condition (-1±69 kcal·day^{-1}). A second possibility is that these divergent responses are instead capturing an acute effect of feeding on heart rate (Matsumoto *et al.*, 2001; Walhin *et al.*, 2013). The proposed increase in response to feeding could cause overestimation during overfeeding and perhaps underestimation in response to fasting, which does align with the patterns shown in Figure 5.9a. Although, if true, this effect would be expected to manifest as differences in moderate-vigorous intensity activity on fed and fasted days. This is because the branched-equation model from which these estimates

are derived assigns a larger weighting to heart rate data at these higher intensities, meaning this acute feeding effect would be amplified there (Brage *et al.*, 2004). Consequently, this is difficult to reconcile with the present pattern in which lower intensity activities seem to be more responsive to acute feeding, as at these intensities the estimates are determined almost entirely by accelerometry data.

This therefore proposes that imposing calorie restriction through complete alternate-day fasting may lead to greater adaptive declines in physical activity thermogenesis than alternative methods. Consequently, such dietary strategies should place emphasis on maintaining levels of physical activity given its importance to long-term weight loss maintenance (Elfhag and Rossner, 2005; Thomas *et al.*, 2014). The reduction incurred was equivalent to approximately 30 minutes of walking per day (Ainsworth *et al.*, 2011), thereby providing a useful prescription which could be easily self-monitored using wearable technologies (Chowdhury *et al.*, 2017). Although the proposed confounding influences are difficult to align with the changes seen, they should nonetheless be kept in mind and highlight the need to replicate this finding using alternative methods. This is particularly pertinent in light of the findings of Muller *et al.* (2015); following 3 weeks of daily calorie restriction, they observed a reduction in the energetic cost of physical activity whilst physically active behaviours themselves were unchanged. If present here, such an adaptive increase in efficiency would not be captured by combined heart rate/accelerometry methods. The use of doubly-labelled water stands as the most obvious alternative for capturing such free-living data (Brage *et al.*, 2015), and this would overcome the influence of acute feeding state on heart rate to isolate a behavioural adaptation of some degree. However, this is instead prone to confounding influences arising from changes in substrate metabolism in such an extreme feeding model (Hall *et al.*, 2018), and would not provide the resolution needed to examine temporal changes in activity patterns.

Shifting to focus on changes in body composition, another key finding emerging from the present study is that fat mass was reduced to a greater degree by daily calorie restriction when compared to intermittent fasting, despite similar overall weight losses. In part, this is likely to be mediated by the larger reduction in fat intake with daily calorie restriction when compared to the two intermittent fasting conditions. This is supported by Hall *et al.* (2015), who observed that dietary fat restriction results in more

pronounced negative fat balance than dietary carbohydrate restriction, even when matched for energy intake. However, given that the discrepancy in the reduction in fat intake between the two energy-restricted groups was only was only 14 grams per day in absolute terms, this is unlikely to be the sole explanation (Galgani and Ravussin, 2008). Instead, the explanation for this disparity may lie in the proposed regulatory pathways that underpin rates of muscle protein synthesis and breakdown, given that skeletal muscle mass constitutes a substantial fraction of whole-body protein stores (Milan *et al.*, 2015; Abdulla *et al.*, 2016).

In response to prolonged fasting, reductions in muscle protein synthesis have been observed (Felig, 1975; Vendelbo *et al.*, 2014), which shifts the dynamic equilibrium in which this tissue exists in favour of muscle protein breakdown (Atherton and Smith, 2012; Dickinson and Rasmussen, 2013; Tipton, Hamilton and Gallagher, 2018). Consequently, the residual amino acids resulting from this imbalance can instead be utilised in gluconeogenesis, allowing protein to make a meaningful contribution to whole-body energy metabolism and reducing reliance on endogenous fat stores (Owen *et al.*, 1979; Carlson, Snead and Campbell, 1994; Soeters *et al.*, 2012). In contrast, the maintenance of typical meal patterns in the daily calorie restriction group is likely to have provided a regular influx of amino acids to stimulate muscle protein synthesis despite the imposed energy restriction (Areta *et al.*, 2013), resulting in fewer amino acids being available for use in gluconeogenic pathways.

Collectively, this reduction in protein synthesis and increased use of amino acids in energy-producing pathways when combining this approach to intermittent fasting with calorie restriction may also explain the contrasts with prior literature. Contrary to the present observation that fat mass decreases more readily in response to daily calorie restriction, in a systematic review of randomised-controlled trials, Varady (2011) concluded that intermittent and continuous approaches to calorie restriction are equally effective in reducing body mass and fat mass, but that intermittent calorie restriction may offer enhanced retention of lean mass. A similar conclusion was also drawn by a more recent review comparing intermittent approaches with very-low calorie dieting (Alhamdan *et al.*, 2016). However, of the seven trials of intermittent approaches included in the Varady (2011) review, only two utilised the complete alternate-day approach as was employed in the current study. Instead, the majority of these studies

utilised a modified alternate-day approach (Patterson and Sears, 2017), in which participants were prescribed 15-25% of their habitual intake during fasting periods. Even a low dose of amino acids such as this can stimulate muscle protein synthesis (Areta *et al.*, 2013), thereby encouraging the incorporation of amino acids into skeletal muscle tissue. The distinct effect of fasting observed in the present study may therefore depend on the imposition of fasting inferring a complete absence of such anabolic signals as opposed to a mere reduction.

It is therefore understandable why the present data regarding changes in fat mass with intermittent fasting show better agreement with the findings of Heilbronn, Smith, *et al.* (2005), who applied a 24-hour complete fast on alternate days for 3 weeks. Specifically, fat mass decreased by 0.8 kg whilst lean mass decreased by 0.6 kg, which shows good agreement with the 0.8 kg decreases in both fat and lean mass seen in response to the intermittent fasting with calorie restriction diet in the present study. Unfortunately, that study did not feature a comparative daily restriction arm, but the review of Varady (2011) suggests that short-term continuous energy restriction typically decreases fat mass by ~10% and lean mass by ~1%, which contrasts well with the respective 9.7% and 0.2% reductions seen in response to the daily calorie restriction group in the present study. It is also worthy of note that the lack of change in body mass and composition in response to intermittent fasting without calorie restriction affirms the previous findings of both Halberg *et al.* (2005) and Soeters *et al.* (2009), who applied a 20-hour complete fast on alternate days for two weeks.

The stability of resting metabolic rate in response to all three interventions when adjusted for changes in body mass was also unexpected, particularly in the daily calorie restriction group. This is because adjusting resting metabolic rate for changes in body mass aims to isolate adaptive responses, which have been consistently reported following daily calorie restriction of varying degrees and durations (Redman *et al.*, 2009; Rosenbaum and Leibel, 2010; Martin *et al.*, 2011; Redman and Ravussin, 2011; Dhurandhar *et al.*, 2015). Although it could be argued that the current intervention duration was insufficient to invoke such an adaptive response, Muller *et al.* (2015) observed adaptive declines in resting energy expenditure following just one week of 50% calorie restriction, which remained significant after three weeks of calorie restriction following adjustment for changes in body composition. The scale of this

change was also similar to that observed by Friedlander *et al.* (2005) following 3 weeks of 40% calorie restriction, on which the power calculation for this study was based.

This would appear to suggest that the relative stability of resting metabolic rate in the present study can therefore be ascribed to the lesser degree of calorie restriction imposed, reaching 27% in the daily restriction group. This is reflected by the smaller reductions in body mass, decreasing by 2.7% from pre-intervention values in the present study compared to the 5.4% and 4.8% reductions observed by Muller *et al.* (2015) and Friedlander *et al.* (2005), respectively. However, the observed changes in fat mass in the present study were also broadly paralleled by changes in leptin concentration, which decreased by 42% and 22% in the daily calorie restriction and intermittent fasting with calorie restriction groups, respectively. Given suggestions that the adipokine leptin plays a prominent role in initiating the adaptive changes in energy expenditure often seen with energy restriction (Rosenbaum *et al.*, 2005; Rosenbaum, Hirsch, *et al.*, 2008; Kluge *et al.*, 2010; Mullur, Liu and Brent, 2014; Morton *et al.*, 2015), it would be reasonable to expect that a change of this magnitude would invoke an adaptive response of some degree.

Although this outcome therefore seems somewhat discordant with current understanding, a similar observation was made by Heilbronn et al. (2005) in response to their study of complete alternate-day fasting, which invoked similar changes in both body mass and composition. Consequently, this instead points to the notion of a leptin threshold, wherein circulating concentrations need to decrease below a critical level in order to activate specific responses (Dardeno et al., 2010; Rosenbaum et al., 2014). In light of this, the degree of weight loss achieved may need to be considered when examining these adaptive changes in future studies. However, whilst increasing the duration of the intervention could have potentially established meaningful effects on resting metabolic rate, this would not be advisable based on participant feedback. As such, if future studies were to explore this further, increasing the degree of caloric restriction imposed by the energy-restricted conditions would be the best course.

Lastly, both fasting and postprandial health outcomes were unaffected by all three interventions. This stands in stark contrast to the few randomised–controlled trials conducted thus far, which identify improvements in fasted metabolic health outcomes

that are similar but not superior to those arising from daily calorie restriction (Barnosky *et al.*, 2014; Carter, Clifton and Keogh, 2016; Conley *et al.*, 2018). Two studies by Harvie *et al.* (2011, 2013) suggest that intermittent approaches may actually offer greater improvements in insulin resistance. Focusing instead on postprandial changes, Antoni, Johnston, *et al.* (2018) observed greater reductions in lipaemia and C-peptide concentration in response to intermittent energy restriction when compared to continuous. As discussed earlier for resting metabolic rate, upon considering the comparatively short duration and minimal weight losses in the present study, the lack of an effect on metabolic health is perhaps not surprising, given that weight losses of 5% or more are believed to be necessary to improve biomarkers of disease risk (Williamson, Bray and Ryan, 2015). This possibility is reinforced by the agreement of the present findings with those of Heilbronn, Smith, *et al.* (2005) and Soeters *et al.* (2009), who employed similar durations of intermittent fasting and observed similarly null results.

These recurrent issues of intervention duration and weight loss magnitude were not simple oversights. A key part of the rationale for this study was rooted in using prolonged fasting periods to provide more opportunities for clearance of surplus lipids implicated in hyperlipidaemia and insulin resistance, particularly those in ectopic stores (McArdle *et al.*, 2013; Rutkowski, Stern and Scherer, 2015; Wensveen *et al.*, 2015; Spalding *et al.*, 2017). As such, the study was designed to capture this therapeutic potential, in which intermittent fasting could reasonably be expected to offer improvements in health outcomes beyond those attributable to weight loss alone (Section 2.3.2). The lack of an effect on metabolic health therefore raises a different consideration: the capacity for improvement from baseline. Considering the relatively healthy metabolic profile of this lean cohort at baseline, it stands to reason that surplus lipids are unlikely to be compromising their metabolic health to a great degree, meaning that the benefits they stand to gain from this routine extension of fasting are constrained (Sparks *et al.*, 2017).

Collectively, this suggests that imposing an energy restriction through a complete alternate-day approach to intermittent fasting in lean individuals may attenuate the decline in fat mass and prompt more profound adaptive declines in physical activity thermogenesis. Coupling this with the lack of an effect on metabolic health outcomes

means that such approaches are unlikely to be beneficial in this population. However, there is a definite need to explore these facets of intermittent fasting in the context of more substantial energy deficits and clinical populations, to fully unmask the therapeutic potential of this particular format.

Chapter 6: Impact of intermittent fasting on energy balance and associated health outcomes in overweight and obese adults.

6.1 – Introduction

With over 50% of the UK population currently classified as overweight or obese (NHS Digital, 2017), finding more effective strategies to help manage the condition and the accompanying metabolic dysfunction remains imperative (Wang *et al.*, 2011). Intermittent fasting is a dietary strategy in which typical patterns of food and drink consumption are punctuated by scheduled periods of energy restriction or abstinence from all energy-providing nutrients. Work by various groups has consistently shown that such an approach is associated with reductions in body mass and improvements in markers of metabolic health in obese participants (Johnson *et al.*, 2007; Varady, Bhutani, *et al.*, 2009; Bhutani *et al.*, 2010; Varady, 2011; Harvie *et al.*, 2011; Barnosky *et al.*, 2014; Headland *et al.*, 2016). However, the majority of studies conducted thus far utilise a modified approach, in which participants are permitted to consume 15-25% of habitual energy intake during fasted periods (Patterson and Sears, 2017). This dramatically reduces the resulting uninterrupted fasting interval, meaning that these studies may not fully capture the therapeutic potential of these diets in resolving metabolic dysfunction (McQuaid *et al.*, 2011; Soeters *et al.*, 2012; Rutkowski, Stern and Scherer, 2015; Anton *et al.*, 2018). Comparatively, with complete alternate-day fasting, the uninterrupted fasting period is extended to 20-24 hours on every other day (Patterson and Sears, 2017). In providing a protracted fasting opportunity, this latter format may constitute a more effecaious approach in this context (Anton *et al.*, 2018), yet studies of complete alternate-day fasting are both scarce and contradictory.

A particularly pertinent question relates to the impact of baseline adiposity and metabolic health, which may explain some of the disparate findings in the prior literature. The study of Heilbronn and colleagues applied a 3-week complete alternate-day fasting intervention to a cohort of 16 non-obese adults and observed sexually-dimorphic responses (Heilbronn, Civitarese, *et al.*, 2005; Heilbronn, Smith, *et al.*,

2005). Men experienced improvements in fasted triglyceride concentrations and postprandial insulin sensitivity, whilst women saw increases in HDL cholesterol concentration and reductions in postprandial glucose tolerance (Heilbronn, Civitarese, *et al.*, 2005; Heilbronn, Smith, *et al.*, 2005). However, beyond the difference in sex, women also had a more favourable metabolic profile at baseline, including better blood lipid profile and lower glucose concentrations (Heilbronn, Civitarese, *et al.*, 2005; Heilbronn, Smith, *et al.*, 2005). Consequently, it can be argued that the group with worse metabolic health benefitted more substantially from the intervention (Sparks *et al.*, 2017). Such a proposition also helps to reconcile the conflicting results of Halberg *et al.* (2005) and Soeters *et al.* (2009), considering that the respective body fat percentages of their cohorts were 20.1% and 14.8%. Therefore, the lean nature of participants in most prior studies of complete alternate-day fasting may not have allowed the therapeutic potential of this dieting modality to be fully elucidated.

To date, only Catenacci *et al.* (2016) have explored the effects of complete alternate-day fasting in obese adults, contrasting it against daily calorie restriction in an 8-week randomised-controlled trial. The two diets were matched for macronutrient balance but not energy intake, with both energy intake and body mass being reduced to a greater extent by the intermittent fasting condition. This was also accompanied by reductions in fasting glucose concentration following intermittent restriction, although fasting lipid profile was similarly improved by both interventions and dynamic tests of metabolic control were unaffected. However, comparisons were compromised by baseline differences, with those in the daily restriction group presenting with higher body mass and fasting insulin concentration, thus necessitating further investigation.

Another intriguing consideration in overweight and obese populations is the adaptive changes in energy expenditure that may arise in response to intermittent fasting. Although Chapter 5 identified a reduction in physical activity energy expenditure upon combining intermittent fasting with calorie restriction, this may be unique to the lean cohort. For instance, Betts *et al.* (2014) established that extending the overnight fast to midday in lean adults resulted in adaptive declines in 24-hour physical activity energy expenditure. However, an ensuing study in obese adults revealed that 24-hour physical activity thermogenesis was not affected by extended morning fasting (Chowdhury *et al.*, 2016). This notion is consistent with prior suggestions that obesity occurs as a

result of an inability to adequately align energy intake with energy expenditure, meaning that baseline adiposity may actually reflect a fundamental difference in how energy balance is regulated (Hill, Wyatt and Peters, 2012; Church and Martin, 2018). Consequently, establishing how intermittent fasting modifies energy use in overweight and obese adults also stands as a key question, which needs to be addressed.

To build upon this limited understanding of how complete alternate-day fasting may impact upon energy balance and health in overweight and obese adults, the present study has three core objectives:

1. To establish whether intermittent fasting elicits compensatory changes in the components of energy balance in overweight/obese adults, and to compare these against those arising from energy-matched daily calorie restriction.

2. To examine the effect of intermittent fasting on postprandial metabolism in overweight/obese adults relative to energy-matched daily calorie restriction.

3. To explore whether hypocaloric intermittent fasting affects the components of energy balance and postprandial metabolism independently from chronic energy imbalance in overweight/obese adults, by contrasting against eucaloric intermittent fasting.

Based on the literature discussed thus far, it was hypothesised that postprandial metabolism (i.e. glycaemia, insulinaemia) would be improved to a greater extent by the condition combining intermittent fasting with calorie restriction. This was predicated on the interaction between the favourable effects of calorie restriction (Larson-Meyer *et al.*, 2006; Ravussin *et al.*, 2015) and the provision of more opportunities for clearance of deleterious excess lipids (McQuaid *et al.*, 2011; Soeters *et al.*, 2012; Rutkowski, Stern and Scherer, 2015; Anton *et al.*, 2018). Focusing instead on energy expenditure, it was anticipated that resting metabolic rate, postprandial thermogenesis and physical activity energy expenditure would be unaffected by the two intermittent fasting conditions (Catenacci *et al.*, 2016; Chowdhury *et al.*, 2016), but reduced by the daily calorie restriction intervention (Rosenbaum, Hirsch, *et al.*, 2008; Martin *et al.*, 2011; Muller *et al.*, 2015).

6.2 – Methods

The methodology employed in this experiment was identical to the randomised-controlled trial detailed in Chapter 5 of this thesis. The only difference between the two resided in the inclusion criteria relating to body mass index (BMI) and fat mass index (FMI). In the present study, participants were initially classified as overweight/obese and recruited on the premise of a BMI value greater than 25.0 $kg \cdot m^{-2}$. This was subsequently confirmed using FMI based on data emerging from the pre-intervention dual energy x-ray absorptiometry (DEXA) scan, with values $\geq$7.5 $kg \cdot m^{-2}$ and $\geq$11.0 $kg \cdot m^{-2}$ classified as overweight/obese for men and women, respectively. All other aspects of eligibility, the experimental protocol (**Figure 6.1**), the dietary interventions (**Table 6.1**) and the approach to statistical analysis were unchanged. The protocol for this study was approved by the NHS Research Ethics Committee (reference: 15/SW/0007) and all participants provided written informed consent prior to any data being collected.

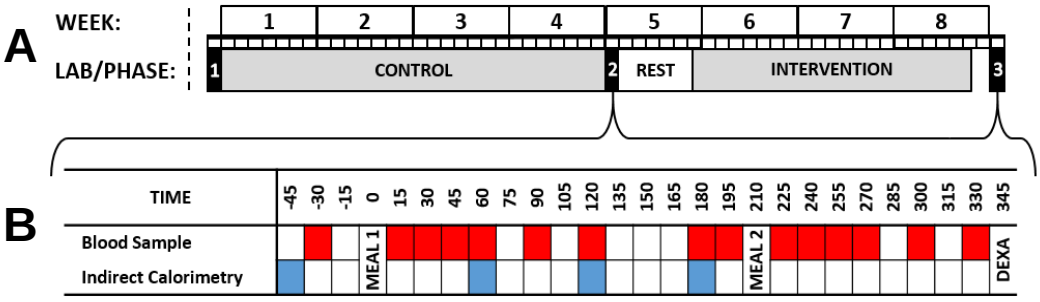

Figure 6.1: Schematic of the 8-week study design (A) and the sampling intervals for laboratory sessions 2 and 3 (B). *Abbreviations: DEXA = Dual Energy X-ray Absorptiometry.*

Table 6.1: Intervention arms employed in the study protocol

Intervention	Description
Daily calorie restriction (75:75)	Reduce normal intake by 25% every day
Intermittent fasting with calorie restriction (0:150)	Alternate between 24-hour periods of fasting and feeding (transitioning at 15:00), with 150% of normal intake on fed days.
Intermittent fasting without calorie restriction (0:200)	Alternate between 24-hour periods of fasting and feeding (transitioning at 15:00), with 200% of normal intake on fed days.

6.3 – Results

6.3.1 – Participants

The baseline characteristics of the participants in each intervention arm are shown in **Table 6.2**. In total, 26 participants provided informed consent for their participation with two withdrawing prior to the baseline lab session due to scheduling conflicts and the possibility of allocation to the 0:200 condition. Of the 24 who completed the baseline lab session, a further four withdrew during the control phase. Cited reasons for withdrawal at this stage were, time pressures (2), medical issues deemed unrelated to the study (1) and a desire to change diet/exercise habits (1). Two participants were also excluded during the control period; one for changes in medication use and one due to changes in body mass that exceeded the permissible range. Post-randomisation, only one participant withdrew from the study. This was in the 0:150 intervention and was due to illness which was deemed unrelated to the study (i.e. flu-like symptoms). Unfortunately, cannulation difficulties meant that postprandial blood samples were only obtained from three of the five participants randomised to the 0:150 group, whilst fasted blood samples were only available for four.

Table 6.2: Participant characteristics for the three intervention arms. Data are presented as mean ± SD.

Variable	Diet Allocation		
	75:75	**0:150** [1]	**0:200**
n	6	5	6
Age (years)	44 ± 8	48 ± 13	49 ± 13
Female (n)	5	4	4
Height (m)	1.714 ± 0.119	1.693 ± 0.066	1.703 ± 0.074
Body Mass (kg)	98.7 ± 16.2	93.2 ± 7.0	85.4 ± 9.4
BMI ($kg \cdot m^{-2}$)	33.6 ± 4.9	32.6 ± 3.7	29.4 ± 1.2
FMI ($kg \cdot m^{-2}$)	14.13 ± 2.62	13.11 ± 3.17	11.06 ± 1.83
PAL [2]	1.51 ± 0.13	1.55 ± 0.16	1.61 ± 0.20
RMR ($kcal \cdot day^{-1}$)	1711 ± 266	1671 ± 145	1500 ± 165
Fasting Plasma Glucose ($mmol \cdot l^{-1}$)	5.45 ± 0.40	6.05 ± 1.14	5.37 ± 0.66
Fasting Plasma Insulin ($pmol \cdot l^{-1}$)	42.61 ± 19.94	71.36 ± 19.22	40.72 ± 27.46
HOMA-IR [3]	1.74 ± 0.86	3.24 ± 1.09	1.57 ± 0.98
Plasma Total Cholesterol ($mmol \cdot l^{-1}$)	4.75 ± 0.69	5.00 ± 1.22	4.92 ± 0.98
Plasma LDL Cholesterol ($mmol \cdot l^{-1}$)	3.17 ± 0.71	3.35 ± 0.55	3.31 ± 1.14
Plasma HDL Cholesterol ($mmol \cdot l^{-1}$)	1.39 ± 0.38	1.29 ± 0.30	1.32 ± 0.24
Fasting Plasma Triacylglycerol ($mmol \cdot l^{-1}$)	1.37 ± 1.18	1.36 ± 0.59	1.19 ± 0.35

[1] FMI and fasting biochemistry parameters based on n = 4 for the 0:150 group.

[2] PAL = Total Daily Energy Expenditure/RMR

[3] HOMA-IR = (fasted insulin $\mu IU \cdot ml^{-1}$ * fasted glucose $mmol \cdot l^{-1}$)/22.5

Abbreviations: BMI = Body Mass Index; FMI = Fat Mass Index; PAL = Physical Activity Level; RMR = Resting Metabolic Rate; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; LDL= Low-Density Lipoprotein; HDL = High-Density Lipoprotein

6.3.2 – Body Composition

Changes in body mass and composition are shown in **Table 6.3**. Body mass was stable throughout the control phase in all three groups in accordance with the requirements of the study (75:75 = +0.1±0.5 kg, 0:150 = +0.0±0.5 kg, 0:200 = -0.1±0.7 kg). In response to the intervention, an interaction effect (group*time, $p<0.01$) was apparent for body mass, with the decreases seen in response to the 75:75 and 0:150 conditions differing from the 0:200 group (Δ-between, $p=0.02$ vs 75:75, $p=0.01$ vs 0:150), in which body mass was unchanged. However, the decreases in body mass seen within the two energy-restricted groups were not different from one another (Δ-between, $p=0.60$). This pattern was also mirrored by changes in BMI (group*time, $p<0.01$), whilst no meaningful changes were apparent in measurements of waist circumference (group*time, $p=0.30$).

Interestingly, despite these changes in body mass, no group-level differences emerged for measures of fat mass (group*time, $p=0.19$) or lean mass (group*time, $p=0.11$), although time effects were evident for both. Based on the data shown in **Table 6.3**, the time effect for fat mass (time, $p=0.01$) appears to have been driven primarily by a decrease in the 75:75 group, whilst the time effect for lean mass (time, $p<0.01$) can be attributed to decreases seen in response to the two energy-restricted conditions. Both fat mass and lean mass were comparatively stable in the 0:200 group despite the lack of an interaction. A similar pattern for time but not interaction effects was also apparent for both FMI (time, $p=0.01$) and visceral fat mass (time, $p=0.08$).

Table 6.3: Body composition outcomes. Data are mean (SD) at pre- and post-intervention, with delta change and 95% confidence intervals (CI) for the response within each group.

Outcome	75:75 (n=6)			0:150 (n=5)[1]			0:200 (n=6)			Two-way ANOVA		
	PRE	POST	Δ from PRE	PRE	POST	Δ from PRE	PRE	POST	Δ from PRE	Interaction	Time	Group
Body Mass (kg)	98.8 (16.3)	96.0 (15.9)	**-2.8 *[C] (-3.9, -1.7)**	93.2 (6.8)	90.7 (7.0)	**-2.5 *[C] (-2.8, -2.1)**	85.4 (8.9)	84.9 (8.3)	-0.5 [A,B] (-1.2, 0.3)	**<0.01**	**<0.01**	0.22
Body Mass Index (kg·m^{-2})	33.6 (4.9)	32.7 (5.0)	**-0.9 *[C] (-1.3, -0.6)**	32.6 (3.6)	31.8 (3.6)	**-0.9 *[C] (-1.0, -0.7)**	29.4 (1.1)	29.2 (1.0)	-0.1 [A,B] (-0.4, 0.1)	**<0.01**	**<0.01**	0.19
Waist Circumference (cm)	103.7 (12.4)	104.3 (11.6)	0.6 (-3.8, 5.0)	105.2 (8.7)	102.5 (8.2)	-2.7 (-4.2, -1.1)	102.9 (6.5)	100.3 (6.9)	-2.6 (-5.1, -0.1)	0.30	0.12	0.88
Fat Mass (kg)	40.2 (5.4)	38.8 (5.6)	-1.4 (-2.0, -0.7)	37.1 (6.8)	36.4 (7.4)	-0.8 (-1.9, 0.4)	31.5 (4.5)	31.2 (3.8)	-0.2 (-1.1, 0.6)	0.19	**0.01**	0.06
Fat Mass Index (kg·m^{-2})	13.8 (2.6)	13.4 (2.7)	-0.5 (-0.7, -0.2)	12.8 (3.1)	12.6 (3.2)	-0.3 (-0.7, 0.1)	10.9 (1.9)	10.8 (1.6)	-0.1 (-0.4, 0.2)	0.21	**0.01**	0.19
Lean Mass (kg)	58.6 (13.2)	57.2 (13.1)	-1.4 (-2.2, -0.6)	56.5 (3.9)	54.7 (3.1)	-1.8 (-2.8, -0.9)	53.9 (9.5)	53.7 (8.6)	-0.2 (-1.4, 1.0)	0.11	**<0.01**	0.78
Visceral Fat Mass (g)	835.6 (273.5)	788.2 (304.3)	-47.4 (-106.2, 11.5)	688.6 (264.7)	679.0 (243.3)	-9.5 (-67.2, 48.1)	742.1 (225.2)	715.6 (243.6)	-26.6 (-55.9, 2.8)	0.60	0.08	0.73

[1] Dual-Energy X-ray Absorptiometry data based on n=4 due to data loss

* denotes $p \leq 0.05$ for within-group time effect (paired t-test)

[A] denotes $p \leq 0.05$ for between-group change score vs. 75:75 (independent samples t-test)

[B] denotes $p \leq 0.05$ for between-group change score vs. 0:150 (independent samples t-test)

[C] denotes $p \leq 0.05$ for between-group change score vs. 0:200 (independent samples t-test)

6.3.3 – Energy Intake

Energy intake, and the contributions of the four macronutrients to this, is illustrated in **Figure 6.2**. The change in energy intake from the control to the intervention phase differed between groups (group*time, $p<0.01$). This highlighted a similar reduction in the 75:75 and 0:150 groups (75:75 = -573±184 kcal·day^{-1}, 0:150 = -678±430 kcal·day^{-1}; Δ-between, $p=0.63$), which contrasted with the maintenance of energy intake in the 0:200 group (+92±180 kcal·day^{-1}; Δ-between, $p<0.01$ vs 75:75, $p=0.03$ vs 0:150).

Upon examining the changes in macronutrient consumption driving these reductions, the change in absolute carbohydrate (group*time, $p=0.01$), fat (group*time, $p<0.01$) and protein intake (group*time, $p=0.01$) was considered meaningful. Focusing initially on the 75:75 group, carbohydrate and fat intake were reduced by 60±28 g·day^{-1} and 31±8 g·day^{-1}, respectively, whilst there was a trend for reduced protein intake (-13±13 g·day^{-1}). Conversely, the 0:150 group reduced both fat (-24±17 g·day^{-1}) and protein consumption (-20±8 g·day^{-1}), which was accompanied by a trend for reduced carbohydrate intake (-88±79 g·day^{-1}). However, none of these reductions differed between the two energy-restricted conditions (Δ-between, all $p\geq0.31$), with significance only established for comparisons against the 0:200 group in which macronutrient intake was unaltered. Specifically, fat intake was reduced in both groups relative to the 0:200 condition (Δ-between, $p<0.01$ vs 75:75, $p=0.02$ vs 0:150), whilst the decrease in carbohydrate and protein intake was only meaningfully different from the 0:200 group for the 75:75 (Δ-between, $p<0.01$ vs 0:200) and 0:150 (Δ-between, $p=0.01$ vs 0:200) groups, respectively. There were no time, group or interaction effects for alcohol intake.

These changes in macronutrient intake resulted in between-group differences in macronutrient balance, with changes apparent for protein-derived energy intake (group*time, $p=0.05$) and fat-derived energy intake (group*time, $p=0.06$). This reflected that the reduction in fat-derived energy intake in the 75:75 diet (-4±3%) tended to differ from the stability seen in response to the 0:150 diet (+2±4%; Δ-between, $p=0.06$ vs 75:75). This was compensated for by an increase in protein-derived energy intake in the 75:75 group (+2±1%), which contrasted with the stability of the 0:200 group (-1±2%; Δ-between, $p=0.06$ vs 75:75). Collectively, this suggests

that fat intake was decreased more readily in response to the 75:75 diet, whilst macronutrient balance was preserved in the two intermittent fasting interventions.

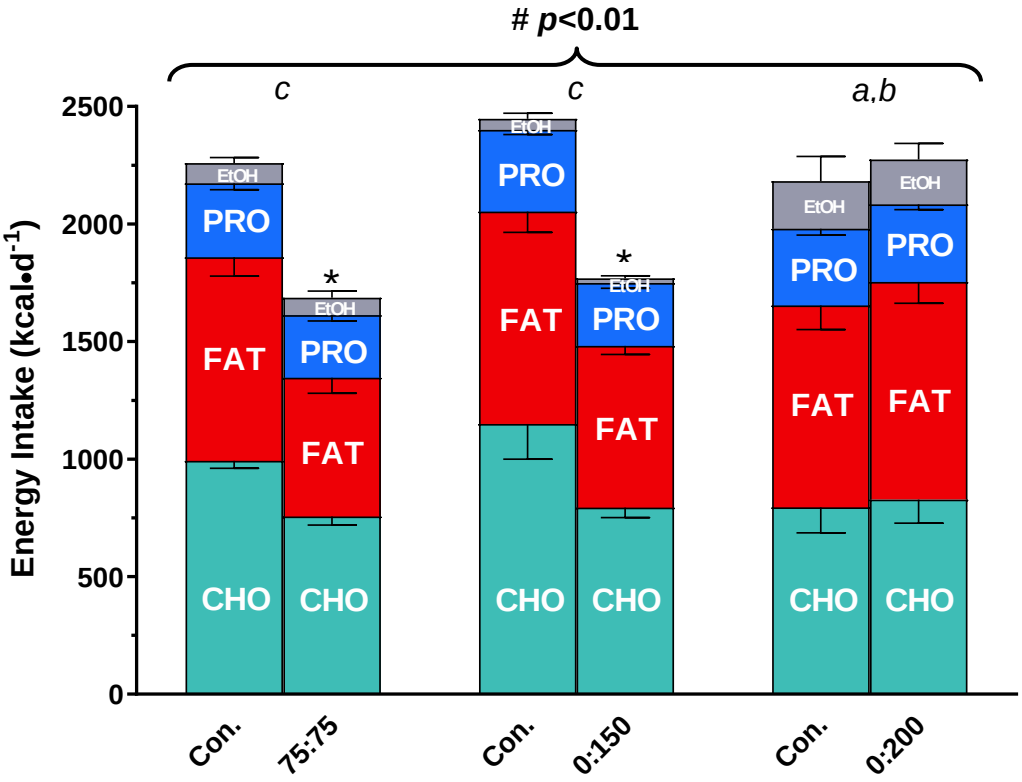

Figure 6.2: Energy and macronutrient intake during the control and intervention phases in the daily calorie restriction (75:75), intermittent fasting with calorie restriction (0:150) and intermittent fasting without calorie restriction groups (0:200). Energy derived from the respective macronutrients was derived by multiplying the reported intake in grams by the accompanying Atwater general factor. # denotes $p \leq 0.05$ for group*time interaction (two-way ANOVA). * denotes $p \leq 0.05$ for within-group time effect (paired t-test). a, b and c denote $p \leq 0.05$ for between-group change score vs. 75:75, 0:150 and 0:200, respectively (independent t-test). Data are presented as mean and SEM. Abbreviations: Con = control; CHO = carbohydrate; FAT = lipid; PRO = protein; EtOH = alcohol.

6.3.4 – Energy Expenditure

Total daily energy expenditure during the control and intervention phases in each of the three groups is shown in **Figure 6.3**, as a sum of resting metabolic rate, diet-induced thermogenesis and physical activity thermogenesis. No meaningful interaction effects were apparent (group*time, p=0.58), with only the time effect achieving significance (time, p=0.02). Although this seemed to be driven by a tendency for energy expenditure to decrease more readily in response to the 0:150 diet (-106±97 kcal·day^{-1}), all three groups followed a similar pattern.

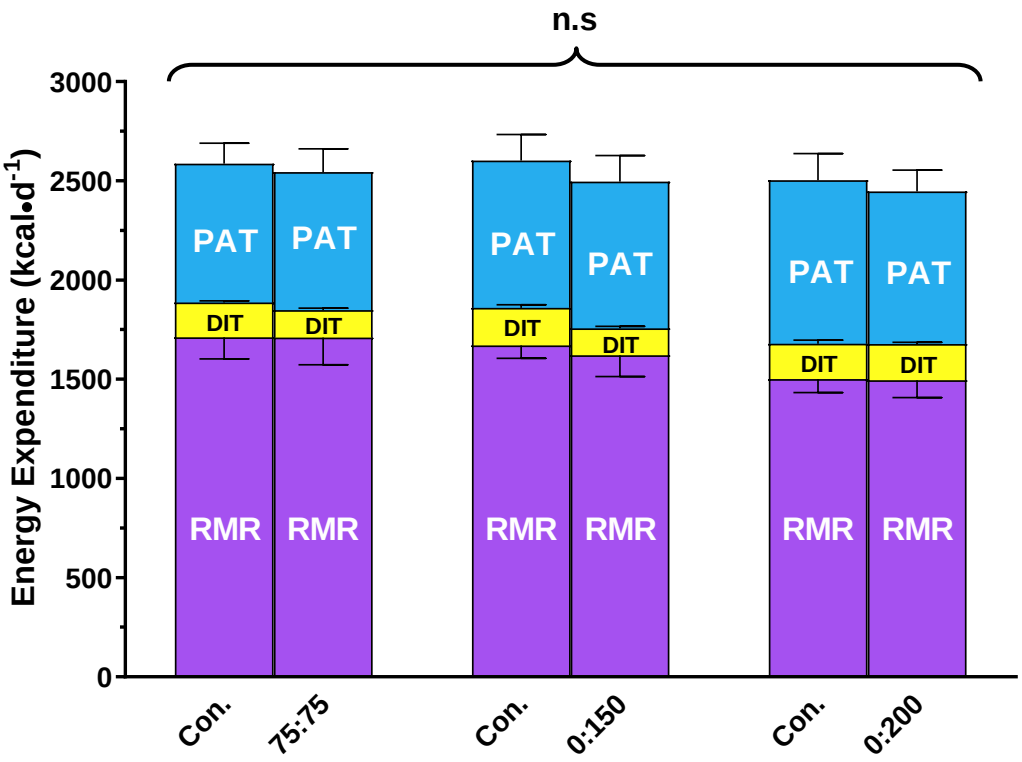

Figure 6.3: Total daily energy expenditure as a product of the three main components during the control and intervention phases in the daily calorie restriction (75:75), intermittent fasting with calorie restriction (0:150) and intermittent fasting without calorie restriction groups (0:200). RMR was measured via indirect calorimetry during the pre- and post-intervention lab sessions, DIT was estimated by multiplying reported intakes by the equivalents proposed by Westerterp (2004), and PAT was measured using Actiheart™ monitors. # denotes $p \leq 0.05$ for group*time interaction (two-way ANOVA). * denotes $p \leq 0.05$ for within-group time effect (paired t-test). a, b and c denote $p \leq 0.05$ for between-group change score vs. 75:75, 0:150 and 0:200, respectively (independent t-test). Data are presented as mean and SEM. Abbreviations: Con = control; RMR = resting metabolic rate; DIT = diet-induced thermogenesis; PAT = physical activity thermogenesis.

6.3.5 – Energy Balance

For clarity, the energy balance data discussed thus far are summarised in **Figure 6.4**. This serves to better visualise the effect of the three interventions on energy intake and the resultant impacts upon the components of energy expenditure, which was a primary outcome in this study. However, direct comparisons between intake and expenditure data were deemed inappropriate in light of the different measurement techniques employed and the associated limitations (Hall *et al.*, 2012).

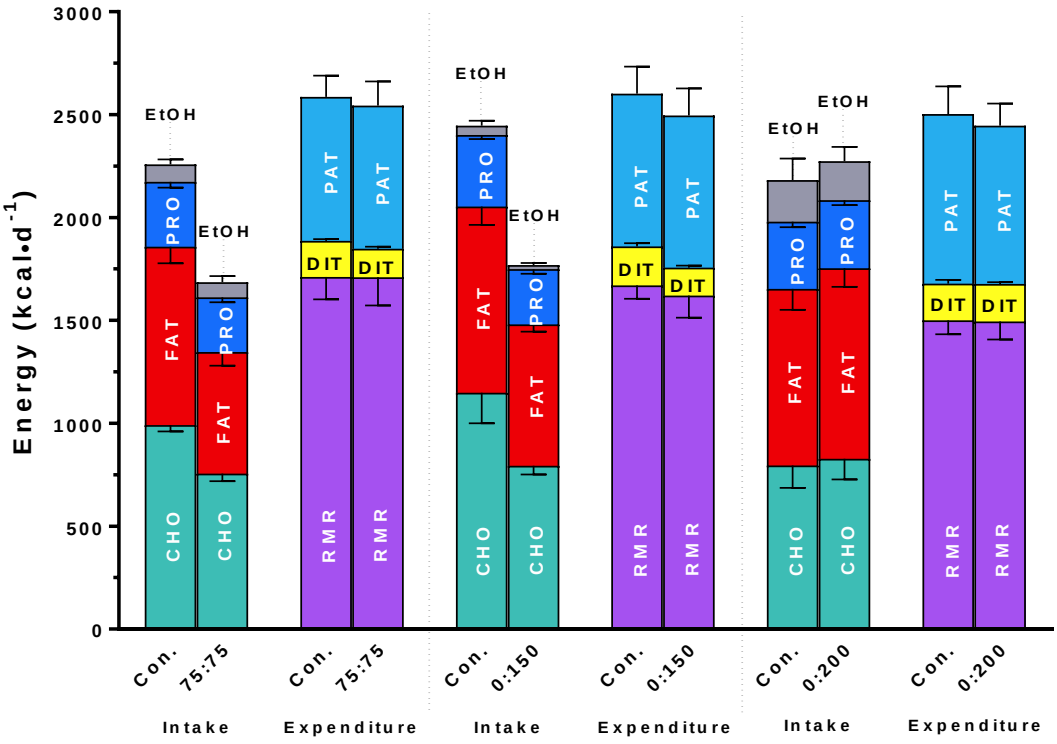

Figure 6.4: Components of energy intake and energy expenditure measured during the control and intervention phases in the daily calorie restriction (75:75), intermittent fasting with calorie restriction (0:150) and intermittent fasting without calorie restriction groups (0:200). Data are presented as mean and SEM. Abbreviations: Con = control; CHO = carbohydrate; FAT = lipid, PRO = protein; EtOH = alcohol; RMR = resting metabolic rate; DIT = diet-induced thermogenesis; PAT = physical activity thermogenesis.

6.3.6 – Metabolic Rate

Following adjustment for changes in body mass, resting metabolic rate was unchanged by the three interventions (75:75 = +0.49±1.49 kcal·kg^{-1}·day^{-1}, 0:150 = -0.14±1.29 kcal·kg^{-1}·day^{-1}, 0:200 = +0.00±1.20 kcal·kg^{-1}·day^{-1}), characterising neither an interaction (group*time, p=0.69) nor a time effect (time, p=0.74). Furthermore, although cumulative substrate oxidation rate tended to differ between groups (group*time, p=0.07), this was not attributable to specific changes in either carbohydrate (group*time, p=0.09) or fat oxidation (group*time, p=0.09) in isolation. However, **Figure 6.5** does suggest that the two energy-restricted conditions may be exerting differential effects on substrate oxidation, with the change in respiratory exchange ratio approaching significance for the interaction (75:75 = +0.05±0.10, 0:150 = -0.06±0.08, 0:200 = +0.01±0.03; group*time, p=0.08).

Conversely, in the postprandial state, the change in metabolic rate seen in response to the intervention tended to differ across diet allocations when averaged across the 3-hour measurement window (group*time, p=0.06). This trend seemed to be driven primarily by an increase in the 0:150 group relative to a slight decrease in the 75:75 group (75:75 = -0.19±0.78 kcal·kg^{-1}·day^{-1}, 0:150 = +1.34±1.14 kcal·kg^{-1}·day^{-1}, 0:200 = +0.46±0.95 kcal·kg^{-1}·day^{-1}). However, no further differences were apparent upon examining changes in substrate oxidation **(Figure 6.6)**, including total rates (group*time; p=0.86), as well as carbohydrate (group*time, p=0.95), fat (group*time, p=0.68) and protein (group*time, p=0.94) oxidation. The only effect worthy of mention was a time effect for postprandial carbohydrate oxidation (time, p=0.03), reflecting the increase seen in response to all three interventions.

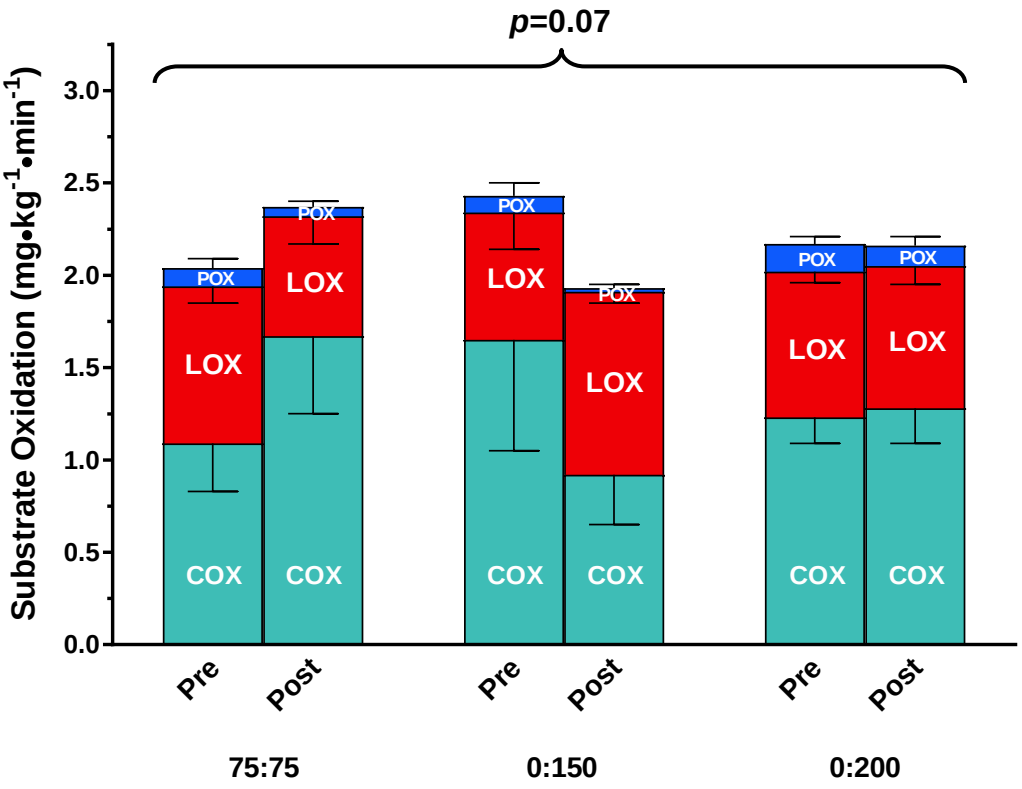

Figure 6.5: Total fasting substrate oxidation as accounted for by each macronutrient at pre- and post-intervention in the daily calorie restriction (75:75), intermittent fasting with calorie restriction (0:150) and intermittent fasting without calorie restriction groups (0:200). # denotes $p \leq 0.05$ for group*time interaction (two-way ANOVA). * denotes $p \leq 0.05$ for within-group time effect (paired t-test). a, b and c denote $p \leq 0.05$ for between-group change score vs. 75:75, 0:150 and 0:200, respectively (independent t-test). Data are presented as mean and SEM. Abbreviations: COX = carbohydrate oxidation; LOX = lipid oxidation; POX = protein oxidation.

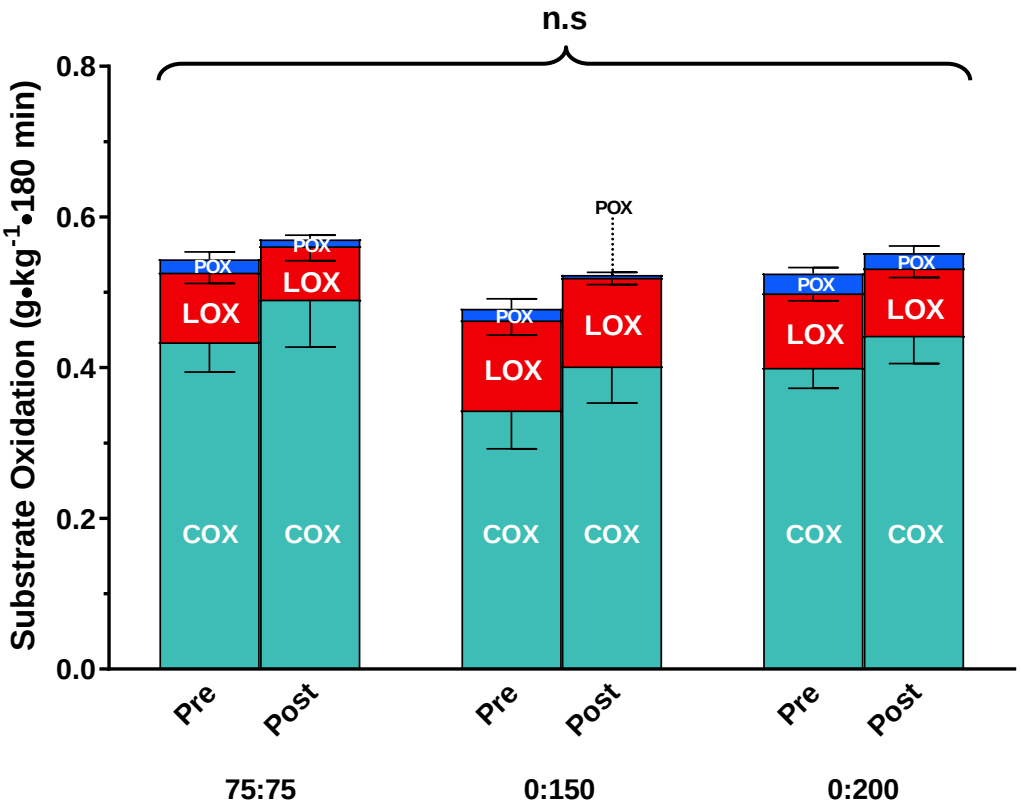

Figure 6.6: Average substrate oxidation throughout the 3-hour postprandial period presented as a sum of measured oxidation of specific macronutrients at pre- and post-intervention in the daily calorie restriction (75:75), intermittent fasting with calorie restriction (0:150) and intermittent fasting without calorie restriction groups (0:200). # denotes $p \leq 0.05$ for group*time interaction (two-way ANOVA). * denotes $p \leq 0.05$ for within-group time effect (paired t-test). a, b and c denote $p \leq 0.05$ for between-group change score vs. 75:75, 0:150 and 0:200, respectively (independent t-test). Data are presented as mean and SEM. Abbreviations: COX = carbohydrate oxidation; LOX = lipid oxidation; POX = protein oxidation.

6.3.7 – Diet-Induced Thermogenesis

Given that diet-induced thermogenesis (**Figure 6.7**) was estimated from reported intakes of the four main macronutrients, the effects of the interventions on this outcome broadly align with those discussed previously for energy intake (Section 6.3.4). The interaction effect (group*time, $p<0.01$) revealed that there was a comparable decrease in diet-induced thermogenesis from the control to the intervention phase in both the 75:75 and 0:150 groups (75:75 = -38±17 kcal·day^{-1}, 0:150 = -53±28 kcal·day^{-1}; Δ-between, $p=0.31$), both of which differed from the 0:200 group (+3±24 kcal·day^{-1}; Δ-between, $p=0.02$ vs 75:75, $p=0.02$ vs 0:150). Accordingly, this was accompanied by differential changes in the thermogenic fraction derived from carbohydrate (group*time, $p=0.01$), fat (group*time; $p<0.01$) and protein (group*time; $p=0.01$) across groups. Briefly, the thermogenic fractions derived from carbohydrate and fat decreased during the 75:75 diet compared to the 0:200 condition (Δ-between, all $p<0.01$), whilst for the 0:150 group these differences were apparent for the reduction in fat and protein thermogenesis relative to the 0:200 condition (Δ-between, all $p\leq0.02$ vs 0:200).

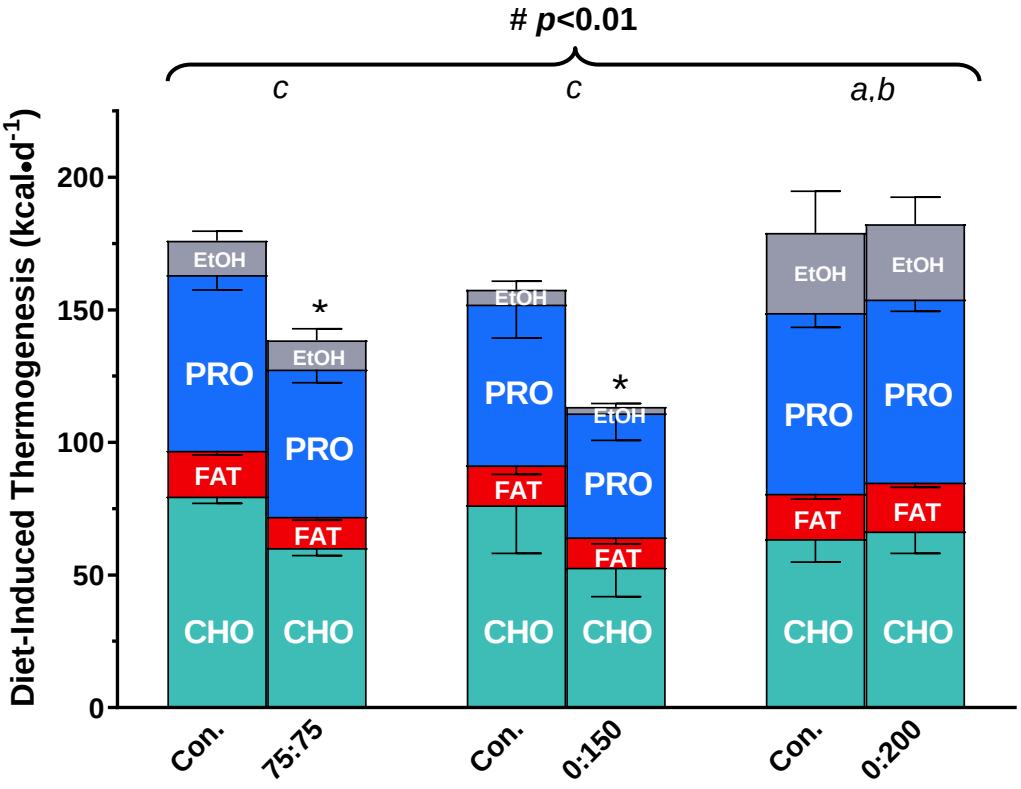

Figure 6.7: Diet induced thermogenesis as a product of the thermogenic fractions of the four main macronutrients during the control and intervention phases for the daily calorie restriction (75:75), intermittent fasting with calorie restriction (0:150) and intermittent fasting without calorie restriction groups (0:200). Estimates were made by multiplying reported intakes in the respective phases by the equivalents proposed by Westerterp (2004). # denotes $p \leq 0.05$ for group*time interaction (two-way ANOVA). * denotes $p \leq 0.05$ for within-group time effect (paired t-test). a, b and c denote $p \leq 0.05$ for between-group change score vs. 75:75, 0:150 and 0:200, respectively (independent t-test). Data are presented as mean and SEM. Abbreviations: Con = control; CHO = carbohydrate; FAT = lipid; PRO = protein; EtOH = alcohol.

6.3.8 – Physical Activity Thermogenesis

Physical activity thermogenesis, as measured by individually-calibrated Actiheart^{TM} monitors, is shown in **Figure 6.8**. This clearly illustrates the largely stable nature of physical activity thermogenesis across the control and intervention phases in all groups, with no time or interaction effects for either the global value or the calories expended in specific intensity thresholds (all $p \geq 0.12$). The figure does appear to suggest a decrease in moderate activity energy expenditure during the 0:200 diet, but this was highly variable between participants (-58±78 kcal·day^{-1}).

When the changes in physical activity thermogenesis seen from the control to the intervention phase (**Figure 6.9a**) were plotted separately for fasted and fed cycles in the 0:150 and 0:200 groups (**Figure 6.9b**), once again no meaningful interactions emerged (group*time, all $p \geq 0.30$). Although a clear pattern is apparent upon visual inspection of the data, with physical activity decreasing during fasted periods and increasing during fed periods (**Figure 6.9**), no time effect was apparent. However, several time effects did approach significance for the calories expended at specific intensities of activity, which does allude to differential activity patterns on fasted and fed days. This was most apparent for the calories expended through vigorous activities (time, $p < 0.01$) and sedentary activities (time, $p = 0.08$), which tended to be lower on fasted days but preserved on fed days in both the 0:150 and 0:200 groups. The figure also suggests a similar pattern for light activity, but this did not achieve significance for a time effect (time, $p = 0.09$).

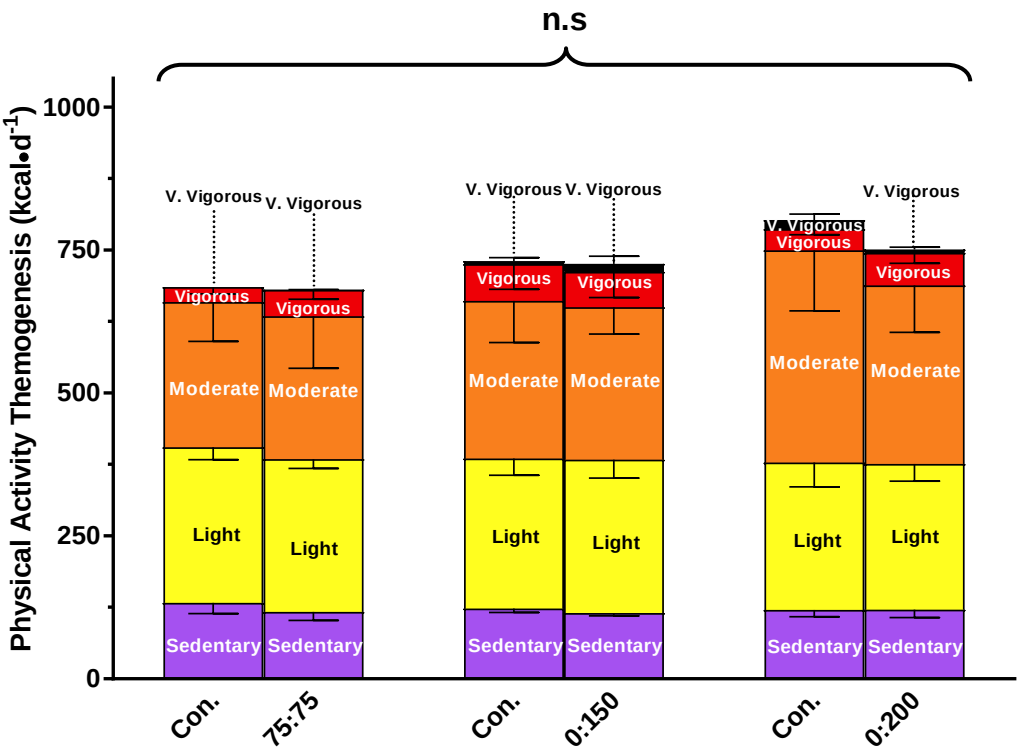

Figure 6.8: Physical activity thermogenesis presented as a sum of the kilocalories expended at differing intensities of activity during the control and intervention phases for the daily calorie restriction (75:75), intermittent fasting with calorie restriction (0:150) and intermittent fasting without calorie restriction groups (0:200). Intensity thresholds were defined as multiples of resting metabolic rate (i.e. METs), as measured at pre-intervention, using the following ranges: sedentary = ≤1.5 METs, light = 1.5-2.9 METs, moderate = 3.0-5.9 METs, vigorous = 6.0-10.1 METs, v.vigorous = ≥10.2 METs. # denotes $p \leq 0.05$ for group*time interaction (two-way ANOVA). * denotes $p \leq 0.05$ for within-group time effect (paired t-test). a, b and c denote $p \leq 0.05$ for between-group change score vs. 75:75, 0:150 and 0:200, respectively (independent t-test). Data are presented as mean and SEM. Abbreviations: Con = control; v.vigorous = very vigorous.

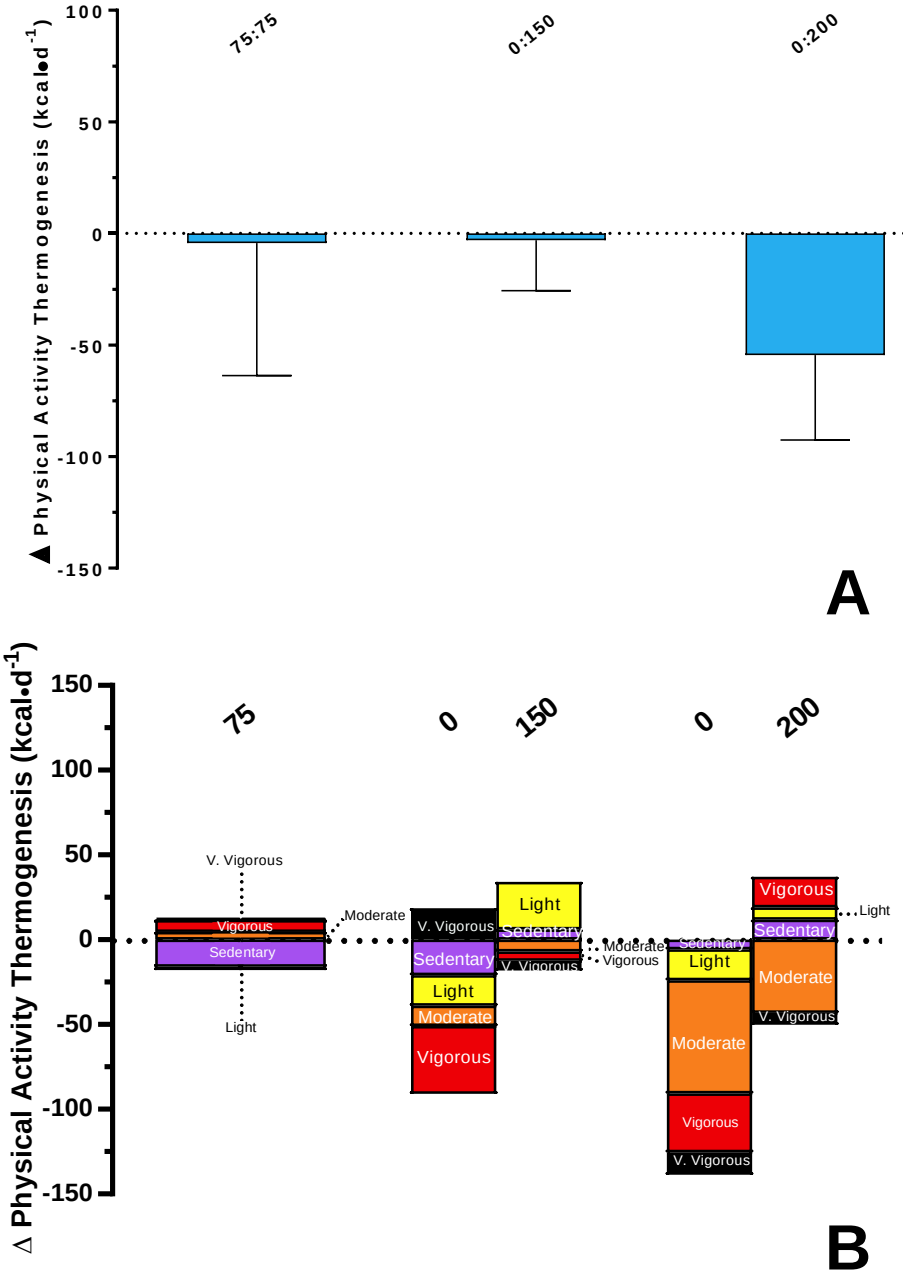

Figure 6.9: Change in physical activity thermogenesis from the control to the intervention phase (A) as a sum of the kilocalories expended at differing intensities of activity during fasted and fed periods (B) for intermittent fasting with calorie restriction (0:150) and intermittent fasting without calorie restriction groups (0:200). Intensity thresholds were defined as multiples of resting metabolic rate (i.e. METs), as measured prior to the intervention, using the following ranges: sedentary = ≤1.5 METs, light = 1.5-2.9 METs, moderate = 3.0-5.9 METs, vigorous = 6.0-10.1 METs, v.vigorous = ≥10.2 METs. # denotes $p \leq 0.05$ for group*time interaction (two-way ANOVA). * denotes $p \leq 0.05$ for within-group time effect (paired t-test). a, b and c

denote $p \leq 0.05$ for between-group change score vs. 75:75, 0:150 and 0:200, respectively (independent t-test). Data are presented as mean and SEM. Abbreviations: Con = control; v.vigorous = very vigorous.

6.3.9 – Fasting Biochemistry

All fasting biochemistry outcomes are shown in **Table 6.4**. Fasting blood lipids appeared to be more heavily impacted by the interventions than fasting glycaemia and insulinaemia, which were largely stable. Changes in total cholesterol neared significance for an interaction (group*time, $p=0.08$), which was mainly driven by a decrease in LDL cholesterol in response to the 0:150 diet. Plasma triacylglycerol concentrations were also differentially affected by the interventions (group*time, $p=0.04$), highlighting the increase in the 0:200 group relative to the other groups, although the specific within-group change in 0:200 was not different from either the 75:75 or 0:150 groups. The change in plasma glycerol concentration was also different across groups (group*time, $p=0.05$), specifically the 75:75 and 0:150 conditions (Δ-between, $p=0.03$), but this should be viewed conservatively due to the variable performance of the assay in this cohort. Intrestingly, the change in plasma leptin concentration was not different across groups (group*time, $p=0.60$), with only a time effect emerging (time, $p=0.04$), which was driven primarily by decreases in response to the two energy-restricted conditions.

Table 6.4: Fasting and postprandial biochemistry outcomes. Data are mean (SD) at pre- and post-intervention, with delta change and 95% confidence intervals (CI) for the response within each group.

Outcome	75:75 (n=6)			0:150 (n=4)[2]			0:200 (n=6)			Two-way ANOVA		
	PRE	POST	Δ from PRE	PRE	POST	Δ from PRE	PRE	POST	Δ from PRE	Interaction	Time	Group
Plasma Glucose												
Fasted (mmol·l^{-1})	5.36 (0.64)	5.57 (0.43)	0.21 (-0.14, 0.55)	6.11 (1.10)	6.30 (0.80)	0.19 (–0.44, 0.81)	5.52 (0.88)	5.64 (0.54)	0.12 (-0.30, 0.54)	0.91	0.10	0.28
iAUC (mmol·l^{-1}·330 min)	269 (67)	250 (81)	-18 (-93, 57)	370 (102)	253 (82)	-118 (-575, 339)	337 (143)	309 (196)	-28 (-175, 119)	0.52	0.14	0.59
Meal 1 Peak (mmol·l^{-1})	**8.17** [B] **(1.31)**	7.93 (1.18)	-0.25 (-0.59, 0.10)	**10.07** [A] **(0.52)**	9.25 (0.83)	-0.82 (-2.43, 0.79)	8.56 (1.32)	8.82 (1.36)	0.26 (-0.46, 0.98)	**0.05**	0.10	0.20
Meal 2 Peak (mmol·l^{-1})	8.09 (1.23)	8.14 (1.23)	0.05 (-0.90, 1.00)	8.53 (0.63)	7.86 (0.80)	-0.67 (-4.08, 2.75)	8.15 (1.08)	8.15 (1.36)	0.00 (-0.49, 0.48)	0.48	0.39	0.99
Meal 1 Time to Peak (min)	33 (11)	35 (12)	3 (-16, 21)	45 (15)	40 (17)	-5 (-62, 52)	38 (13)	30 (9)	-8 (-27, 12)	0.65	0.53	0.28
Meal 2 Time to Peak (min)	20 (8)	33 (6)	13 (1, 24)	55 (38)	60 (30)	5 (-17, 27)	55 (34)	40 (26)	-15 (-62, 32)	0.30	0.92	0.08
Plasma Insulin												
Fasted (pmol·l^{-1})	34.03 (22.47)	43.82 (24.74)	9.79 (-0.80, 20.38)	69.14 (26.58)	66.76 (38.86)	-2.37 (-29.99, 25.24)	34.70 (12.18)	33.70 (8.70)	-1.01 (-11.28, 9.27)	0.23	0.50	0.08
iAUC (nmol·l^{-1}·330 min)	80.03 (59.90)	86.27 (49.00)	6.23 (-17.63, 30.09)	104.02 (13.22)	79.25 (15.59)	-24.78 [C] (-53.16, 3.60)	68.44 (36.63)	76.07 (42.58)	7.62 [B] (-4.86, 20.11)	**0.04**	0.45	0.81
Meal 1 Peak (pmol·l^{-1})	593.09 (428.29)	614.51 (452.97)	21.42 (-67.95, 110.79)	721.18 (92.64)	717.55 (246.32)	-3.63 (-398.40, 391.15)	554.80 (298.73)	555.83 (259.28)	1.03 (-102.66, 104.72)	0.92	0.83	0.80
Meal 2 Peak (pmol·l^{-1})	694.38 (557.55)	843.05 (459.79)	148.67 (-158.59, 455.92)	683.92 (271.11)	451.02 (259.99)	-232.90 (-645.49, 179.69)	425.15 (199.60)	527.22 (203.75)	102.07 (53.89, 150.24)	**0.05**	0.92	0.38
Meal 1 Time to Peak (min)	33 (15)	35 (8)	3 (-13, 18)	55 (9)	50 (9)	-5 (-27, 17)	35 (12)	43 (15)	8 (-6, 21)	0.42	0.65	0.07
Meal 2 Time to Peak (min)	30 (16)	30 (9)	0 (-20, 20)	35 (17)	25 (9)	-10 (-67, 47)	43 (28)	48 (33)	5 (-28, 38)	0.71	0.81	0.33
HOMA-IR[1]	1.42 (1.02)	1.84 (1.10)	0.42 (-0.05, 0.90)	3.28 (1.61)	3.27 (2.02)	-0.01 (-1.53, 1.50)	1.39 (0.48)	1.39 (0.33)	0.01 (-0.44, 0.46)	0.42	0.38	**0.04**
Plasma Total Cholesterol												
Fasted (mmol·l^{-1})	4.80 (0.70)	4.57 (0.69)	-0.24 (-0.65, 0.18)	4.74 (0.59)	4.11 (0.56)	-0.64 (-1.01, -0.26)	4.98 (1.12)	5.06 (0.59)	0.08 (–0.51, 0.67)	0.08	**0.04**	0.45

Table 6.4 cont.: Fasting and postprandial biochemistry outcomes. Data are mean (SD) at pre- and post-intervention, with delta change and 95% confidence intervals (CI) for the response within each group.

Outcome	75:75 (n=12)			0:150 (n=12)			0:200 (n=12)			Two-way ANOVA		
	PRE	POST	Δ from PRE	PRE	POST	Δ from PRE	PRE	POST	Δ from PRE	Interaction	Time	Group
Plasma HDL Cholesterol												
Fasted (mmol·l^{-1})	4.80 (0.70)	4.57 (0.69)	-0.24 (-0.65, 0.18)	4.74 (0.59)	4.11 (0.56)	-0.64 (-1.01, -0.26)	4.98 (1.12)	5.06 (0.59)	0.08 (–0.51, 0.67)	0.08	**0.04**	0.45
Plasma LDL Cholesterol												
Fasted (mmol·l^{-1})	3.29 (0.70)	3.05 (0.64)	-0.24 (-0.57, 0.09)	3.57 (0.40)	2.96 (0.33)	-0.62 (-1.00, -0.24)	3.58 (1.47)	3.48 (1.00)	-0.10 (-0.67, 0.47)	0.17	**0.01**	0.76
Plasma Triacylglycerol												
Fasted (mmol·l^{-1})	1.23 (0.73)	1.16 (0.59)	-0.07 (-0.24, 0.10)	1.16 (0.48)	0.97 (0.31)	-0.19 (-0.57, 0.19)	1.15 (0.33)	1.27 (0.41)	0.12 (-0.02, 0.26)	**0.04**	0.32	0.89
Total AUC (mmol·l^{-1}·330 min)	497 (301)	507 (238)	10 (-105, 125)	497 (285)	367 (153)	-131 (-653, 392)	466 (169)	525 (208)	60 (-18, 137)	0.13	0.55	0.90
Plasma NEFA												
Fasted (mmol·l^{-1})	0.34 (0.18)	0.35 (0.14)	0.02 (-0.19, 0.22)	0.49 (0.19)	0.53 (0.18)	0.04 (-0.16, 0.24)	0.36 (0.17)	0.40 (0.22)	0.04 (-0.06, 0.13)	0.95	0.44	0.38
Total AUC (mmol·l^{-1}·330 min)	57 (31)	50 (19)	-6 (-30, 18)	47 (27)	50 (25)	3 (-5, 11)	44 (9)	40 (9)	-4 (-17, 9)	0.73	0.62	0.58
Plasma Glycerol												
Fasted (mmol·l^{-1})	0.07 (0.05)	0.05 (0.03)	-0.02 [B] (-0.05,0.00)	0.04 (0.03)	0.06 (0.02)	0.03 [A] (-0.01,0.06)	0.05 (0.03)	0.04 (0.02)	-0.00 (-0.02,0.02)	**0.05**	0.85	0.50
Total AUC (mmol·l^{-1}·330 min)	15.11 (4.41)	14.41 (4.32)	-0.70 (-3.24, 1.84)	15.76 (5.00)	15.62 (8.97)	-0.14 (-10.11, 9.84)	11.97 (6.38)	14.01 (6.07)	2.04 (-0.50, 4.59)	0.29	0.31	0.61
Plasma Leptin												
Fasted (ng·ml^{-1})	41.71 (16.61)	37.22 (16.66)	-4.49 (-9.59, 0.61)	45.34 (26.40)	41.48 (23.36)	-3.87 (-12.27, 4.54)	34.90 (19.96)	33.60 (20.27)	-1.30 (-7.93, 5.36)	0.60	**0.04**	0.77

[1] HOMA-IR = (fasted insulin μIU·ml^{-1} * fasted glucose mmol·l^{-1})/22.5

[2] n=3 for all non-fasting measures

[A] denotes significantly different from 75:75 according to post-hoc independent samples t-test ($p \leq 0.05$)

[B] denotes significantly different from 0:150 according to post-hoc independent samples t-test ($p \leq 0.05$)

[C] denotes significantly different from 0:200 according to post-hoc independent samples t-test ($p \leq 0.05$)

Abbreviations: AUC = Area Under Curve; iAUC = Incremental Area Under Curve; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; LDL= Low-Density Lipoprotein; HDL = High-Density Lipoprotein; NEFA = Non-Esterified Fatty Acids

6.3.10 – Postprandial Glucose

The postprandial glucose response to the sequential meal tests ingested before and after the 20-day interventions is shown in **Figure 6.10**. Within this, only the change in peak concentration following meal 1 characterised an interaction (group*time, p=0.05), which was driven by baseline differences in pre-intervention values (75:75 = 8.17±1.31 mmol·l^{-1}, 0:150 = 10.07±0.52 mmol·l^{-1}; Δ-between, p=0.05). This was also accompanied by a time effect for incremental area under the meal 1 glucose curve (time, p=0.03), which was primarily accounted for by decreases in response to both the 75:75 and 0:150 conditions (75:75 = -28±22 mmol·l^{-1}·180min, 0:150 = -101±49 mmol·l^{-1}·180min).

6.3.11 – Postprandial Insulin

The postprandial insulin response to the sequential meal tests ingested before and after the 20-day interventions is shown in **Figure 6.11**. The change in incremental area under curve for the 5.5-hour postprandial period was different between the three dietary conditions (group*time, p=0.04). This was due to a tendency to decrease in response to the 0:150 condition when contrasted against the slight increases in both the 75:75 (Δ-between, p=0.06 vs 0:150) and 0:200 conditions (Δ-between, p=0.05 vs 0:150). However, this may reflect regression to the mean given that the incremental area under curve was highest in the 0:150 group prior to the intervention, despite not being significantly different (**Table 6.4**). It is also worthy of note that this effect was not apparent when the two meals were analysed separately. Beyond this, the change in peak insulin concentration following meal 2 was also different between groups (group*time, p=0.05). Although the source of this variance was not apparent upon exploring specific comparisons, the figure would suggest that this was driven by an increase in the 75:75 group and a decrease in the 0:150 group.

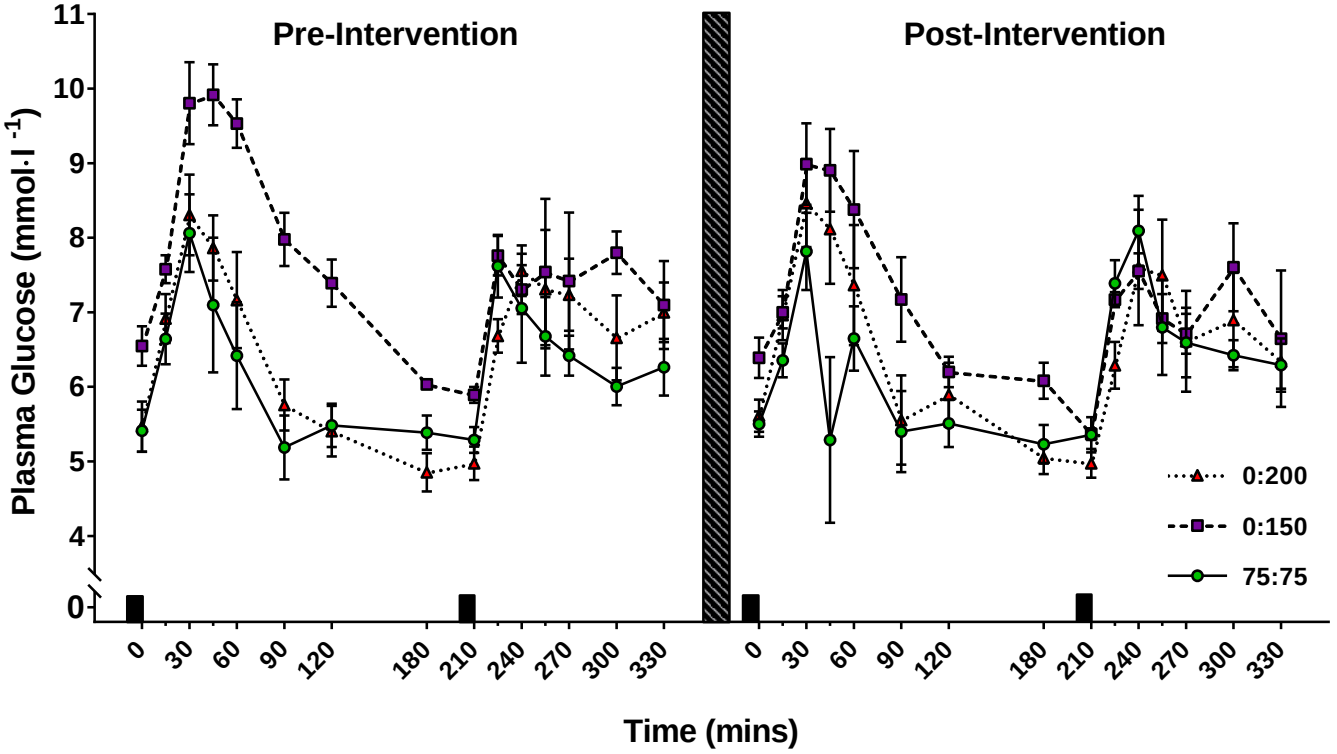

Figure 6.10: Postprandial plasma glucose profiles in response to two sequential meal tests conducted at pre- and post-intervention in the daily calorie restriction (75:75), intermittent fasting with calorie restriction (0:150) and intermittent fasting without calorie restriction (0:200) groups. The white box on the x-axis represents the 20-day intervention. The black boxes on the x-axis denote the timing of prescribed meals. No interaction effects were identified by a three-way ANOVA (group*time*timepoint; p=0.47). Data are presented as mean ± SEM.

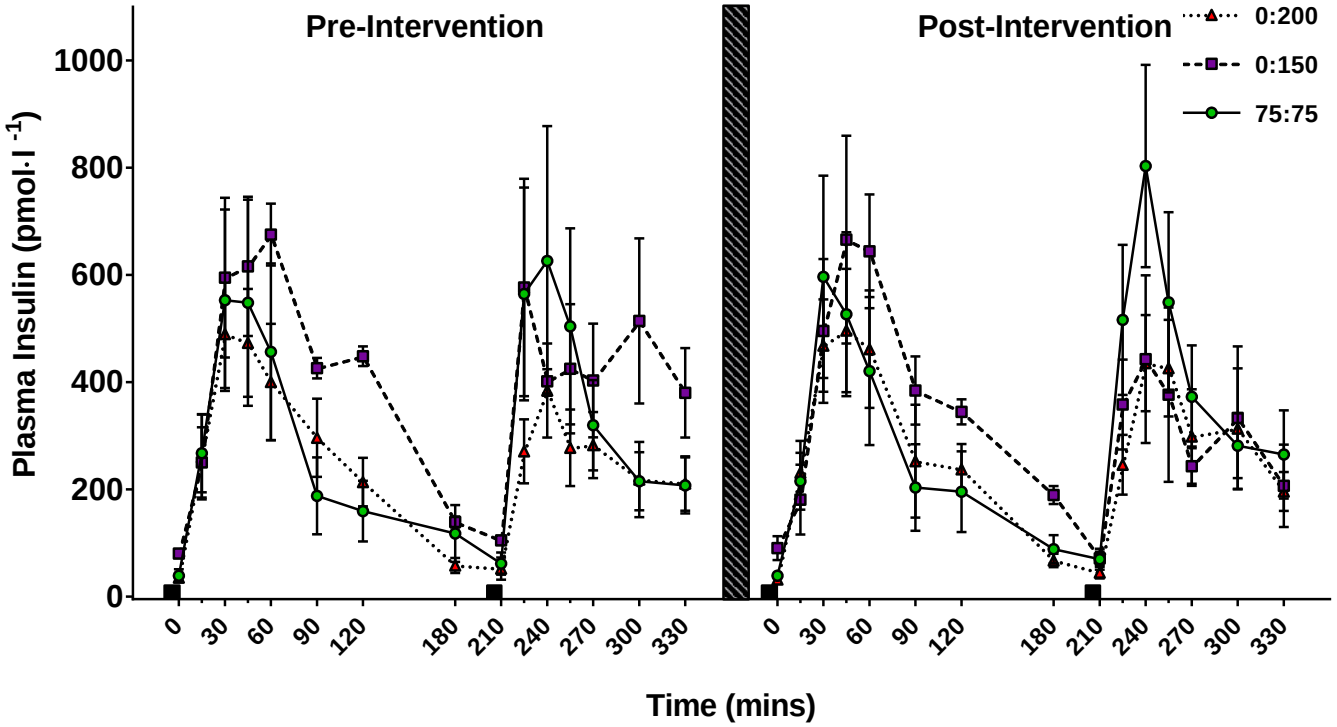

Figure 6.11: Postprandial plasma insulin profiles in response to two sequential meal tests conducted at pre- and post-intervention in the daily calorie restriction (75:75), intermittent fasting with calorie restriction (0:150) and intermittent fasting without calorie restriction (0:200) groups. The white box on the x-axis represents the 20-day intervention. The black boxes on the x-axis denote the timing of prescribed meals. No interaction effects were identified by a three-way ANOVA (group*time*timepoint; p=0.61). Data are presented as mean ± SEM.

6.3.12 – Postprandial Triacylglycerol

The postprandial triacylglycerol response to the sequential meal tests ingested before and after the 20-day interventions is shown in **Figure 6.12**. Although the graph would appear to suggest a decrease in postprandial concentrations following the 0:150 diet, no interaction, time or group effects emerged for any aspect of this profile (**Table 6.4**). When the two meals were analysed separately however, there was a trend for differences in total area under curve following meal 1 (group*time, p=0.06), which is most likely capturing the reduction in postprandial lipaemia following the 0:150 diet (75:75 = -14±22 mmol·l^{-1}·180min, 0:150 = -36±53 mmol·l^{-1}·180min, 0:200 = +24±34 mmol·l^{-1}·180min).

6.3.13 – Postprandial NEFA

The postprandial NEFA response to the sequential meal tests ingested before and after the 20-day interventions is shown in **Figure 6.13**. No interaction, time or group effects emerged for any aspect of this profile (**Table 6.4**) and this was unaffected when these outcomes were determined separately for each meal.

6.3.14 – Postprandial Glycerol

In terms of of the postprandial glycerol response to the sequential meal tests ingested before and after the 20-day interventions, the only meaningful effect was an interaction for meal 2 total area under curve (group*time, p=0.01). This highlighted a decrease in total area under curve following meal 2 in the 0:150 group (-2400±305 μmol·l^{-1}·120min), contrasting with both the 75:75 group (-438±1235 μmol·l^{-1}·120min; p=0.02 vs 0:150) and the 0:200 group (+733±939 μmol·l^{-1}·120min; p<0.01 vs 0:150). However, this may be a result of the variable performance of this particular assay and as such should be viewed cautiously.

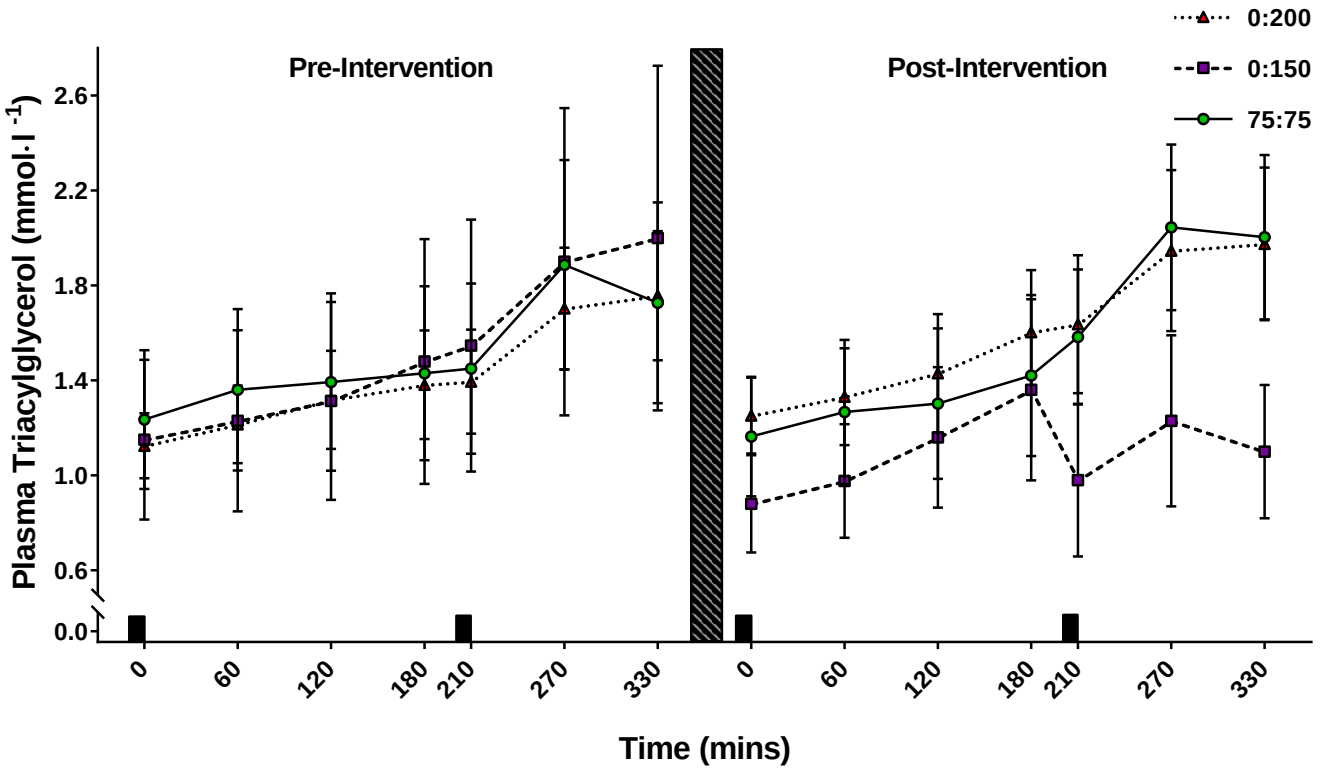

Figure 6.12: Postprandial plasma triacylglycerol profiles in response to two sequential meal tests conducted at pre- and post-intervention in the daily calorie restriction (75:75), intermittent fasting with calorie restriction (0:150) and intermittent fasting without calorie restriction (0:200) groups. The white box on the x-axis represents the 20-day intervention. The black boxes on the x-axis denote the timing of prescribed meals. No interaction effects were identified by a three-way ANOVA (group*time*timepoint; p=0.85). Data are presented as mean ± SEM.

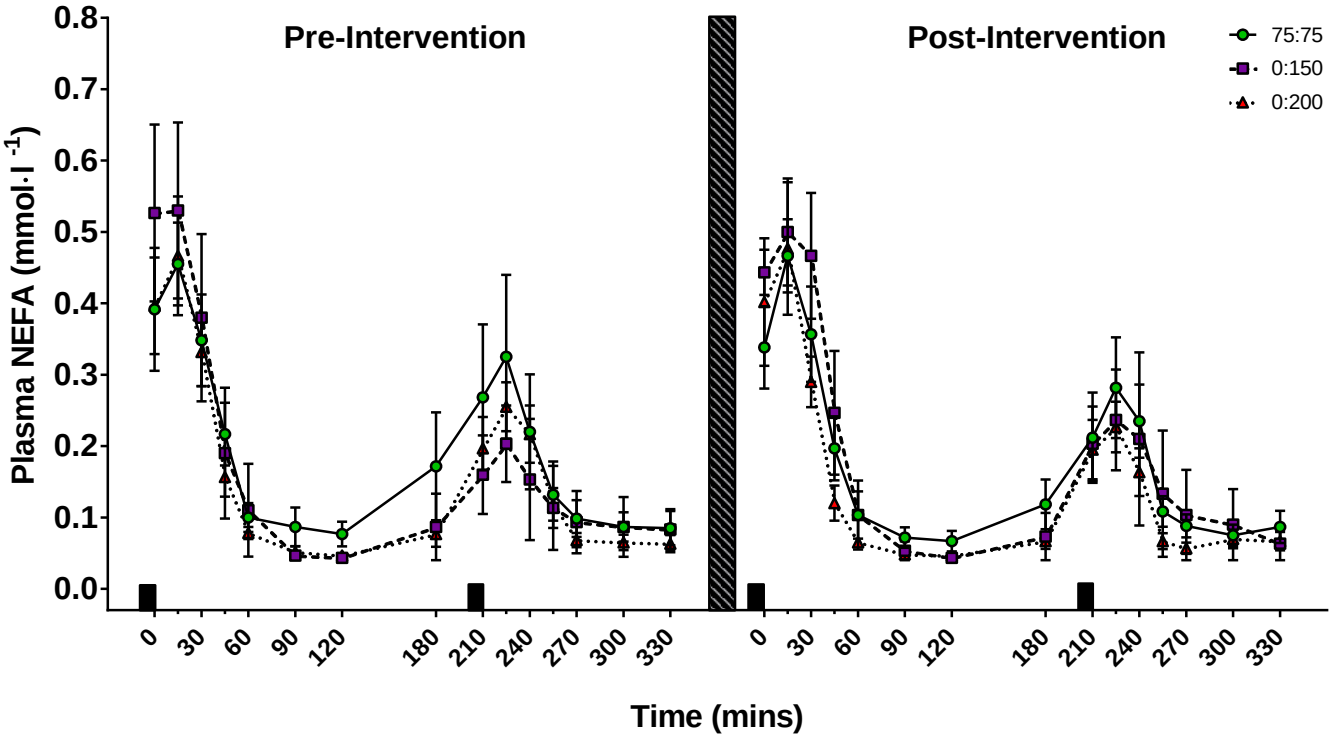

Figure 6.13: Postprandial plasma NEFA profiles in response to two sequential meal tests conducted at pre- and post-intervention in the daily calorie restriction (75:75), intermittent fasting with calorie restriction (0:150) and intermittent fasting without calorie restriction (0:200) groups. The white box on the x-axis represents the 20-day intervention. The black boxes on the x-axis denote the timing of prescribed meals. No interaction effects were identified by a three-way ANOVA (group*time*timepoint; p=0.68). Data are presented as mean ± SEM.

6.4 – Discussion

The present study established the effects of a diet combining intermittent fasting with calorie restriction on energy expenditure and metabolic health in overweight and obese adults, compared to energy-matched (i.e. hypocaloric) daily calorie restriction and intermittent fasting *without* calorie restriction. In accordance with these prescriptions, energy intake was successfully reduced to a similarly meaningful degree in the two energy-restricted conditions but maintained in the intermittent fasting without calorie restriction group. This was mirrored by the resultant decreases in body mass in the two energy-restricted groups, although fat mass appeared to decrease more readily in response to daily calorie restriction than when combining fasting with calorie restriction. However, other than the decrease in diet-induced thermogenesis arising from the reductions in energy intake, energy expenditure was largely unaltered in all three groups. The only exception to this was a reduction in the calories expended through sedentary and vigorous activities on fasted days when compared to fed days in the two intermittent fasting conditions. Most interestingly however, participants in the condition combining intermittent fasting and calorie restriction experienced reductions in postprandial insulin area under curve, differing from both comparative groups which were largely unchanged. This was also accompanied by a more lipolytic profile following the intervention, with apparent reductions in fasted respiratory exchange ratio, fasted LDL cholesterol concentration and postprandial triacylglycerol concentration, coupled with increases in fasted glycerol concentration. However, these outcomes should be treated with caution considering the sample size, particularly in the intermittent fasting with calorie restriction group.

The reduction in postprandial insulin concentrations seen following intermittent fasting with calorie restriction is a key finding emerging from the present study. This change is consistent with enhanced insulin sensitivity given the stability of glycaemic responses. This suggests that neither short-term calorie restriction nor routine extension of fasting is sufficient to modify insulin responses in isolation, but rather that the combined influence of fasting and calorie restriction is required for the effect. Furthermore, as analysing the two postprandial periods separately did not attribute this improvement to a specific meal, it seems that this response persists across sequential eating occasions and may therefore be extended or amplified with the inclusion of a

third meal. Although this finding should certainly be treated cautiously given the sample size for these postprandial biochemical outcomes, it was consistent across all participants in this group and shows reasonable agreement with the prior literature in non-obese adults (Halberg *et al.*, 2005; Heilbronn, Civitarese, *et al.*, 2005). Contrary to these observations, Catenacci *et al.* (2016) did not establish a meaningful change in insulin sensitivity following 8-weeks of complete alternate-day fasting in obese adults. However, Catenacci *et al.* (2016) also found no effect of daily calorie restriction on insulin sensitivity, which contradicts the majority of prior literature (Larson-Meyer *et al.*, 2006; Weiss *et al.*, 2006; Redman and Ravussin, 2011; Johnson *et al.*, 2016), even when the intervention is of a comparable duration (Antoni, Johnston, *et al.*, 2018). Consequently, this suggests that combining intermittent fasting with calorie restriction may more readily improve insulin sensitivity than either calorie restriction or routine fasting extension in isolation. This also highlights the need for dynamic tests of glycaemic and insulinaemic responses in future studies, given the stability of fasted measures of glucose and insulin concentration.

As described earlier, a key part of the rationale for intermittent fasting is enhancing opportunities for surplus lipid clearance to alleviate the metabolic dysfunction arising from chronic overnutrition (Emberson *et al.*, 2002; Mattson *et al.*, 2014). In the present study, the reduction in postprandial insulin concentration seen following intermittent fasting with calorie restriction was also accompanied by reductions in LDL cholesterol and postprandial triacylglycerol concentrations. Furthermore, this group also characterised a tendency for reduced respiratory exchange ratio, changes which collectively point to the induction of an enhanced lipolytic environment. This may therefore provide a mechanistic underpinning for the apparent improvements in insulinaemia. Although speculative, prior reports of increased carnitine palmitoyltransferase (CPT) 1 and sirtuin (SIRT) 1 expression in skeletal muscle tissue following complete alternate-day fasting do support this notion (Halberg *et al.*, 2005; Heilbronn, Civitarese, *et al.*, 2005). This is in light of suggestions that CPT1 is rate-limiting in mitochondrial beta-oxidation (Schreurs, Kuipers and van der Leij, 2010), whilst SIRT1 has been implicated in enhancing skeletal muscle insulin sensitivity (Hesselink, Schrauwen-Hinderling and Schrauwen, 2016). The study of Halberg *et al.* (2005) also observed increases in NEFA and glycerol concentrations during fasted periods, illustrating the onset of a lipolytic state with less than 20 hours of fasting.

Despite the contrary findings for insulin sensitivity, Catenacci *et al.* (2016) also observed a reduction in fasted triacylglycerol concentration with complete alternate-day fasting, an effect that was not apparent in response to daily calorie restriction, lending further credence to the current observations. Although there is certainly a need to explore this proposition further, this study provides compelling support for the induction of an enhanced lipolytic environment and accompanying improvements in metabolic health upon combining intermittent fasting with calorie restriction on a short-term basis.

Although body mass decreased similarly in response to both daily calorie restriction and intermittent fasting with calorie restriction, accompanying changes in fat mass and lean mass were not established by conventional hypothesis testing. This highlights the issue of sampling variance in the present study as opposed to a genuine lack of effect; upon querying the raw values, a pattern emerged in which fat mass appeared to decrease only in response to daily calorie restriction, whilst lean mass was reduced by both the energy-restricted conditions. In terms of fat mass, this conflicts with the observations of Catenacci *et al.* (2016), in which fat mass was reduced to a similar degree by complete alternate-day fasting and daily calorie restriction. However, better agreement is found upon contrasting against the results from lean adults described in Chapter 5, wherein fat mass was reduced to a greater degree by daily calorie restriction when compared to intermittent fasting. Conversely, the tendency for lean mass to be reduced by both energy-restricted conditions in the present study aligns with the findings of Catenacci *et al.* (2016), but not with the findings in lean adults in Chapter 5, where only intermittent fasting combined with calorie restriction seemed to reduce lean mass.

An explanation to reconcile these divergent outcomes for body composition is not immediately apparent, but it most likely reflects interactions within a broader range of lifestyle factors beyond the temporal and quantitative restrictions imposed on energy intake. For instance, physical activity and protein balance are also heavily implicated in the regulation of skeletal muscle mass (Dickinson and Rasmussen, 2013; Milan *et al.*, 2015; Ravussin *et al.*, 2015; Abdulla *et al.*, 2016; Tipton, Hamilton and Gallagher, 2018), whilst dietary fat restriction results in more negative fat balance overall when compared to restriction of other macronutrients (Hall *et al.*, 2015). As such, to optimise

the changes in body composition arising from these interventions, due consideration should be given to these factors, particularly protein intake and physical activity during intermittent fasting given the reductions in lean mass observed.

Focusing instead upon the components of energy expenditure, resting metabolic rate was not altered by any of the interventions, suggesting that neither short-term intermittent fasting nor short-term calorie restriction results in metabolic adaptation in overweight and obese adults. This is similar to the observations in lean adults described in Chapter 5, and as such is likely attributable to the relatively minor weight losses incurred. In this instance, the respective decreases in body mass amounted to 2.8% and 2.6% of pre-intervention values for the daily calorie restriction and intermittent fasting with calorie restriction groups, with only the accompanying reduction in plasma leptin concentration in the daily calorie restriction group approaching a meaningful effect. In agreement with this hypothesis, Catenacci *et al.* (2016) did observe reductions in metabolic rate in response to 8 weeks of daily calorie restriction, reductions that were beyond those predicted by the 6.2% decline in body mass. Interestingly however, in their comparative complete alternate-day fasting group, Catenacci *et al.* (2016) found that the reduction in metabolic rate was explained fully by the accompanying change in body composition. This suggested that no metabolic adaptation had taken place despite the imposition of more severe caloric restriction and greater weight losses of 8.8%. Consequently, this raises an interesting question concerning how these intermittent fasting interventions could be best applied.

The apparent absence of metabolic adaptation in response to both short- and long-term complete alternate-day fasting certainly creates an argument for the use of such approaches in treating obesity (Rosenbaum and Leibel, 2010). However, participant feedback from the present study suggests that such an intense regimen would not be sustainable for more than a matter of weeks, particularly when taken outside a research setting. Instead, these findings point to the potential of an intermittent approach to intermittent fasting, wherein complete alternate-day fasting is implemented for short intervals as opposed to continuously. Such an approach was recently employed to good effect in the context of daily calorie restriction by Byrne *et al.* (2018), separating 2-week periods of daily calorie restriction with 2-week periods in which energy balance was maintained. In a cohort of obese men, this resulted in enhanced loss of fat mass

and less than half the metabolic adaptation when compared to continuous calorie restriction, in what the authors describe as improved weight loss efficiency. Consequently, applying this intermittent approach to intermittent fasting may represent a long-term strategy which results in weight loss and improvements in metabolic health with minimal, if any, metabolic adaptation.

In a similar vein, physical activity energy expenditure was remarkably stable from the control to the intervention phase in all three groups, suggesting that none of the diets elicited behavioural adaptations to the temporal or quantitative restrictions imposed on energy intake in overweight/obese adults. This is in agreement with prior studies of modified alternate-day fasting in obese adults, in which step counts were unaffected by 8 weeks of dieting (Klempel *et al.*, 2010, 2012; Hoddy *et al.*, 2016). However, these trials lacked a comparative arm, making it difficult to disentangle the role of the diet in this null result from the limitations of the measurement techniques employed (Crouter *et al.*, 2003; Corder, Brage and Ekelund, 2007). The outcome in the present study is also contrary to the observed decline in physical activity energy expenditure seen upon combining intermittent fasting with calorie restriction in lean adults (Chapter 5). Although it could be argued that this is due to the lower sample size in the present study failing to isolate the effect in what is a highly variable measurement, it would still be reasonable to expect a quantitative decrease. Instead, in this overweight/obese cohort, physical activity energy expenditure was stable to within 3 $kcal \cdot day^{-1}$ in the intermittent fasting with calorie restriction group, whilst the daily calorie restriction and intermittent fasting without calorie restriction groups were stable to within 4 $kcal \cdot day^{-1}$ and 54 $kcal \cdot day^{-1}$, respectively. Consequently, this disparity with the findings in lean adults instead suggests that behavioural adaptations to intermittent fasting when combined with calorie restriction may be influenced by baseline adiposity. A similar adiposity-dependent effect was observed by Chowdhury *et al.* (2016), where extended morning fasting in obese adults did not alter 24-hour physical activity energy expenditure, which was contrary to their prior findings in lean adults (Betts *et al.*, 2014, 2016). Such an effect could simply be a reflection of lower activity levels in overweight/obese cohorts (Bakrania *et al.*, 2017), meaning there is less capacity for an adaptive decline. Alternatively, overweight/obese individuals may be less responsive to signals of energy availability; for instance, varying degrees of leptin resistance are often seen in overweight/obese individuals (Myers *et al.*, 2010).

It is also worthy of note that patterns of activity were found to differ between fasted and fed periods in the present study, with the energy expended performing sedentary and vigorous intensity activities being lower on fasted days when compared to fed days during intermittent fasting. This is once again concordant with the findings of Chowdhury *et al.* (2016), who found that physical activity thermogenesis was lower during fasted periods, but that this did not extend to the 24-hour measurement window. Given that the same tendency for physical activity to be lower during fasted periods was also apparent for lean individuals in Chapter 5, this may once again be due to an influence of acute feeding state on heart rate, a change in sleep patterns, or a reduction in activity arising from the absence of food preparation. Yet none of these factors would explain the reduction in vigorous activity during fasting in overweight/obese individuals but not in lean, which instead points to a conscious behavioural response in this cohort (i.e. obese participants felt less able to undertake more intense activities during fasting) (Betts *et al.*, 2014). Nonetheless, the consistent observation that behavioural adaptation to fasting of various durations differs between lean and overweight/obese individuals is intriguing, particularly in light of suggestions that metabolic adaptation is not different in these cohorts (Leibel, Rosenbaum and Hirsch, 1995). Such a trend is consistent with the aforementioned hypothesis of differences in how readily individuals might compensate for perturbations in energy balance, which could carry implications for both the onset and persistence of obesity, making this a key area for future research to explore.

As alluded to throughout this discussion, the inflated risk of type II errors is an important consideration in the present study. In Section 5.2.1, *a-priori* power analysis was used to estimate the sample size needed to detect the anticipated effects within the primary outcomes. However, owing to the recruitment challenges faced in this study, the attained sample size was approximately half of what was originally targeted, falling as low as 25% for some postprandial outcomes due to cannulation issues. Consequently, this study was underpowered to detect some of the anticipated effects, meaning that several null hypotheses may have been falsely accepted. This therefore necessitates further research, however, the present data nonetheless provides a useful resource to help inform the design of these studies, as advocated by Hoenig and Heisey (2001). For instance, based on the observed change in total daily energy expenditure in the intermittent fasting with calorie restriction group, 9 participants would be

required to establish this as a meaningful within-group change with 80% power at an alpha level of 0.05. Alternatively, in order to discern meaningful between-group differences in this parameter between the two energy-restricted groups, 48 participants would be required to achieve 80% power when detecting such a difference at an alpha level of 0.05.

Collectively, the data from this experiment reveal that combining intermittent fasting with calorie restriction (i.e. weight-loss) results in improved insulin sensitivity when compared to either intermittent fasting or calorie restriction alone. This may be a result of an enhanced lipolytic state under their combined influence, with accompanying improvements in markers of lipid metabolism. Despite this, fat mass does not seem to decrease as readily in response to intermittent fasting when compared to daily calorie restriction. However, this is likely to be influenced by factors such as protein intake and physical activity, which future studies should seek to manipulate in order to optimise the outcomes. Lastly, neither short-term calorie restriction nor short-term intermittent fasting elicited adaptive declines in energy expenditure. This lends credence to the notion that such short-term approaches could offer enhanced weight loss efficiency relative to the continuous alternatives, and that the behavioural responses to fasting may differ between lean and overweight/obese individuals. However, future work remains a necessity to in order clarify these findings given the inflated risk of error with such small sample sizes and the potential for statistical regression.

Chapter 7: General Discussion

This programme of research set out to understand and evaluate the utility of intermittent fasting as a dietary strategy for improving metabolic health. Initially, this involved quantifying diurnal rhythms in subjective appetite and related regulatory outcomes (Chapter 4), which subsequently informed the design of two intervention studies. These centred upon the application of a short-term complete alternate-day fasting intervention to a cohort of lean adults (Chapter 5) and then a cohort of overweight/obese adults (Chapter 6). In doing so, this revealed the novel finding that lean individuals combining intermittent fasting with calorie restriction exhibit reduced energy expenditure relative to intermittent fasting or calorie restriction in isolation. This was largely attributable to reductions in lower intensity physical activities during fasted periods. Furthermore, intermittent fasting also resulted in less pronounced declines in fat mass than daily calorie restriction, whilst there were no improvements in any aspect of fasted or postprandial metabolic health. In overweight/obese individuals, a similar pattern was apparent in terms of body composition, yet none of the three interventions altered resting metabolic rate or physical activity thermogenesis. Instead, an attenuated postprandial insulin response was observed following the combined intermittent fasting and calorie restriction intervention, which was accompanied by improvements in fasted and postprandial lipid concentrations. Collectively, these observations yield insights spanning three broad domains worthy of further discussion, namely: body composition, physical activity thermogenesis and metabolic health.

Amongst the most interesting contrasts emerging from these experiments was the observation that, under conditions of energy restriction, fat mass decreased more readily in response to daily calorie restriction than intermittent fasting. In the lean cohort, greater declines in fat mass were seen in the daily calorie restriction group relative to the intermittent fasting condition (75:75 = -1.8±0.8 kg, 0:150 = -0.8±0.9 kg, 0:200 = -0.1±0.7 kg). Comparatively, in the overweight/obese cohort, fat mass was only meaningfully reduced by the daily calorie restriction condition (75:75 = -1.4±0.8 kg, 0:150 = -0.8±1.2 kg, 0:200 = -0.2±1.0 kg). Although differences in fat balance will contribute to this variance, they cannot quantitively explain disparities of this

magnitude (Hall *et al.*, 2015). Instead, the stark contrast with the outcomes of intermittent fasting studies employing a modified alternate-day approach implicates the extended fasting period as the driving factor (Varady, 2011; Alhamdan *et al.*, 2016).

Considering that the rationale behind extending the fasting interval to 24-hours in the present experiments was to provide more opportunities for lipolysis and energy efflux from adipose tissue (Ruge *et al.*, 2009; McQuaid *et al.*, 2011; Frayn, 2016), this attenuated decline in fat mass seems somewhat discordant. There can be little doubt that the fasting durations employed in the current experiments were sufficient to induce meaningful increases in lipolysis. For instance, Halberg *et al.* (2005) noted increases in circulating concentrations of both non-esterified fatty acids and glycerol after 19 hours of fasting when compared to those seen after a typical overnight fast. As such, it seems unlikely that the smaller reductions in fat mass seen with intermittent fasting are rooted in failure to stimulate lipolytic pathways. Instead, the smaller decline highlights that over the longer-term, substrate-specific metabolism is altered to regulate endogenous carbohydrate and protein stores in the first instance, which then changes as the fasting period extends beyond a certain point (Galgani and Ravussin, 2008; Soeters *et al.*, 2012).

Protein stores within skeletal muscle are often cited as being more readily conserved than endogenous fat stores during acute periods of fasting courtesy of their functional role (Vendelbo *et al.*, 2014), yet following an overnight fast there is an increase in amino acid efflux from muscle tissue (Felig, 1975). This suggests that when the low amino acid and insulin concentrations that accompany the fasted state persist for 8-12 hours, the dynamic equilibrium in which skeletal muscle exists shifts in favour of net muscle protein breakdown (Atherton and Smith, 2012; Dickinson and Rasmussen, 2013; Tipton, Hamilton and Gallagher, 2018). Although there are limited data to support an exaggeration of this catabolic state when the fasting duration is extended to 24 hours, it seems reasonable to suspect so. A recent study by Vendelbo *et al.* (2014) showed that fasting for 72 hours doubled the rate of amino acid efflux from skeletal muscle when compared to a 10-hour fast; this was associated with reduced mTOR activity and protein synthesis, whilst muscle protein breakdown remained relatively constant. These liberated amino acids can subsequently be utilised in gluconeogenic

pathways to spare the comparatively limited glycogen stores within the body (Owen *et al.*, 1979; Carlson, Snead and Campbell, 1994), thereby allowing protein to contribute to whole-body energy metabolism during prolonged periods of fasting.

As a result of the above processes, the divergent effects of intermittent fasting on body composition are perhaps best explained by reframing the question, asking instead why daily calorie restriction resulted in maintenance of lean mass whilst intermittent fasting seemed to decrease it more readily. Both conditions led to a similar reduction in energy intake, which is overcome by the combined degradation of endogenous carbohydrate, fat and protein stores to varying extents (Galgani and Ravussin, 2008; Hill, Wyatt and Peters, 2012; Thompson *et al.*, 2012; Birsoy, Festuccia and Laplante, 2013; Adeva-Andany *et al.*, 2016; Rostom and Shine, 2018). Carbohydrate and fat stores respond on a relatively acute basis to meet energy demands, whilst protein shows a more latent response which is proposed to be contingent on a degree of hepatic glycogen depletion (Owen *et al.*, 1979; Carlson, Snead and Campbell, 1994). Given that habitual meal patterns are maintained with daily calorie restriction, it seems likely that this latent protein response is less often invoked, given that the fasting period is still regularly punctuated by nutrient influx and insulin secretion. This regular re-feeding not only permits the replenishment of glycogen stores, but also stimulates mTOR activity and the restoration of synthetic pathways in skeletal muscle tissue (Dickinson and Rasmussen, 2013; Adeva-Andany *et al.*, 2016). In lay terms, the preservation of meal patterns with daily calorie restriction minimises the opportunities for protein to make a meaningful contribution to resolving the energy deficit, placing a greater onus on degradation of carbohydrate and fat stores which are involved in the acute response to fasting. The same effect may therefore be induced if the fasting period is punctuated by permitted intake, as in the modified alternate-day approaches employed by others (Varady, 2011; Barnosky *et al.*, 2014; Patterson and Sears, 2017), thus also explaining the maintenance of lean mass in these instances. However, it seems that extending this fasting period to 24-hours allows the rate of muscle protein breakdown to exceed the rate of muscle protein synthesis. The resultant surplus of amino acids can therefore make a meaningful contribution to energy metabolism via gluconeogenesis instead, reducing the reliance upon endogenous fat stores within adipose tissue.

The above interpretation does, however, leave two unexplained observations: the retention of lean mass during intermittent fasting without calorie restriction and the apparent decrease in lean mass during daily calorie restriction in overweight/obese adults but not in lean. Given the above discussion of energy metabolism during prolonged fasting, it would be anticipated that the imposition of identical fasting durations in the intermittent fasting without calorie restriction groups would also lead to a loss of lean mass. Instead, this suggests that it is combining prolonged fasting intervals with a state of negative energy balance that fosters this state. Therefore, incorporating strategies that favour more positive protein balance may help to facilitate preferential decreases in fat mass as opposed to lean mass during such diets (e.g. increased protein intake, resistance training). In a similar vein, the reduction in lean mass in response to daily calorie restriction in overweight/obese adults but not in lean may be explained by the underpinning physiology of protein storage (Section 2.1.4). Specifically, differences in protein intake and physical activity are likely candidates to explain this contrast, rather than differential physiological regulation of energy metabolism in these two cohorts. Data from the daily calorie restriction group in the two cohorts pertaining to these parameters are shown in **Table 7.1**.

Table 7.1: Determinants of protein balance in the daily calorie restriction group in lean and overweight/obese cohorts. Data are presented as mean ± SD.

Outcome	Lean			Overweight/Obese		
	Control	Intervention	Change	Control	Intervention	Change
Lean Mass (kg)	53.6 ± 10.5	53.5 ± 10.2	-0.1 ± 0.8	58.6 ± 13.2	57.2 ± 13.1	-1.4 ± 1.0
Protein Intake ($g \cdot kg^{-1} \cdot day^{-1}$)	1.2 ± 0.3	0.9 ± 0.2	-0.3 ± 0.2	1.1 ± 0.3	0.9 ± 0.3	-0.2 ± 0.2
PAT ($kcal \cdot day^{-1}$)	892 ± 301	893 ± 342	+1 ± 126	700 ± 250	695 ± 289	-5 ± 145
Sedentary ($kcal \cdot day^{-1}$)	123 ± 32	119 ± 22	-5 ± 18	133 ± 46	117 ± 37	-16 ± 25
Light ($kcal \cdot day^{-1}$)	282 ± 66	294 ± 91	+12 ± 52	272 ± 53	267 ± 39	-5 ± 55
Moderate ($kcal \cdot day^{-1}$)	317 ± 161	331 ± 179	+14 ± 61	254 ± 169	250 ± 223	-4 ± 69
Vigorous ($kcal \cdot day^{-1}$)	84 ± 57	73 ± 63	-11 ± 37	26 ± 28	46 ± 40	+20 ± 40
Very Vigorous ($kcal \cdot day^{-1}$)	67 ± 83	53 ± 72	-14 ± 52	0 ± 0	0 ± 1	0 ± 1

Although protein intake during the intervention phase was well below the 1.2 $g \cdot kg^{-1} \cdot day^{-1}$ proposed to be necessary for accretion of muscle mass during calorie restriction (Santarpia, Contaldo and Pasanisi, 2017), this was similar across groups. Instead, the most obvious contrast emerging from these data is that physical activity thermogenesis was approximately 200 $kcal \cdot day^{-1}$ lower in both the control and intervention phases in the overweight/obese group when compared to the lean. Furthermore, only 37% of this daily expenditure was accumulated at moderate-vigorous intensities in

overweight/obese participants, which contrasts with the 48% seen in lean individuals. Given that physical activity thermogenesis is likely to play a role in lean mass retention (Ravussin *et al.*, 2015), along with the role of more demanding exercise in enhancing muscle protein synthesis in response to feeding (Drummond *et al.*, 2011; Dickinson and Rasmussen, 2013), these differences in physical activity may well be driving the differing effects of daily calorie restriction on lean mass between cohorts. This is further supported by the maintenance of lean mass during weight loss via calorie restriction when combined with resistance training (Clark, 2015).

According to the above explanation, complete alternate-day fasting may result in more negative protein balance and less negative fat balance than daily calorie restriction, due primarily to the effects of extended fasting on muscle protein synthesis. However, the maintenance of lean mass in the eucaloric interventions and the disparate effects of daily calorie restriction on lean mass in the two cohorts suggests that these changes could be modified by key regulators of protein balance, particularly physical activity/exercise and protein intake. The question now stands as to whether these factors can be better utilised in the design of future interventions to bring about more favourable changes in body composition. Partnering complete alternate-day fasting with a resistance training programme could be one such strategy, although the training would need to be undertaken during fed periods to foster an acute anabolic state as opposed to accelerating post-exercise skeletal muscle catabolism (Tipton, Hamilton and Gallagher, 2018). Although no such studies have been undertaken as yet, some insights can be gleaned from the study of Tinsley *et al.* (2017). This applied a time-restricted feeding protocol alongside a resistance-training programme in a cohort of lean adults over 8 weeks. Time-restricted feeding involved restricting *ad libitum* energy intake to a 4-hour window in the evening on four days per week, whilst on the remaining three days food intake was *ad libitum* all day. On these three days, participants also undertook a resistance training programme targeting muscle hypertrophy. The net effect of this was a reduction in energy intake of approximately 370 kcal per day which was driven by reductions on time-restricted days, although no reductions in lean mass or fat mass were apparent. The maintenance of fat mass was surprising, but this may well reflect the relatively small calorie restriction imposed of ~15%. Nonetheless, lean mass was also maintained despite the imposition of mild calorie restriction and 20-hour fasts on 3 days per week. Consequently, this suggests

that, if incorporated appropriately, resistance training does have the potential to offset the decrease in lean mass associated with prolonged fasting periods.

An alternative solution to encourage lean mass retention is to introduce protein supplementation during fasted periods to stimulate muscle protein synthesis. However, it is currently unclear whether this effect of protein supplementation can be achieved without also disrupting the systemic lipolytic state that may drive improvements in health (Acheson *et al.*, 2011). Insights can be gleaned from the study of Katsanos *et al.* (2009), which examined the effect of ingesting an amino acid bolus on plasma insulin concentrations and muscle protein synthesis. Three-fold increases in insulin were apparent at 15 and 30 minutes post-ingestion, which resolved to fasted values within 60 minutes, but this was also accompanied by increases in fractional synthetic rate. It seems likely that even this small insulin response will suppress lipolytic activity to some degree (Hickner *et al.*, 1999; Stumvoll *et al.*, 2000), but the accompanying increase in muscle protein synthesis may not be predicated on the insulinaemic response (Robinson *et al.*, 2014). This means that in principle, this insulinaemic effect could be ameliorated whilst preserving the stimulatory effect on protein synthesis with ingestion of a more carefully selected set of amino acids (van Loon *et al.*, 2000; Gannon and Nuttall, 2010; Dickinson and Rasmussen, 2013).

Although the efficacy of these solutions will need to be explored by future research, they nonetheless highlight the important role that lifestyle factors such as macronutrient balance and physical activity have to play in modifying the effects of such nutritional interventions. In light of this, the effects that intermittent fasting exerted upon physical activity thermogenesis in Chapters 5 and 6 are pertinent points of discussion. Establishing whether intermittent fasting invokes the same behavioural adaptations that often accompany periods of daily calorie restriction (Martin *et al.*, 2011) was a core objective of this thesis, courtesy of their proposed importance to long-term weight loss maintenance (Elfhag and Rossner, 2005; Wing and Phelan, 2005; Thomas *et al.*, 2014). This line of enquiry yielded perhaps the most striking outcomes of this series of studies.

In the lean cohort, combining intermittent fasting with calorie restriction (i.e. weight-loss) reduced daily physical activity thermogenesis, an effect that was not apparent

with either calorie restriction or intermittent fasting in isolation. This pointed to an interactive effect of the two dimensions, suggesting that calorie restriction and intermittent fasting exert small but separate effects on physically active behaviours which amounted to a meaningful change when combined. When stratified into fed and fasted cycles, this decrease was attributable to a reduction in physical activity thermogenesis during fasted periods rather than fed periods, a pattern which was driven primarily by changes in the calories expended performing sedentary and light activities. This adds further confidence that the observation is real and is at least partly attributable to fasting. Conversely, in the overweight/obese cohort, physical activity thermogenesis was unresponsive to all three interventions, thus introducing the notion that such behavioural adaptations may either be influenced by baseline adiposity, or by some other factor that is also causally related to both physical activity and adiposity. However, reverse causality is unlikely given that a propensity to reduce activity levels to minimise the net deficit and preserve body mass should then be *more* not less evident amongst overweight/obese individuals. Although the same pattern emerged in lean and overweight/obese groups for physical activity to be lower during fasted cycles, the most apparent decline for overweight/obese participants was in the calories expended performing vigorous activities rather than light. Given that reductions in physical activity thermogenesis have been consistently observed in response to extended periods of daily calorie restriction (Weyer *et al.*, 2000; Martin *et al.*, 2011), the novel observation emerging from Chapters 5 and 6 is that combining this with prolonged fasting seems to exert an interactive effect on this outcome. This could be the product of three possible explanations: an adaptive decline in physical activity during prolonged fasting; a confounding influence of the intervention on physically active behaviours; or a confounding influence of the intervention on the measurement technique employed to quantify physical activity.

Collectively, the evidence available does point to the induction of a genuine decline in physical activity during fasted periods in the current experiments, rather than an artefactual finding. Firstly, the distinct activity patterns on fasting periods when compared to fed periods suggest that the acute feeding state is driving this reduction, as if it was an accumulated physiological response it would affect fasted and fed cycles equally (Betts *et al.*, 2014). Such an outcome may be reasonably expected given the understandably non-blinded trial design, perhaps simply reflecting a conscious

decision to avoid physical activity during periods of perceived energy insufficiency. However, were this the case, then it would arguably be more likely that the calories expended at moderate-vigorous intensities would be reduced, and that this effect would be consistent across the cohorts. In fact, this reduction in moderate-vigorous intensity activity could be anticipated to be more apparent in the lean cohort, as they expended a higher fraction of their daily calories through these more intense activities during the control phase (**Table 7.1**). Yet, in the lean cohort, the decrease in physical activity thermogenesis during fasting was driven by sedentary and light activities, a pattern which is consistent with a reduction in spontaneous rather than deliberate activity (Betts *et al.*, 2014). Comparatively, in the overweight cohort, the reduction in vigorous activity was the driving factor, which might be more consistent with a conscious decision (Betts *et al.*, 2014).

It is this discrepancy between cohorts that lies at the heart of the justification for an adaptive response, as it suggests that the decline is influenced by adiposity, which is difficult to reconcile with the other two proposed explanations. It seems reasonable to expect that the onset of major challenges to energy homeostasis (i.e. prolonged fasting) would stimulate more pronounced adaptive changes in regulatory pathways in those with lower levels of stored energy. Certainly, such behavioural adaptations in spontaneous physical activity have been noted at the lower extremes of adiposity (Carrera *et al.*, 2012), whilst these results also concur with the findings from studies of extended morning fasting (Betts *et al.*, 2016). However, there is a definite need to explore this proposition further, particularly given that similar adiposity-dependent effects do not seem to be apparent for adaptive responses to daily calorie restriction (Leibel, Rosenbaum and Hirsch, 1995).

An alternative explanation of these divergent activity patterns during fed and fasted cycles is that the intermittent fasting interventions encouraged behavioural modifications which led to the reduction in activity. At the most simplistic level, this might be considered in terms of the energetic cost of food preparation. The very act of fasting reduces the need for food preparation, which is ascribed an intensity of 2.0-2.5 METs (Ainsworth *et al.*, 2011). This therefore aligns with the light intensity range and fits with the reduction in light activity that drove the reductions in physical activity on fasted days in the lean intermittent fasting with calorie restriction group, which totalled

-54±71 kcal·day^{-1}. By crude inference, a reduction in physical activity thermogenesis of this magnitude is equivalent to approximately 21 minutes of food preparation per day. The assumption this then makes is that these 21 minutes are instead spent being completely rested, given that the calories expended performing sedentary activities were also reduced and any activities at higher intensities would offset this reduction in activity more readily (Thompson, Peacock and Betts, 2014). This seems unlikely but, even if considered to be true, a comparable reduction in light activity would still be expected in the intermittent fasting without calorie restriction group, which was not apparent (-1±69 kcal·day^{-1}). In a similar vein, the lack of breakfast during fasted periods could have allowed participants to get up later, thereby eliminating some low-level activity. Whilst data on sleep patterns were not collected in the present study to support or refute this suggestion, such an effect was not observed by Betts *et al.* (2014) or Chowdhury *et al.* (2016) in their studies of extended morning fasting. Furthermore, this would once again be expected to exert a similar influence in the two intermittent fasting groups, which was not the case. However, it is worthy of note that, whether conscious or not, such changes in behaviour are nonetheless an adaptation to prolonged fasting. Therefore, there would still be a need to monitor and maintain physical activity levels in order to enhance the effects of such interventions.

Another possibility to explain the difference in activity levels is that the interventions may have confounded the method used to quantify physical activity (i.e. combined heart rate/accelerometry). This is based on the observation that feeding affects heart rate; for instance, in response to the consumption of a mixed-meal containing 480 kcal, Matsumoto *et al.* (2001) observed an increase in heart rate of 4-5 bpm in the ensuing 35-minute postprandial period. Consequently, the postprandial increase in heart rate during overfeeding may lead to small inaccuracies in the measurements on fed days (Walhin *et al.*, 2013). This certainly fits with the pattern seen between fasting and feeding cycles in all the intermittent fasting groups, although the counterpoint once again rests in the intensity thresholds. The branched-equation model used to derive these estimates applies different weightings to the two data types (i.e. heart-rate *versus* accelerometry) at different intensities (Brage *et al.*, 2004). At lower intensities, the model bases the estimates predominantly upon accelerometry data, whilst higher intensities rely more heavily upon heart rate (Brage *et al.*, 2004). Consequently, if this impact of acute feeding state on heart rate was the driving factor behind these patterns,

it would be most apparent at the higher intensities of activity, not the lower as was typically seen.

This also raises the question of whether combined heart/accelerometry was the most appropriate technique for quantifying these changes. For instance, Muller *et al.* (2015) used this approach to quantify adaptive changes in physical activity during 3 weeks of 50% daily calorie restriction. Although no reductions were apparent in the resultant estimates of physical activity thermogenesis, a laboratory-based protocol revealed an adaptive increase in skeletal muscle work efficiency, meaning that the same physical activity had a lower energy cost following daily calorie restriction. As such, it is possible that the present experiments may underestimate the change in physical activity thermogenesis. In light of this, the most obvious alternative is the use of doubly-labelled water. This offers similarly free-living data, but in quantifying the rate of isotopic oxygen depletion it may better capture such reduced oxidative costs more effectively. This would also overcome the potentially confounding influence of feeding-induced increases in heart rate, thereby isolating behavioural adaptation of some nature as the cause. However, whilst these are certainly strengths, doubly-labelled water is instead prone to inaccuracy in terms of changes in respiratory exchange ratio from fasted to fed cycles and in response to the intervention (Schmidt *et al.*, 2013; Hall *et al.*, 2018), whilst also missing important temporal fluctuations in the intensity and pattern of daily activities (Katzmarzyk, 2010). As such, it seems that there is no perfect approach to capture free-living data in this context, and the resolution of data offered by the Actiheart^{TM} does at least permit closer scrutiny of these potential confounding influences.

Ultimately, to conclude that these confounding influences are therefore not contributing to the observed changes in physical activity based on these arguments would be radical, but equally to conclude that they are driving these activity patterns is overtly conservative. Certainly, the divergent modifications in activity that emerged across both interventions and cohorts do make a strong case for the induction of an adaptive response to the acute feeding state. This is further supported by the similar conclusions drawn by the aforementioned studies on extended morning fasting, which implicated the postprandial rise in plasma glucose concentrations as the signal controlling the onset of spontaneous physical activity (Betts *et al.*, 2016). Although

such a mechanism would certainly be invoked here, without continuous glucose monitoring it is difficult to support or refute this based on the available data. An alternative hypothesis is that hepatic carbohydrate status may dictate this response instead (Müller, Enderle and Bosy-Westphal, 2016). In the previous discussion of catabolic responses to fasting, the reduction in protein synthesis and resulting liberation of amino acids for use in energy metabolism following 10-12 hours of fasting was said to be initiated by a degree of hepatic glycogen depletion (Owen *et al.*, 1979; Carlson, Snead and Campbell, 1994). It stands to reason that such depletion may also initiate an adaptive response in energy expenditure to constrain further challenges to glucose homeostasis. However, without accompanying metrics of liver glycogen content, this is once again speculative. Nonetheless, the observation that prolonged fasting periods do elicit a reduction in physical activity thermogenesis is a valuable addition to current understanding, particularly following the prior discussion of the potential impact this can have on lean mass retention and weight loss maintenance. As such, future studies will need to explore this adaptive dimension further, using a combination of free-living and laboratory techniques to provide a more complete view of their scale and origin.

Following on from this discussion, it seems sensible to highlight that while weight loss is often the goal when applying calorie restriction, from a scientific and economic perspective this is driven by the improvements in metabolic health that typically accompany reduced adiposity (Goldstein, 1992; Blackburn, 1995; Hamman *et al.*, 2006; Varady, Tussing, *et al.*, 2009; Wing *et al.*, 2011). Consequently, these adaptive changes in energy use and disparate effects on body composition are secondary considerations to the overall impacts on metabolic health. This is a particularly pertinent point in this case, as a key dimension of this investigation was exploring the potential for intermittent fasting to provide improvements in health that were independent of changes in energy balance (Halberg *et al.*, 2005; Heilbronn, Civitarese, *et al.*, 2005; McArdle *et al.*, 2013; Rutkowski, Stern and Scherer, 2015; Wensveen *et al.*, 2015; Spalding *et al.*, 2017).

In the lean cohort, both fasting and postprandial health outcomes were largely unresponsive to all the interventions. As discussed in Chapter 5, this is most likely to be a consequence of the relatively small weight losses (Varady, Tussing, *et al.*, 2009),

coupled with the seemingly healthy metabolic profile of participants at baseline (Sparks *et al.*, 2017). This suggestion is reinforced by the observed changes in the overweight/obese cohort, wherein LDL cholesterol levels were raised in all three groups prior to the intervention. Following the intermittent fasting with calorie restriction diet in this cohort, postprandial insulin concentrations were reduced when the combined postprandial period of the two meals was considered, suggesting a chronic effect of the intervention which persisted across meals. As was the case with physical activity, the absence of comparable effects in response to either calorie restriction or intermittent fasting in isolation points to interactive effects of these two dimensions, although this most certainly needs to be verified by future studies with greater statistical power. However, this observation also fits with the accompanying reductions in LDL cholesterol and triacylglycerol concentrations in this group who combined fasting with calorie restriction. Coupling these changes with the tendency for respiratory exchange ratio to decrease in this group collectively points to the upregulation of lipolytic pathways (Owen *et al.*, 1979; Carlson, Snead and Campbell, 1994; Soeters *et al.*, 2012) and enhanced clearance of surplus lipids during 24-hour fasting (Emberson *et al.*, 2002; Mattson *et al.*, 2014). Although attributing improvements in insulinaemia to these modifications in fat metabolism would be highly speculative, it is worthy of note that the accumulation of certain lipid species has been implicated in insulin resistance (Rutkowski, Stern and Scherer, 2015; Spalding *et al.*, 2017; Petersen and Shulman, 2018), which the predominance of lipid oxidation that fasting permits could help to clear. Consequently, future studies should seek to explore this proposition further, perhaps including skeletal muscle biopsies to assess changes in intramuscular lipid content.

Do the findings considered thus far therefore mean that reducing energy intake through intermittent fasting is inadvisable unless a degree of metabolic dysfunction is already present? Certainly, the loss of lean mass and reduction in physical activity in the absence of health improvement in the lean cohort are strong arguments for such an advocation. However, there are reasons to suspect that the therapeutic potential of intermittent fasting may be under-estimated by the complete alternate-day approach used in Chapters 5 and 6. The rationale for this particular modality was maximising the uninterrupted fasting period to enhance lipid clearance as described above, but the drawback of this is that it cannot be applied consistently (i.e. on a daily basis). In light

of the understanding of circadian rhythms necessitated by Chapter 4, this is likely to have led to a degree of circadian misalignment.

Shifting feeding behaviour from a 24-hour rhythm to a 48-hour rhythm moves it outside the entrainable range, much alike a forced desynchrony protocol (Pagani *et al.*, 2010; Scheer, Morris and Shea, 2013). Consequently, endogenous rhythms which are dictated by, or responsive to, nutrient influx (i.e. meal timing), are likely to have become disordered as feeding behaviour could not be anticipated. Although not fully elucidated, there is mounting evidence to suggest that such feeding dependent rhythms are present in various dimensions of metabolic control (Johnston, 2014; Johnston *et al.*, 2016), as discussed at length in Section 2.3. For instance, Scheer *et al.* (2009) observed that under conditions of forced desynchrony, misalignment of circadian and behavioural rhythms resulted in deteriorations in glucose tolerance to a degree consistent with the onset of diabetes, despite higher circulating insulin concentrations. This is also supported by the work undertaken in fulfilment of the wider objectives of Chapter 4, wherein genes involved in glucose uptake were downregulated in skeletal muscle in response to circadian disruption, resulting in impaired insulin-stimulated glucose uptake (Perrin *et al.*, 2018). Similar rhythms have also been proposed for lipid metabolism (Gooley, 2016), which it also seems reasonable to speculate are dictated by rhythms in feeding behaviour (Mattson *et al.*, 2014; Longo and Panda, 2016; McGinnis and Young, 2016). Consequently, although the fasting period may well have permitted the predominance of lipolysis and lipid clearance, the inability of processes involved in glucose and lipid homeostasis to anticipate nutrient influx may well have resulted in exaggerated postprandial responses and deleterious lipid deposition. This may therefore represent an inherent weakness of these approaches, the impact of which needs to be established by future work.

Considering this final point, and the wider discussions in this final chapter, it may be that other modalities of intermittent fasting will prove more effective in managing obesity and the accompanying dysfunction; specifically, time-restricted feeding stands as a particularly promising candidate. Although research to substantiate this claim is lacking as the concept remains in its infancy, time-restricted feeding certainly represents the best reconciliation of the various strengths and weaknesses established by these experiments. Firstly, in constraining the feeding window to 10 hours or less

(Patterson and Sears, 2017), this method offers enhanced opportunities for lipolytic predominance compared to typical meal patterns (Antoni, Robertson, *et al.*, 2018; Gabel *et al.*, 2018). Although this falls short of the 24-hour fast achieved in the present study, with time-restricted feeding the stated fasting duration can be applied daily, meaning that overall fasted time may actually be greater. Secondly, the ability to apply the restriction on a daily basis means that feeding behaviour can be anticipated by 24-hour endogenous rhythms, thereby fostering circadian alignment and avoiding the deleterious effects of misalignment on energy metabolism and health. Thirdly, shortening the fasting window should also help to minimise the loss of lean mass, whilst also providing the exogenous influx of carbohydrate that may allow physical activity levels to be maintained (Betts *et al.*, 2016; Müller, Enderle and Bosy-Westphal, 2016). Although there are certainly questions as to how this approach might fit with modern lifestyles (Antoni, Robertson, *et al.*, 2018), early studies of this method are promising, as laid out in Section 2.4.3, with improvements in fat oxidation, reductions in fat mass, and potential impacts on glycaemia (Stote *et al.*, 2007; Moro *et al.*, 2016; Antoni, Robertson, *et al.*, 2018; Sutton *et al.*, 2018).

In a similar vein, it is worthy of note that metabolic control is but one dimension of overall health and wellbeing, so the conclusions drawn on the utility of complete alternate-day fasting may be entirely different if other outcomes and endpoints had been considered. For instance, intermittent fasting has been associated with reductions in inflammation and oxidative stress (Longo and Mattson, 2014). These processes have been implicated in cancer aetiology and the adipocyte dysfunction that precedes observable deteriorations in metabolic control (Reuter *et al.*, 2010; Tripathi and Pandey, 2012; Wensveen *et al.*, 2015; Engin, 2017). As a result, the therapeutic potential of routinely extending fasting intervals may instead lie in preventing obesity-associated dysfunction as opposed to managing it, or perhaps slowing tumour development. The intention of such speculations is not to propose inadequate outcome selection in the present work, but rather to illustrate the dearth of knowledge pertaining to how acute periods of fasting affect human physiology and disease mechanisms.

Much of what is currently known draws from studies of more extreme fasting durations, in the order of days rather than hours (Owen *et al.*, 1979; Carlson, Snead and Campbell, 1994; Soeters *et al.*, 2012; Vendelbo *et al.*, 2014). Such durations are

far removed from the 12- to 24-hour periods that might be reasonably be applied by the general population for the purposes of cardiometabolic health improvement, and the current results relating to body composition eloquently demonstrate the profound impact that extending the fasting interval by a few hours can exert. As such, there needs to be a concerted effort by the research community to establish how acute periods of fasting in the order of 12-24 hours affect human physiology. This will answer fundamental questions, such as which mechanistic pathways are responsive, the optimal duration for enhancing metabolic health, how frequently fasting should be applied, and so on, which will ultimately dictate the most efficacious strategy, which can then be evaluated itself.

In the collective light of the experiments undertaken and these ensuing discussions, it can be concluded that diurnal rhythms in subjective appetite can be employed effectively in the design of intermittent fasting interventions. When applied in the form of energy-restricted complete alternate-day fasting, such diets can elicit improvements in metabolic control in overweight and obese adults when compared to daily calorie restriction, without incurring adaptive declines in energy expenditure. However, in lean adults, these diets do not result in improvements in metabolic health, instead inducing adaptive declines in spontaneous physical activity during fasting. As none of these effects were apparent with either intermittent fasting or calorie restriction in isolation, it seems that the amount and timing of energy intake exert interactive effects on these outcomes. However, intermittent fasting did also result in less pronounced declines in fat mass than daily calorie restriction, an effect that is likely to be driven by reduced muscle protein synthesis during fasting. Therefore, from a practical perspective, short-term complete alternate-day fasting represents an effective dietary intervention for improving health in states where there is a pre-existing degree of metabolic disquiet, but in healthy individuals there seems to be little benefit to be gained. However, routinely extending the fasting interval to 24-hours does negatively impact on lean mass, so those undertaking such diets should place emphasis upon maintaining physical activity levels and possibly supplementing their protein intake.

There is little doubt that temporal restrictions of feeding have potential as strategies for managing obesity and the accompanying dysfunction; after all, it is often said that time heals all wounds. They are likely to induce calorie restriction without the need to

actively monitor dietary intake and there is evidence to support beneficial effects of routine fasting extension. However, in light of the potential for: a) circadian misalignment; b) the loss of lean mass invoked by 24-hour fasting intervals; and c) the suppression of spontaneous physical activity during fasted periods, complete alternate-day fasting seems unlikely to be the optimal format.

References

Abbott, W. G., Howard, B. V, Christin, L., Freymond, D., Lillioja, S., … Ravussin, E. (1988). 'Short-term energy balance: relationship with protein, carbohydrate, and fat balances.', *The American Journal of Physiology*, 255(3), pp. 332–337.

Abdelaal, M., le Roux, C. W. and Docherty, N. G. (2017). 'Morbidity and mortality associated with obesity.', *Annals of translational medicine*, 5(7), p. 161.

Abdulla, H., Smith, K., Atherton, P. J. and Idris, I. (2016). 'Role of insulin in the regulation of human skeletal muscle protein synthesis and breakdown: a systematic review and meta-analysis', *Diabetologia*, 59(1), pp. 44–55.

Abdulla, M., Andersson, I., Asp, N. G., Berthelsen, K., Birkhed, D., … Ockerman, P. A. (1981). 'Nutrient intake and health status of vegans. Chemical analyses of diets using the duplicate portion sampling technique', *The American Journal of Clinical Nutrition*, 34(11), pp. 2464–2477.

Abdullah, A., Peeters, A., de Courten, M. and Stoelwinder, J. (2010). 'The magnitude of association between overweight and obesity and the risk of diabetes: a meta-analysis of prospective cohort studies.', *Diabetes Research and Clinical Practice*, 89(3), pp. 309–19.

Acheson, K. J., Blondel-Lubrano, A., Oguey-Araymon, S., Beaumont, M., Emady-Azar, S., … Bovetto, L. (2011). 'Protein choices targeting thermogenesis and metabolism', *American Journal of Clinical Nutrition*, 93(3), pp. 525–534.

Acheson, K. J., Schutz, Y., Bessard, T., Anantharaman, K., Flatt, J. P. and Jequier, E. (1988). 'Glycogen storage capacity and de novo lipogenesis during massive carbohydrate overfeeding in man.', *American Journal of Clinical Nutrition*, 48(2), pp. 240–247.

Achten, J. and Jeukendrup, A. E. (2003). 'Heart Rate Monitoring', *Sports Medicine*, 33(7), pp. 517–538.

Adab, P., Pallan, M. and Whincup, P. H. (2018). 'Is BMI the best measure of obesity?', *British Medical Journal*, 360, p. k1274.

Adeva-Andany, M. M., González-Lucán, M., Donapetry-García, C., Fernández-Fernández, C. and Ameneiros-Rodríguez, E. (2016). 'Glycogen metabolism in humans', *BBA Clinical*, 5, pp. 85–100.

Agius, L. (2015). 'Role of glycogen phosphorylase in liver glycogen metabolism', *Molecular Aspects of Medicine*, 46, pp. 34–45.

Ahern, A. L., Olson, A. D., Aston, L. M. and Jebb, S. A. (2011). 'Weight Watchers on prescription: An observational study of weight change among adults referred to Weight Watchers by the NHS', *BMC Public Health*, 11(1), p. 434.

Ahima, R. S. and Flier, J. S. (2000). 'Adipose tissue as an endocrine organ.', *Trends in Endocrinology and Metabolism*, 11(8), pp. 327–32.

Ahima, R. S., Qi, Y. and Singhal, N. S. (2006). 'Adipokines that link obesity and diabetes to the hypothalamus.', *Progress in Brain Research*, 153, pp. 155–74.

Ahluwalia, N., Dwyer, J., Terry, A., Moshfegh, A. and Johnson, C. (2016). 'Update on NHANES Dietary Data: Focus on Collection, Release, Analytical Considerations, and Uses to Inform Public Policy', *Advances in Nutrition*, 7(1), pp. 121–134.

Ahmed, M., Gannon, M. C. and Nuttall, F. Q. (1976). 'Postprandial plasma glucose, insulin, glucagon and triglyceride responses to a standard diet in normal subjects', *Diabetologia*, 12(1), pp. 61–67.

Ainsworth, B. E., Haskell, W. L., Herrmann, S. D., Meckes, N., Bassett, D. R., … Leon, A. S. (2011). '2011 Compendium of Physical Activities: a second update of codes and MET values.', *Medicine and science in sports and exercise*, 43(8), pp. 1575–81.

Al-Goblan, A. S., Al-Alfi, M. A. and Khan, M. Z. (2014). 'Mechanism linking diabetes mellitus and obesity.', *Diabetes, metabolic syndrome and obesity*, 7, pp. 587–91.

Al-Naimi, S., Hampton, S. M., Richard, P., Tzung, C. and Morgan, L. M. (2004). 'Postprandial metabolic profiles following meals and snacks eaten during simulated night and day shift work.', *Chronobiology International*, 21(6), pp. 937–47.

Alam, D. S., Hulshof, P. J. M., Roordink, D., Meltzer, M., Yunus, M., … van Raaij, J. M. A. (2005). 'Validity and reproducibility of resting metabolic rate measurements in rural Bangladeshi women: comparison of measurements obtained by Medgem^{TM} and by Deltatrac^{TM} device', *European Journal of Clinical Nutrition*, 59(5), pp. 651–657.

Alberti, K. G. and Zimmet, P. Z. (1998). 'Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation.', *Diabetic Medicine*, 15(7), pp. 539–53.

Albrecht, U. (2012). 'Timing to perfection: the biology of central and peripheral circadian clocks.', *Neuron*, 74(2), pp. 246–60.

Albrecht, U. (2017). 'The circadian clock, metabolism and obesity', *Obesity Reviews*, 18, pp. 25–33.

Alhamdan, B. A., Garcia-Alvarez, A., Alzahrnai, A. H., Karanxha, J., Stretchberry, D. R., … Cheskin, L. J. (2016). 'Alternate-day versus daily energy restriction diets: which is more effective for weight loss? A systematic review and meta-analysis.', *Obesity Science and Practice*, 2(3), pp. 293–302.

Almiron-Roig, E., Solis-Trapala, I., Dodd, J. and Jebb, S. A. (2013). 'Estimating food portions: Influence of unit number, meal type and energy density', *Appetite*, 71, pp. 95–103.

Almundarij, T. I., Gavini, C. K. and Novak, C. M. (2017). 'Suppressed sympathetic outflow to skeletal muscle, muscle thermogenesis, and activity energy expenditure with calorie restriction.', *Physiological Reports*, 5(4), p. e13171.

Andreoli, A., Scalzo, G., Masala, S., Tarantino, U. and Guglielmi, G. (2009). 'Body composition assessment by dual-energy X-ray absorptiometry (DXA)', *La radiologia medica*, 114(2), pp. 286–300.

Di Angelantonio, E., Bhupathiraju, S. N., Wormser, D., Gao, P., Kaptoge, S., … Hu,

F. B. (2016). ‘Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents’, *The Lancet*, 388(10046), pp. 776–786.

Anton, S. D., Moehl, K., Donahoo, W. T., Marosi, K., Lee, S. A., … Mattson, M. P. (2018). ‘Flipping the Metabolic Switch: Understanding and Applying the Health Benefits of Fasting.’, *Obesity*, 26(2), pp. 254–268.

Antoni, R., Johnston, K. L., Collins, A. L. and Robertson, M. D. (2017). ‘Effects of intermittent fasting on glucose and lipid metabolism’, *Proceedings of the Nutrition Society*, 76(3), pp. 361–368.

Antoni, R., Johnston, K. L., Collins, A. L. and Robertson, M. D. (2018). ‘Intermittent v. continuous energy restriction: differential effects on postprandial glucose and lipid metabolism following matched weight loss in overweight/obese participants’, *British Journal of Nutrition*, 119(5), pp. 507–516.

Antoni, R., Robertson, T. M., Robertson, M. D. and Johnston, J. D. (2018). ‘A pilot feasibility study exploring the effects of a moderate time-restricted feeding intervention on energy intake, adiposity and metabolic physiology in free-living human subjects’, *Journal of Nutritional Science*, 7, p. e22.

Arble, D. M., Bass, J., Laposky, A. D., Vitaterna, M. H. and Turek, F. W. (2009). ‘Circadian timing of food intake contributes to weight gain.’, *Obesity (Silver Spring, Md.)*, 17(11), pp. 2100–2.

Archer, S. N., Laing, E. E., Möller-Levet, C. S., van der Veen, D. R., Bucca, G., … Dijk, D.-J. (2014). ‘Mistimed sleep disrupts circadian regulation of the human transcriptome.’, *Proceedings of the National Academy of Sciences of the United States of America*, 111(6), pp. E682-91.

Areta, J. L., Burke, L. M., Ross, M. L., Camera, D. M., West, D. W. D., … Coffey, V. G. (2013). ‘Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis’, *The Journal of Physiology*, 591(9), pp. 2319–2331.

Armstrong, L. E. (2005). ‘Hydration assessment techniques.’, *Nutrition Reviews*, 63(6 Pt 2), pp. S40-54.

Arner, E., Westermark, P. O., Spalding, K. L., Britton, T., Ryden, M., … Arner, P. (2010). ‘Adipocyte Turnover: Relevance to Human Adipose Tissue Morphology’, *Diabetes*, 59(1), pp. 105–109.

Ashwell, M., Gunn, P. and Gibson, S. (2012). ‘Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis.’, *Obesity Reviews*, 13(3), pp. 275–86.

Atherton, P. J. and Smith, K. (2012). ‘Muscle protein synthesis in response to nutrition and exercise.’, *The Journal of physiology*, 590(5), pp. 1049–57.

Atkinson, G. (2002). ‘Analysis of repeated measurements in physical therapy research: multiple comparisons amongst level means and multi-factorial designs’, *Physical Therapy in Sport*, 3(4), pp. 191–203.

Austin, J. and Marks, D. (2009). 'Hormonal regulators of appetite.', *International journal of pediatric endocrinology*, 2009, p. 141753.

Aydin, S. (2015). 'A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA', *Peptides*, 72, pp. 4–15.

Bäckhed, F. (2009). 'Changes in Intestinal Microflora in Obesity: Cause or Consequence?', *Journal of Pediatric Gastroenterology and Nutrition*, 48(Suppl 2), pp. S56–S57.

Bagger, J. I., Holst, J. J., Hartmann, B., Andersen, B., Knop, F. K. and Vilsbøll, T. (2015). 'Effect of Oxyntomodulin, Glucagon, GLP-1, and Combined Glucagon +GLP-1 Infusion on Food Intake, Appetite, and Resting Energy Expenditure', *The Journal of Clinical Endocrinology and Metabolism*, 100(12), pp. 4541–4552.

Baicy, K., London, E. D., Monterosso, J., Wong, M.-L., Delibasi, T., … Licinio, J. (2007). 'Leptin replacement alters brain response to food cues in genetically leptin-deficient adults.', *Proceedings of the National Academy of Sciences of the United States of America*, 104(46), pp. 18276–9.

Baile, C. A. and Forbes, J. M. (1974). 'Control of Feed Intake and Regulation of Energy Balance in Ruminants', *Physiological Reviews*, 54(1), pp. 160–214.

Bakrania, K., Yates, T., Edwardson, C. L., Bodicoat, D. H., Esliger, D. W., … Khunti, K. (2017). 'Associations of moderate-to-vigorous-intensity physical activity and body mass index with glycated haemoglobin within the general population: a cross-sectional analysis of the 2008 Health Survey for England', *BMJ Open*, 7(4), p. e014456.

Bandín, C., Scheer, F. A. J. L., Luque, A. J., Ávila-Gandía, V., Zamora, S., … Garaulet, M. (2015). 'Meal timing affects glucose tolerance, substrate oxidation and circadian-related variables: A randomized, crossover trial', *International Journal of Obesity*, 39(5), pp. 828–833.

Barnosky, A. R., Hoddy, K. K., Unterman, T. G. and Varady, K. A. (2014). 'Intermittent fasting vs daily calorie restriction for type 2 diabetes prevention: a review of human findings', *Translational Research*, 164(4), pp. 302–311.

Barte, J. C. M., Ter Bogt, N. C. W., Bogers, R. P., Teixeira, P. J., Blissmer, B., … Bemelmans, W. J. E. (2010). 'Maintenance of weight loss after lifestyle interventions for overweight and obesity, a systematic review', *Obesity Reviews*, 11(12), pp. 899–906.

Batterham, R. L., Le Roux, C. W., Cohen, M. A., Park, A. J., Ellis, S. M., … Bloom, S. R. (2003). 'Pancreatic Polypeptide Reduces Appetite and Food Intake in Humans', *The Journal of Clinical Endocrinology and Metabolism*, 88(8), pp. 3989–3992.

Benloucif, S., Burgess, H. J., Klerman, E. B., Lewy, A. J., Middleton, B., … Revell, V. L. (2008). 'Measuring melatonin in humans.', *Journal of Clinical Sleep Medicine*, 4(1), pp. 66–9.

Berg, A. H. and Scherer, P. E. (2005). 'Adipose Tissue, Inflammation, and Cardiovascular Disease', *Circulation Research*, 96(9), pp. 939–949.

Bergström, J. (1962). 'Muscle Electrolytes in Man Determined by Neutron Activation

Analysis on Needle Biopsy Specimens: A Study on Normal Subjects, Kidney Patients, and Patients with Chronic Diarrhoea', *Scandinavian Journal of Clinical and Laboratory Investigation*, 14(Supplement 68), p. 110.

Betts, J. A., Chowdhury, E. A., Gonzalez, J. T., Richardson, J. D., Tsintzas, K. and Thompson, D. (2016). 'Is breakfast the most important meal of the day?', *Proceedings of the Nutrition Society*, 75(4), pp. 464–474.

Betts, J. A., Richardson, J. D., Chowdhury, E. A., Holman, G. D., Tsintzas, K. and Thompson, D. (2014). 'The causal role of breakfast in energy balance and health: a randomized controlled trial in lean adults.', *The American Journal of Clinical Nutrition*, 100(2), pp. 539–47.

Bhutani, S., Klempel, M. C., Berger, R. A. and Varady, K. A. (2010). 'Improvements in Coronary Heart Disease Risk Indicators by Alternate-Day Fasting Involve Adipose Tissue Modulations', *Obesity*, 18(11), pp. 2152–2159.

Bhutani, S., Racine, N., Shriver, T. and Schoeller, D. A. (2015). 'Special Considerations for Measuring Energy Expenditure with Doubly Labeled Water under Atypical Conditions.', *Journal of Obesity and Weight Loss Therapy*, 5(Suppl 5), p. 2.

Birsoy, K., Festuccia, W. T. and Laplante, M. (2013). 'A comparative perspective on lipid storage in animals', *Journal of Cell Science*, 126(7), pp. 1541–1552.

Black, A. E., Welch, A. A. and Bingham, S. A. (2000). 'Validation of dietary intakes measured by diet history against 24 h urinary nitrogen excretion and energy expenditure measured by the doubly-labelled water method in middle-aged women.', *The British journal of nutrition*, 83(4), pp. 341–54.

Blackburn, G. (1995). 'Effect of degree of weight loss on health benefits.', *Obesity research*, 3 Suppl 2, p. 211s–216s.

Blüher, S., Shah, S. and Mantzoros, C. S. (2009). 'Leptin deficiency: clinical implications and opportunities for therapeutic interventions.', *Journal of investigative medicine : the official publication of the American Federation for Clinical Research*, 57(7), pp. 784–8.

Bo, S., Fadda, M., Castiglione, A., Ciccone, G., De Francesco, A., … Broglio, F. (2015). 'Is the timing of caloric intake associated with variation in diet-induced thermogenesis and in the metabolic pattern? A randomized cross-over study', *International Journal of Obesity*, 39(12), pp. 1689–1695.

Boden, G., Chen, X., Mozzoli, M. and Ryan, I. (1996). 'Effect of fasting on serum leptin in normal human subjects.', *The Journal of Clinical Endocrinology and Metabolism*, 81(9), pp. 3419–3423.

Boelen, A., Wiersinga, W. M. and Fliers, E. (2008). 'Fasting-induced changes in the hypothalamus-pituitary-thyroid axis.', *Thyroid*, 18(2), pp. 123–9.

Borai, A., Livingstone, C., Kaddam, I. and Ferns, G. (2011). 'Selection of the appropriate method for the assessment of insulin resistance.', *BMC Medical Research Methodology*, 11(1), p. 158.

Borina, E., Pellegrino, M. A., D'Antona, G. and Bottinelli, R. (2010). 'Myosin and

actin content of human skeletal muscle fibers following 35 days bed rest', *Scandinavian Journal of Medicine and Science in Sports*, 20(1), pp. 65–73.

Bot, S. D. M. and Hollander, A. P. (2000). 'The relationship between heart rate and oxygen uptake during non-steady state exercise', *Ergonomics*, 43(10), pp. 1578–1592.

Brage, S., Brage, N., Franks, P., Ekelund, U. and Wareham, N. (2005). 'Reliability and validity of the combined heart rate and movement sensor Actiheart', *European Journal of Clinical Nutrition*, 59(4), pp. 561–570.

Brage, S., Brage, N., Franks, P. W., Ekelund, U., Wong, M.-Y., … Wareham, N. J. (2004). 'Branched equation modeling of simultaneous accelerometry and heart rate monitoring improves estimate of directly measured physical activity energy expenditure', *Journal of Applied Physiology*, 96(1), pp. 343–351.

Brage, S., Ekelund, U., Brage, N., Hennings, M. A., Froberg, K., … Wareham, N. J. (2007). 'Hierarchy of individual calibration levels for heart rate and accelerometry to measure physical activity.', *Journal of Applied Physiology*, 103(2), pp. 682–92.

Brage, S., Westgate, K., Franks, P. W., Stegle, O., Wright, A., … Wareham, N. J. (2015). 'Estimation of Free-Living Energy Expenditure by Heart Rate and Movement Sensing: A Doubly-Labelled Water Study', *PLOS ONE*. Edited by F. Criscuolo, 10(9), p. e0137206.

Bray, G. A., Frühbeck, G., Ryan, D. H. and Wilding, J. P. H. (2016). 'Management of obesity', *Lancet*, 387(10031), pp. 1947–1956.

Bray, M. S., Tsai, J.-Y., Villegas-Montoya, C., Boland, B. B., Blasier, Z., … Young, M. E. (2010). 'Time-of-day-dependent dietary fat consumption influences multiple cardiometabolic syndrome parameters in mice.', *International Journal of Obesity*, 34(11), pp. 1589–98.

Brennan, I. M., Little, T. J., Feltrin, K. L., Smout, A. J. P. M., Wishart, J. M., … Feinle-Bisset, C. (2008). 'Dose-dependent effects of cholecystokinin-8 on antropyloroduodenal motility, gastrointestinal hormones, appetite, and energy intake in healthy men', *American Journal of Physiology - Endocrinology and Metabolism*, 295(6), pp. E1487–E1494.

Brennan, I. M., Luscombe-Marsh, N. D., Seimon, R. V., Otto, B., Horowitz, M., … Feinle-Bisset, C. (2012). 'Effects of fat, protein, and carbohydrate and protein load on appetite, plasma cholecystokinin, peptide YY, and ghrelin, and energy intake in lean and obese men', *American Journal of Physiology - Gastrointestinal and Liver Physiology*, 303(1), pp. G129–G140.

Breslin, P. A. S. (2013). 'An Evolutionary Perspective on Food and Human Taste', *Current Biology*, 23(9), pp. R409–R418.

Brewer, P. D., Habtemichael, E. N., Romenskaia, I., Mastick, C. C. and Coster, A. C. F. (2014). 'Insulin-regulated Glut4 translocation: membrane protein trafficking with six distinctive steps.', *The Journal of biological chemistry*, 289(25), pp. 17280–98.

Brillon, D. J., Zheng, B., Campbell, R. G. and Matthews, D. E. (1995). 'Effect of cortisol on energy expenditure and amino acid metabolism in humans', *American Journal of Physiology-Endocrinology and Metabolism*, 268(3), pp. E501–E513.

Brown, S. A. (2014). 'Circadian clock-mediated control of stem cell division and differentiation: beyond night and day.', *Development*, 141(16), pp. 3105–11.

Budnik, A. and Henneberg, M. (2017). 'Worldwide Increase of Obesity Is Related to the Reduced Opportunity for Natural Selection', *PLOS ONE*. Edited by C. S. Rosenfeld, 12(1), p. e0170098.

Buhr, E. D. and Takahashi, J. S. (2013). 'Molecular Components of the Mammalian Circadian Clock', in *Handbook of experimental pharmacology*, pp. 3–27.

Burton, P. and Lightowler, H. J. (2008). 'The impact of freezing and toasting on the glycaemic response of white bread', *European Journal of Clinical Nutrition*, 62(5), pp. 594–599.

Butland, B., Jebb, S., Kopelman, P., McPherson, K., Thomas, S., … Parry, V. (2007). *Foresight - Tackling obesities: Future choices - Project report*.

Butler, P. J., Green, J. A., Boyd, I. L. and Speakman, J. R. (2004). 'Measuring metabolic rate in the field: the pros and cons of the doubly labelled water and heart rate methods', *Functional Ecology*, 18(2), pp. 168–183.

Buysse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R. and Kupfer, D. J. (1989). 'The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research', *Psychiatry Research*, 28(2), pp. 193–213.

Byrne, N. M., Sainsbury, A., King, N. A., Hills, A. P. and Wood, R. E. (2018). 'Intermittent energy restriction improves weight loss efficiency in obese men: the MATADOR study', *International Journal of Obesity*, 42(2), pp. 129–138.

Cable, E. J., Onishi, K. G. and Prendergast, B. J. (2017). 'Circadian rhythms accelerate wound healing in female Siberian hamsters', *Physiology and Behavior*, 171, pp. 165–174.

Cailotto, C., La Fleur, S. E., Van Heijningen, C., Wortel, J., Kalsbeek, A., … Buijs, R. M. (2005). 'The suprachiasmatic nucleus controls the daily variation of plasma glucose via the autonomic output to the liver: are the clock genes involved?', *European Journal of Neuroscience*, 22(10), pp. 2531–2540.

Camps, S. G., Verhoef, S. P. and Westerterp, K. R. (2013). 'Weight loss, weight maintenance, and adaptive thermogenesis', *The American Journal of Clinical Nutrition*, 97(5), pp. 990–994.

Canuto, R., da Silva Garcez, A., Kac, G., de Lira, P. I. C. and Olinto, M. T. A. (2017). 'Eating frequency and weight and body composition: a systematic review of observational studies', *Public Health Nutrition*, 20(12), pp. 2079–2095.

Cardoso, L., Rodrigues, D., Gomes, L. and Carrilho, F. (2017). 'Short- and long-term mortality after bariatric surgery: A systematic review and meta-analysis', *Diabetes, Obesity and Metabolism*, 19(9), pp. 1223–1232.

Carlson, M. G., Snead, W. L. and Campbell, P. J. (1994). 'Fuel and energy metabolism in fasting humans', *The American Journal of Clinical Nutrition*, 60(1), pp. 29–36.

Carlson, O., Martin, B., Stote, K. S., Golden, E., Maudsley, S., … Mattson, M. P.

(2007). ‘Impact of reduced meal frequency without caloric restriction on glucose regulation in healthy, normal-weight middle-aged men and women.’, *Metabolism - Clinical and Experimental*, 56(12), pp. 1729–34.

Carnell, S., Grillot, C., Ungredda, T., Ellis, S., Mehta, N., … Geliebter, A. (2018). ‘Morning and afternoon appetite and gut hormone responses to meal and stress challenges in obese individuals with and without binge eating disorder’, *International Journal of Obesity*, 42(4), pp. 841–849.

Carrasco-Benso, M. P., Rivero-Gutierrez, B., Lopez-Minguez, J., Anzola, A., Diez-Noguera, A., … Garaulet, M. (2016). ‘Human adipose tissue expresses intrinsic circadian rhythm in insulin sensitivity’, *The FASEB Journal*, 30(9), pp. 3117–3123.

Carrera, O., Adan, R. A. H., Gutierrez, E., Danner, U. N., Hoek, H. W., … Kas, M. J. H. (2012). ‘Hyperactivity in Anorexia Nervosa: Warming Up Not Just Burning-Off Calories’, *PLoS ONE*. Edited by M. Botbol, 7(7), p. e41851.

Carter, S., Clifton, P. M. and Keogh, J. B. (2016). ‘The effects of intermittent compared to continuous energy restriction on glycaemic control in type 2 diabetes; a pragmatic pilot trial’, *Diabetes Research and Clinical Practice*, 122, pp. 106–112.

Catellier, D. J., Hannan, P. J., Murray, D. M., Addy, C. L., Conway, T. L., … Rice, J. C. (2005). ‘Imputation of missing data when measuring physical activity by accelerometry.’, *Medicine and Science in Sports and Exercise*, 37(11 Suppl), pp. S555-62.

Catenacci, V. A., Pan, Z., Ostendorf, D., Brannon, S., Gozansky, W. S., … Troy Donahoo, W. (2016). ‘A randomized pilot study comparing zero-calorie alternate-day fasting to daily caloric restriction in adults with obesity’, *Obesity*, 24(9), pp. 1874–1883.

Chakravarthy, M. V and Booth, F. W. (2004). ‘Eating, exercise, and “thrifty” genotypes: connecting the dots toward an evolutionary understanding of modern chronic diseases.’, *Journal of Applied Physiology*, 96(1), pp. 3–10.

Chambers, L., McCrickerd, K. and Yeomans, M. R. (2015). ‘Optimising foods for satiety’, *Trends in Food Science and Technology*, 41(2), pp. 149–160.

Chan, J. L., Heist, K., DePaoli, A. M., Veldhuis, J. D., Mantzoros, C. S., … Fekete, C. (2003). ‘The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men’, *Journal of Clinical Investigation*, 111(9), pp. 1409–1421.

Chandarana, K., Drew, M. E., Emmanuel, J., Karra, E., Gelegen, C., … Batterham, R. L. (2009). ‘Subject Standardization, Acclimatization, and Sample Processing Affect Gut Hormone Levels and Appetite in Humans’, *Gastroenterology*, 136(7), pp. 2115–2126.

Chaudhri, O., Small, C. and Bloom, S. (2006). ‘Gastrointestinal hormones regulating appetite.’, *Philosophical Transactions of the Royal Society of London - Series B - Biological sciences*, 361(1471), pp. 1187–209.

Chiou, Y.-Y., Yang, Y., Rashid, N., Ye, R., Selby, C. P. and Sancar, A. (2016). ‘Mammalian Period represses and de-represses transcription by displacing CLOCK-

BMAL1 from promoters in a Cryptochrome-dependent manner.', *Proceedings of the National Academy of Sciences of the United States of America*, 113(41), pp. E6072–E6079.

Cho, K. W. and Lumeng, C. N. (2011). 'SIRT1: A Guardian at the Gates of Adipose Tissue Inflammation', *Diabetes*, 60(12), pp. 3100–3102.

Choe, S. S., Huh, J. Y., Hwang, I. J., Kim, J. I. and Kim, J. B. (2016). 'Adipose Tissue Remodeling: Its Role in Energy Metabolism and Metabolic Disorders.', *Frontiers in endocrinology*, 7, p. 30.

Chow, C. C. and Hall, K. D. (2014). 'Short and long-term energy intake patterns and their implications for human body weight regulation.', *Physiology and Behavior*, 134, pp. 60–65.

Chowdhury, E. A., Richardson, J. D., Holman, G. D., Tsintzas, K., Thompson, D. and Betts, J. A. (2016). 'The causal role of breakfast in energy balance and health: a randomized controlled trial in obese adults.', *The American journal of clinical nutrition*, 103(3), pp. 747–56.

Chowdhury, E. A., Western, M. J., Nightingale, T. E., Peacock, O. J. and Thompson, D. (2017). 'Assessment of laboratory and daily energy expenditure estimates from consumer multi-sensor physical activity monitors', *PLOS ONE*. Edited by L. P. Ardigò, 12(2), p. e0171720.

Christiansen, J. J., Djurhuus, C. B., Gravholt, C. H., Iversen, P., Christiansen, J. S., … Møller, N. (2007). 'Effects of Cortisol on Carbohydrate, Lipid, and Protein Metabolism: Studies of Acute Cortisol Withdrawal in Adrenocortical Failure', *The Journal of Clinical Endocrinology and Metabolism*, 92(9), pp. 3553–3559.

Church, T. and Martin, C. K. (2018). 'The Obesity Epidemic: A Consequence of Reduced Energy Expenditure and the Uncoupling of Energy Intake?', *Obesity*, 26(1), pp. 14–16.

Cipolla-Neto, J., Amaral, F. G., Afeche, S. C., Tan, D. X. and Reiter, R. J. (2014). 'Melatonin, energy metabolism, and obesity: a review', *Journal of Pineal Research*, 56(4), pp. 371–381.

Clark, J. E. (2015). 'Diet, exercise or diet with exercise: comparing the effectiveness of treatment options for weight-loss and changes in fitness for adults (18-65 years old) who are overfat, or obese; systematic review and meta-analysis.', *Journal of diabetes and metabolic disorders*, 14, p. 31.

Clark, L., Fairhurst, C. and Torgerson, D. J. (2016). 'Allocation concealment in randomised controlled trials: are we getting better?', *British Medical Journal*, 355, p. i5663.

Coelho, M., Oliveira, T. and Fernandes, R. (2013). 'Biochemistry of adipose tissue: an endocrine organ.', *Archives of Medical Science*, 9(2), pp. 191–200.

Cohen, M. A., Ellis, S. M., Le Roux, C. W., Batterham, R. L., Park, A., … Bloom, S. R. (2003). 'Oxyntomodulin Suppresses Appetite and Reduces Food Intake in Humans', *The Journal of Clinical Endocrinology and Metabolism*, 88(10), pp. 4696–4701.

Coker, R. H., Williams, R. H., Yeo, S. E., Kortebein, P. M., Bodenner, D. L., … Evans, W. J. (2009). 'The Impact of Exercise Training Compared to Caloric Restriction on Hepatic and Peripheral Insulin Resistance in Obesity', *Journal of Clinical Endocrinology and Metabolism*, 94(11), pp. 4258–4266.

Coleman, R. A. and Lee, D. P. (2004). 'Enzymes of triacylglycerol synthesis and their regulation', *Progress in Lipid Research*, 43(2), pp. 134–176.

Coleman, R. A. and Mashek, D. G. (2011). 'Mammalian triacylglycerol metabolism: synthesis, lipolysis, and signaling.', *Chemical reviews*, 111(10), pp. 6359–86.

Compher, C., Frankenfield, D., Keim, N. and Roth-Yousey, L. (2006). 'Best Practice Methods to Apply to Measurement of Resting Metabolic Rate in Adults: A Systematic Review', *Journal of the American Dietetic Association*, 106(6), pp. 881–903.

Conley, M., Le Fevre, L., Haywood, C. and Proietto, J. (2018). 'Is two days of intermittent energy restriction per week a feasible weight loss approach in obese males? A randomised pilot study', *Nutrition and Dietetics*, 75(1), pp. 65–72.

Cook, A., Pryer, J. and Shetty, P. (2000). 'The problem of accuracy in dietary surveys. Analysis of the over 65 UK National Diet and Nutrition Survey', *Journal of Epidemiology and Community Health*, 54, pp. 611–616.

Coomans, C. P., van den Berg, S. A. A., Lucassen, E. A., Houben, T., Pronk, A. C. M., … Meijer, J. H. (2013). 'The suprachiasmatic nucleus controls circadian energy metabolism and hepatic insulin sensitivity.', *Diabetes*, 62(4), pp. 1102–8.

Copeland, K. C., Kenney, F. A. and Nair, K. S. (1992). 'Heated dorsal hand vein sampling for metabolic studies: a reappraisal.', *The American journal of physiology*, 263(5 Pt 1), pp. E1010-4.

Coppack, S. W., Fisher, R. M., Gibbons, G. F., Humphreys, S. M., McDonough, M. J., … Frayn, K. N. (1990). 'Postprandial substrate deposition in human forearm and adipose tissues in vivo.', *Clinical Science*, 79(4), pp. 339–48.

Cordain, L., Eaton, S. B., Sebastian, A., Mann, N., Lindeberg, S., … Brand-Miller, J. (2005). 'Origins and evolution of the Western diet: health implications for the 21st century.', *The American journal of clinical nutrition*, 81(2), pp. 341–54.

Corder, K., Brage, S. and Ekelund, U. (2007). 'Accelerometers and pedometers: methodology and clinical application.', *Current Opinion in Clinical Nutrition and Metabolic Care*, 10(5), pp. 597–603.

Cornelissen, G. (2014). 'Cosinor-based rhythmometry.', *Theoretical Biology and Medical Modelling*, 11, p. 16.

Del Corral, P., Bryan, D. R., Garvey, W. T., Gower, B. A. and Hunter, G. R. (2011). 'Dietary adherence during weight loss predicts weight regain.', *Obesity*, 19(6), pp. 1177–81.

Courcoulas, A. P., Yanovski, S. Z., Bonds, D., Eggerman, T. L., Horlick, M., … Arterburn, D. E. (2014). 'Long-term outcomes of bariatric surgery: a National Institutes of Health symposium.', *JAMA surgery*, 149(12), pp. 1323–9.

Crespo, C., Perianes Cachero, A., Puebla Jiménez, L., Barrios, V. and Arilla Ferreiro, E. (2014). 'Peptides and food intake.', *Frontiers in endocrinology*, 5, p. 58.

Crouter, S. E., Schneider, P. L., Karabulut, M. and Bassett, D. R. (2003). 'Validity of 10 electronic pedometers for measuring steps, distance, and energy cost.', *Medicine and Science in Sports and Exercise*, 35(8), pp. 1455–1460.

Cummings, D. E., Purnell, J. Q., Frayo, R. S., Schmidova, K., Wisse, B. E. and Weigle, D. S. (2001). 'A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans.', *Diabetes*, 50(8), pp. 1714–9.

Cuthbertson, D. J., Steele, T., Wilding, J. P., Halford, J. C., Harrold, J. A., … Karpe, F. (2017). 'What have human experimental overfeeding studies taught us about adipose tissue expansion and susceptibility to obesity and metabolic complications?', *International Journal of Obesity*, 41(6), pp. 853–865.

Czech, M. P., Tencerova, M., Pedersen, D. J. and Aouadi, M. (2013). 'Insulin signalling mechanisms for triacylglycerol storage', *Diabetologia*, 56(5), pp. 949–964.

Dali-Youcef, N., Mecili, M., Ricci, R. and Andrès, E. (2013). 'Metabolic inflammation: Connecting obesity and insulin resistance', *Annals of Medicine*, 45(3), pp. 242–253.

Dallongeville, J., Hecquet, B., Lebel, P., Edme Â, J.-L., Le Fur, C., … Romon, M. (1998). 'Short term response of circulating leptin to feeding and fasting in man: influence of circadian cycle', *International Journal of Obesity*, 22, pp. 728–733.

Damiola, F., Le Minh, N., Preitner, N., Kornmann, B., Fleury-Olela, F. and Schibler, U. (2000). 'Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus.', *Genes and Development*, 14(23), pp. 2950–61.

Dang, F., Sun, X., Ma, X., Wu, R., Zhang, D., … Liu, Y. (2016). 'Insulin post-transcriptionally modulates Bmal1 protein to affect the hepatic circadian clock', *Nature Communications*, 7, p. 12696.

Dansinger, M. L., Gleason, J. A., Griffith, J. L., Selker, H. P., Schaefer, E. J., … J, K. (2005). 'Comparison of the Atkins, Ornish, Weight Watchers, and Zone Diets for Weight Loss and Heart Disease Risk Reduction', *JAMA*, 293(1), p. 43.

Das, S. K., Gilhooly, C. H., Golden, J. K., Pittas, A. G., Fuss, P. J., … Roberts, S. B. (2007). 'Long-term effects of 2 energy-restricted diets differing in glycemic load on dietary adherence, body composition, and metabolism in CALERIE: a 1-y randomized controlled trial', *The American Journal of Clinical Nutrition*, 85(4), pp. 1023–1030.

Das, S. K., Roberts, S. B., Bhapkar, M. V, Villareal, D. T., Fontana, L., … CALERIE-2 Study Group (2017). 'Body-composition changes in the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE)-2 study: a 2-y randomized controlled trial of calorie restriction in nonobese humans.', *The American journal of clinical nutrition*, 105(4), pp. 913–927.

Dattilo, M., Crispim, C. A., Zimberg, I. Z., Tufik, S. and de Mello, M. T. (2011). 'Meal distribution across the day and its relationship with body composition', *Biological Rhythm Research*, 42(2), pp. 119–129.

Degen, L., Oesch, S., Casanova, M., Graf, S., Ketterer, S., … Beglinger, C. (2005). 'Effect of peptide YY3-36 on food intake in humans.', *Gastroenterology*, 129(5), pp. 1430–6.

Delzenne, N., Blundell, J., Brouns, F., Cunningham, K., De Graaf, K., … Westerterp-Plantenga, M. (2010). 'Gastrointestinal targets of appetite regulation in humans', *Obesity Reviews*, 11(3), pp. 234–250.

Dhurandhar, N. V, Schoeller, D., Brown, A. W., Heymsfield, S. B., Thomas, D., … Energy Balance Measurement Working Group (2015). 'Energy balance measurement: when something is not better than nothing', *International Journal of Obesity*, 39(7), pp. 1109–1113.

Dibner, C. and Schibler, U. (2018). 'Body clocks: Time for the Nobel Prize', *Acta Physiologica*, 222(2), p. e13024.

Dibner, C., Schibler, U. and Albrecht, U. (2010). 'The Mammalian Circadian Timing System: Organization and Coordination of Central and Peripheral Clocks', *Annual Review of Physiology*, 72(1), pp. 517–549.

Dickinson, J. M. and Rasmussen, B. B. (2013). 'Amino acid transporters in the regulation of human skeletal muscle protein metabolism', *Current Opinion in Clinical Nutrition and Metabolic Care*, 16(6), pp. 638–644.

Dideriksen, K., Reitelseder, S. and Holm, L. (2013). 'Influence of amino acids, dietary protein, and physical activity on muscle mass development in humans.', *Nutrients*, 5(3), pp. 852–76.

Dierickx, P., Van Laake, L. W. and Geijsen, N. (2018). 'Circadian clocks: from stem cells to tissue homeostasis and regeneration.', *EMBO reports*, 19(1), pp. 18–28.

Dimitriadis, G., Mitrou, P., Lambadiari, V., Maratou, E. and Raptis, S. A. (2011). 'Insulin effects in muscle and adipose tissue', *Diabetes Research and Clinical Practice*, 93, pp. S52–S59.

Dobbins, M., Decorby, K. and Choi, B. C. K. (2013). 'The Association between Obesity and Cancer Risk: A Meta-Analysis of Observational Studies from 1985 to 2011', *ISRN Preventive Medicine*, 2013, pp. 1–16.

Douglas, G. G. (1911). 'A method for determining the total respiratory exchange in man', *Proceedings of the Physiological Society*, pp. 17–22.

Drummond, M. J., Fry, C. S., Glynn, E. L., Timmerman, K. L., Dickinson, J. M., … Rasmussen, B. B. (2011). 'Skeletal muscle amino acid transporter expression is increased in young and older adults following resistance exercise', *Journal of Applied Physiology*, 111(1), pp. 135–142.

Duffy, J. F. and Dijk, D.-J. (2002). 'Getting Through to Circadian Oscillators: Why Use Constant Routines?', *Journal of Biological Rhythms*, 17(1), pp. 4–13.

Duncan, M., Griffith, M., Rutter, H. and Goldacre, M. J. (2010). 'Certification of obesity as a cause of death in England 1979-2006.', *European journal of public health*, 20(6), pp. 671–5.

Duncan, R. E., Ahmadian, M., Jaworski, K., Sarkadi-Nagy, E. and Sul, H. S. (2007). 'Regulation of Lipolysis in Adipocytes', *Annual Review of Nutrition*, 27(1), pp. 79–101.

Dzaja, A., Dalal, M. A., Himmerich, H., Uhr, M., Pollmächer, T. and Schuld, A. (2004). 'Sleep enhances nocturnal plasma ghrelin levels in healthy subjects', *American Journal of Physiology-Endocrinology and Metabolism*, 286(6), pp. E963–E967.

Eaton, S. B., Konner, M. and Shostak, M. (1988). 'Stone agers in the fast lane: chronic degenerative diseases in evolutionary perspective.', *The American Journal of Medicine*, 84(4), pp. 739–749.

Eckel-Mahan, K. and Sassone-Corsi, P. (2013). 'Metabolism and the circadian clock converge', *Physiological Reviews*, 93, pp. 107–135.

Eder, K., Baffy, N., Andras, A., Ae, F. and Fulop, A. K. (2009). 'The major inflammatory mediator interleukin-6 and obesity', *Inflammation Research*, 58, pp. 727–736.

Edinburgh, R. M., Hengist, A., Smith, H. A., Betts, J. A., Thompson, D., … Gonzalez, J. T. (2017). 'Prior exercise alters the difference between arterialised and venous glycaemia: implications for blood sampling procedures', *British Journal of Nutrition*, 117(10), pp. 1414–1421.

Ekmekcioglu, C. and Touitou, Y. (2011). 'Chronobiological aspects of food intake and metabolism and their relevance on energy balance and weight regulation', *Obesity Reviews*, 12(1), pp. 14–25.

Elfhag, K. and Rossner, S. (2005). 'Who succeeds in maintaining weight loss? A conceptual review of factors associated with weight loss maintenance and weight regain', *Obesity Reviews*, 6(1), pp. 67–85.

Emberson, J., Whincup, P., Walker, M., Thomas, M. and Alberti, K. (2002). 'Biochemical measures in a population-based study: effect of fasting duration and time of day', *Annals of Clinical Biochemistry*, 39(5), pp. 493–501.

Engin, A. (2017). 'Adipose Tissue Hypoxia in Obesity and Its Impact on Preadipocytes and Macrophages: Hypoxia Hypothesis', in *Advances in experimental medicine and biology*, pp. 305–326.

Esmaili, S., Xu, A. and George, J. (2014). 'The multifaceted and controversial immunometabolic actions of adiponectin', *Trends in Endocrinology and Metabolism*, 25(9), pp. 444–451.

Espelund, U., Hansen, T. K., Højlund, K., Beck-Nielsen, H., Clausen, J. T., … Frystyk, J. (2005). 'Fasting Unmasks a Strong Inverse Association between Ghrelin and Cortisol in Serum: Studies in Obese and Normal-Weight Subjects', *The Journal of Clinical Endocrinology and Metabolism*, 90(2), pp. 741–746.

Evans, K., Clark, M. L. and Frayn, K. N. (1999). 'Effects of an oral and intravenous fat load on adipose tissue and forearm lipid metabolism.', *The American journal of physiology*, 276(2 Pt 1), pp. E241-8.

Fabry, P., Fodor, J., Hejl, Z., Braun, T. and Zvolankova, K. (1964). 'The frequency of

meals. Its relation to overweight, hypercholesterolaemia, and decreased glucose-tolerance.', *Lancet*, 2, pp. 614–615.

Fairburn, C. G. (2008). *Cognitive Behavior Therapy and Eating Disorders*. New York: USA.

Farooqi, N., Slinde, F., Håglin, L. and Sandström, T. (2013). 'Validation of SenseWear Armband and ActiHeart monitors for assessments of daily energy expenditure in free-living women with chronic obstructive pulmonary disease.', *Physiological reports*, 1(6), p. e00150.

Fasshauer, M. and Paschke, R. (2003). 'Regulation of adipocytokines and insulin resistance.', *Diabetologia*, 46, pp. 1594–1603.

Felig, P. (1975). 'Amino Acid Metabolism in Man', *Annual Review of Biochemistry*, 44(1), pp. 933–955.

Ferrannini, E. (1988). 'The theoretical bases of indirect calorimetry: A review', *Metabolism*, 37(3), pp. 287–301.

de Ferranti, S. and Mozaffarian, D. (2008). 'The Perfect Storm: Obesity, Adipocyte Dysfunction, and Metabolic Consequences', *Clinical Chemistry*, 54(6), pp. 945–955.

Flatt, J. P. (1995). 'Use and storage of carbohydrate and fat', *The American Journal of Clinical Nutrition*, 61(4 Suppl.), p. 952S–959S.

Flegal, K. M., Kit, B. K., Orpana, H., Graubard, B. I., I, J., … KF, F. (2013). 'Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis.', *JAMA*, 309(1), pp. 71–82.

Flegal, K. M., Shepherd, J. A., Looker, A. C., Graubard, B. I., Borrud, L. G., … Schenker, N. (2009). 'Comparisons of percentage body fat, body mass index, waist circumference, and waist-stature ratio in adults', *The American Journal of Clinical Nutrition*, 89(2), pp. 500–508.

Flier, J. S. and Maratos-Flier, E. (2017). 'Leptin's Physiologic Role: Does the Emperor of Energy Balance Have No Clothes?', *Cell Metabolism*, 26(1), pp. 24–26.

Flint, A., Raben, A., Astrup, A. and Holst, J. J. (1998). 'Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans.', *The Journal of clinical investigation*, 101(3), pp. 515–20.

Flint, A., Raben, A., Ersbøll, A., Holst, J. and Astrup, A. (2001). 'The effect of physiological levels of glucagon-like peptide-1 on appetite, gastric emptying, energy and substrate metabolism in obesity', *International Journal of Obesity*, 25(6), pp. 781–792.

Fontana, J. M., Higgins, J. A., Schuckers, S. C., Bellisle, F., Pan, Z., … Sazonov, E. (2015). 'Energy intake estimation from counts of chews and swallows.', *Appetite*, 85, pp. 14–21.

Forrest, M. D. (2014). 'The sodium-potassium pump is an information processing element in brain computation.', *Frontiers in Physiology*, 5, p. 472.

Franz, M. J., VanWormer, J. J., Crain, A. L., Boucher, J. L., Histon, T., … Pronk, N. P. (2007). 'Weight-Loss Outcomes: A Systematic Review and Meta-Analysis of Weight-Loss Clinical Trials with a Minimum 1-Year Follow-Up', *Journal of the American Dietetic Association*, 107(10), pp. 1755–1767.

Fraser, S., Cowen, P., Franklin, M., Franey, C. and Arendt, J. (1983). 'Direct radioimmunoassay for melatonin in plasma.', *Clinical chemistry*, 29(2), pp. 396–7.

Frayn, K., Bernard, S., Spalding, K. and Arner, P. (2012). 'Adipocyte Triglyceride Turnover Is Independently Associated With Atherogenic Dyslipidemia', *Journal of the American Heart Association*, 1(6), pp. e003467–e003467.

Frayn, K. N. (1983). 'Calculation of substrate oxidation rates in vivo from gaseous exchange.', *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, 55(2), pp. 628–34.

Frayn, K. N. (2010). *Metabolic Regulation: A Human Perspective*. 3rd edn, *Wiley-Blackwell*. 3rd edn. Massachusetts, USA.

Frayn, K. N. (2016). 'Living with lipids: Reflections of a fat physiologist', *Nutrition Bulletin*, 41(2), pp. 104–107.

Frayn, K. N., Coppack, S. W., Humphreys, S. M., Clark, M. L. and Evans, R. D. (1993). 'Periprandial regulation of lipid metabolism in insulin-treated diabetes mellitus.', *Metabolism: Clinical and Experimental*, 42(4), pp. 504–10.

Frayn, K. N., Karpe, F., Fielding, B. A., Macdonald, I. A. and Coppack, S. W. (2003). 'Integrative physiology of human adipose tissue', *International Journal of Obesity*, 27, pp. 875–888.

Freedson, P. S. and Miller, K. (2000). 'Objective monitoring of physical activity using motion sensors and heart rate.', *Research quarterly for exercise and sport*, 71(2 Suppl), pp. S21-9.

Friedlander, A. L., Braun, B., Pollack, M., MacDonald, J. R., Fulco, C. S., … Cymerman, A. (2005). 'Three weeks of caloric restriction alters protein metabolism in normal-weight, young men.', *American Journal of Physiology - Endocrinology and Metabolism*, 289(3), pp. E446-55.

Fujita, S., Rasmussen, B. B., Cadenas, J. G., Grady, J. J. and Volpi, E. (2006). 'Effect of insulin on human skeletal muscle protein synthesis is modulated by insulin-induced changes in muscle blood flow and amino acid availability', *American Journal of Physiology-Endocrinology and Metabolism*, 291(4), pp. E745–E754.

Fuller, N. R., Fong, M., Gerofi, J., Ferkh, F., Leung, C., … Caterson, I. D. (2017). 'Comparison of an electronic versus traditional food diary for assessing dietary intake-A validation study.', *Obesity Research and Clinical Practice*, 11(6), pp. 647–654.

Fuster, J. J., Ouchi, N., Gokce, N. and Walsh, K. (2016). 'Obesity-Induced Changes in Adipose Tissue Microenvironment and Their Impact on Cardiovascular Disease', *Circulation Research*, 118(11), pp. 1786–1807.

Fyfe, C. L., Stewart, J., Murison, S. D., Jackson, D. M., Rance, K., … Johnstone, A. M. (2010). 'Evaluating energy intake measurement in free-living subjects: when to

record and for how long?', *Public Health Nutrition*, 13(2), p. 172.

Van Gaal, L. F., Mertens, I. L. and De Block, C. E. (2006). 'Mechanisms linking obesity with cardiovascular disease', *Nature*, 444(7121), pp. 875–880.

Gabel, K., Hoddy, K. K., Haggerty, N., Song, J., Kroeger, C. M., … Varady, K. A. (2018). 'Effects of 8-hour time restricted feeding on body weight and metabolic disease risk factors in obese adults: A pilot study.', *Nutrition and healthy aging*, 4(4), pp. 345–353.

Galgani, J. and Ravussin, E. (2008). 'Energy metabolism, fuel selection and body weight regulation.', *International Journal of Obesity*, 32(Suppl 7), pp. S109–S119.

Gannon, M. C. and Nuttall, F. Q. (2010). 'Amino acid ingestion and glucose metabolism-A review', *IUBMB Life*, 62(9), pp. 660–668.

Garin, M. C., Burns, C. M., Kaul, S. and Cappola, A. R. (2013). 'The Human Experience With Ghrelin Administration', *The Journal of Clinical Endocrinology and Metabolism*, 98(5), pp. 1826–1837.

Garrow, J. S. (1978). *Energy balance and obesity in man*. 2nd edn.

Van Gelder, R. N. and Buhr, E. D. (2016). 'Ocular Photoreception for Circadian Rhythm Entrainment in Mammals', *Annual Review of Vision Science*, 2, pp. 153–169.

Geliebter, A. (1988). 'Gastric distension and gastric capacity in relation to food intake in humans.', *Physiology and Behavior*, 44(4–5), pp. 665–8.

Gerhart-Hines, Z. and Lazar, M. A. (2015). 'Circadian metabolism in the light of evolution.', *Endocrine Reviews*, 36(3), pp. 289–304.

Gibbons, C., Caudwell, P., Finlayson, G., Webb, D.-L., Hellström, P. M., … Blundell, J. E. (2013). 'Comparison of Postprandial Profiles of Ghrelin, Active GLP-1, and Total PYY to Meals Varying in Fat and Carbohydrate and Their Association With Hunger and the Phases of Satiety', *The Journal of Clinical Endocrinology and Metabolism*, 98(5), pp. E847–E855.

Gibson, A. A., Hsu, M. S. H., Rangan, A. M., Seimon, R. V, Lee, C. M. Y., … Sainsbury, A. (2016). 'Accuracy of hands v. household measures as portion size estimation aids.', *Journal of nutritional science*, 5, p. e29.

Gilbert, C. A. and Slingerland, J. M. (2013). 'Cytokines, Obesity, and Cancer: New Insights on Mechanisms Linking Obesity to Cancer Risk and Progression', *Annual Review of Medicine*, 64(1), pp. 45–57.

Gillies, A. R. and Lieber, R. L. (2011). 'Structure and function of the skeletal muscle extracellular matrix', *Muscle and Nerve*, 44(3), pp. 318–331.

Goldberg, I. J., Eckel, R. H. and Abumrad, N. A. (2009). 'Regulation of fatty acid uptake into tissues: lipoprotein lipase- and CD36-mediated pathways.', *Journal of lipid research*, 50(Suppl), pp. S86-90.

Goldsmith, R., Joanisse, D. R., Gallagher, D., Pavlovich, K., Shamoon, E., … Rosenbaum, M. (2010). 'Effects of experimental weight perturbation on skeletal

muscle work efficiency, fuel utilization, and biochemistry in human subjects', *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 298(1), pp. R79–R88.

Goldstein, D. J. (1992). 'Beneficial health effects of modest weight loss.', *International journal of obesity and related metabolic disorders*, 16(6), pp. 397–415.

Gonzalez, J. T. (2014). 'Paradoxical second-meal phenomenon in the acute postexercise period.', *Nutrition*, 30(9), pp. 961–7.

Gonzalez, J. T., Richardson, J. D., Chowdhury, E. A., Koumanov, F., Holman, G. D., … Betts, J. A. (2018). 'Molecular adaptations of adipose tissue to 6 weeks of morning fasting vs. daily breakfast consumption in lean and obese adults', *The Journal of Physiology*, 596(4), pp. 609–622.

Gooley, J. J. (2016). 'Circadian regulation of lipid metabolism', *Proceedings of the Nutrition Society*, 75(4), pp. 440–450.

Gortmaker, S. L., Swinburn, B. A., Levy, D., Carter, R., Mabry, P. L., … Moodie, M. L. (2011). 'Changing the future of obesity: science, policy, and action.', *Lancet*, 378(9793), pp. 838–47.

Gotlieb, N., Rosenne, E., Matzner, P., Shaashua, L., Sorski, L. and Ben-Eliyahu, S. (2015). 'The misleading nature of in vitro and ex vivo findings in studying the impact of stress hormones on NK cell cytotoxicity.', *Brain, behavior, and immunity*, 45, pp. 277–86.

de Graaf, C., Blom, W. A., Smeets, P. A., Stafleu, A. and Hendriks, H. F. (2004). 'Biomarkers of satiation and satiety', *The American Journal of Clinical Nutrition*, 79(6), pp. 946–961.

Grabacka, M., Pierzchalska, M., Dean, M. and Reiss, K. (2016). 'Regulation of Ketone Body Metabolism and the Role of PPARα', *International Journal of Molecular Sciences*, 17(12), p. 2093.

Grange, R. D., Thompson, J. P. and Lambert, D. G. (2014). 'Radioimmunoassay, enzyme and non-enzyme-based immunoassays', *British Journal of Anaesthesia*, 112(2), pp. 213–216.

Green, J., Pollak, C. P. and Smith, G. P. (1987). 'Meal size and intermeal interval in human subjects in time isolation', *Physiology and Behavior*, 41(2), pp. 141–147.

Greenman, Y., Golani, N., Gilad, S., Yaron, M., Limor, R. and Stern, N. (2004). 'Ghrelin secretion is modulated in a nutrient-and gender-specific manner', *Clinical Endocrinology*, 60, pp. 382–388.

Gutzwiller, J. P., Göke, B., Drewe, J., Hildebrand, P., Ketterer, S., … Beglinger, C. (1999). 'Glucagon-like peptide-1: a potent regulator of food intake in humans.', *Gut*, 44(1), pp. 81–6.

Hackett, A. (2011). 'Food Frequency Questionnaires: Simple and cheap, but are they valid?', *Maternal and Child Nutrition*, 7(2), pp. 109–111.

Halberg, N., Henriksen, M., Söderhamn, N., Stallknecht, B., Ploug, T., … Dela, F.

(2005). ‘Effect of intermittent fasting and refeeding on insulin action in healthy men.’, *Journal of Applied Physiology*, 99(6), pp. 2128–2136.

Haldane, J. S. (1912). *Methods of Air Analysis*. London.

Hall, K. D., Bemis, T., Brychta, R., Chen, K. Y., Courville, A., … Yannai, L. (2015). ‘Calorie for Calorie, Dietary Fat Restriction Results in More Body Fat Loss than Carbohydrate Restriction in People with Obesity’, *Cell Metabolism*, 22(3), pp. 427–436.

Hall, K. D., Chandramohan, D., Chow, C. C., Sacks, A. G., Swinburn, B. A., … Swinburn, B. A. (2011). ‘Quantification of the effect of energy imbalance on body weight’, *Lancet*, 378(9793), pp. 826–837.

Hall, K. D. and Chung, S. T. (2018). ‘Low-carbohydrate diets for the treatment of obesity and type 2 diabetes’, *Current Opinion in Clinical Nutrition and Metabolic Care*, 21(4), pp. 308–312.

Hall, K. D., Guo, J., Chen, K. Y., Leibel, R. L., Reitman, M. L., … Ravussin, E. (2018). ‘Potential Bias of Doubly Labeled Water for Measuring Energy Expenditure Differences Between Diets Varying in Carbohydrate’, *bioRxiv*, p. 403931.

Hall, K. D., Heymsfield, S. B., Kemnitz, J. W., Klein, S., Schoeller, D. A. and Speakman, J. R. (2012). ‘Energy balance and its components: implications for body weight regulation.’, *The American journal of clinical nutrition*, 95(4), pp. 989–94.

Hamman, R. F., Wing, R. R., Edelstein, S. L., Lachin, J. M., Bray, G. A., … Wylie-Rosett, J. (2006). ‘Effect of weight loss with lifestyle intervention on risk of diabetes.’, *Diabetes Care*, 29(9), pp. 2102–7.

Han, H.-S., Kang, G., Kim, J. S., Choi, B. H. and Koo, S.-H. (2016). ‘Regulation of glucose metabolism from a liver-centric perspective.’, *Experimental and Molecular Medicine*, 48(3), p. e218.

Harvie, M. N., Pegington, M., Mattson, M. P., Frystyk, J., Dillon, B., … Howell, A. (2011). ‘The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers: a randomized trial in young overweight women.’, *International Journal of Obesity*, 35(5), pp. 714–27.

Harvie, M., Wright, C., Pegington, M., McMullan, D., Mitchell, E., … Howell, A. (2013). ‘The effect of intermittent energy and carbohydrate restriction v. daily energy restriction on weight loss and metabolic disease risk markers in overweight women’, *British Journal of Nutrition*, 110(8), pp. 1534–1547.

Haskell, W. L., Yee, M. C., Evans, A. and Irby, P. J. (1993). ‘Simultaneous measurement of heart rate and body motion to quantitate physical activity.’, *Medicine and science in sports and exercise*, 25(1), pp. 109–15.

Headland, M., Clifton, P. M., Carter, S. and Keogh, J. B. (2016). ‘Weight-Loss Outcomes: A Systematic Review and Meta-Analysis of Intermittent Energy Restriction Trials Lasting a Minimum of 6 Months.’, *Nutrients*, 8(6), p. E354.

Health and Social Care Information Centre (2013). *Statistics on obesity, physical activity and diet: England, 2013*. Leeds. Leeds: Health and Social Care Information

Centre.

Hebert, J. R., Clemow, L., Pbert, L., Ockene, I. S. and Ockene, J. K. (1995). 'Social desirability bias in dietary self-report may compromise the validity of dietary intake measures.', *International journal of epidemiology*, 24(2), pp. 389–98.

Hedrick, V. E., Dietrich, A. M., Estabrooks, P. A., Savla, J., Serrano, E. and Davy, B. M. (2012). 'Dietary biomarkers: advances, limitations and future directions', *Nutrition Journal*, 11(1), p. 109.

Heilbronn, L. K., Civitarese, A. E., Bogacka, I., Smith, S. R., Hulver, M. and Ravussin, E. (2005). 'Glucose Tolerance and Skeletal Muscle Gene Expression in Response to Alternate Day Fasting', *Obesity Research*, 13(3), pp. 574–581.

Heilbronn, L. K., de Jonge, L., Frisard, M. I., DeLany, J. P., Larson-Meyer, D. E., … Ravussin, E. (2006). 'Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial.', *JAMA*, 295(13), pp. 1539–48.

Heilbronn, L. K., Smith, S. R., Martin, C. K., Anton, S. D. and Ravussin, E. (2005). 'Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism.', *The American Journal of Clinical Nutrition*, 81(1), pp. 69–73.

Heiman, M. L., Ahima, R. S., Craft, L. S., Schoner, B., Stephens, T. W. and Flier, J. S. (1997). 'Leptin Inhibition of the Hypothalamic-Pituitary-Adrenal Axis in Response to Stress [1]', *Endocrinology*, 138(9), pp. 3859–3863.

Hellerstein, M. (1999). 'De novo lipogenesis in humans: metabolic and regulatory aspects', *European Journal of Clinical Nutrition*, 53(S1), pp. s53–s65.

Hellerstein, M. K. (2001). 'No common energy currency: de novo lipogenesis as the road less traveled', *The American Journal of Clinical Nutrition*, 74(6), pp. 707–708.

Hellerstein, M. K., Schwarz, J.-M. and Neese, R. A. (1996). 'Regulation of Hepatic De Novo Lipogenesis in Humans', *Annual Review of Nutrition*, 16(1), pp. 523–557.

Henique, C., Mansouri, A., Fumey, G., Lenoir, V., Girard, J., … Cohen, I. (2010). 'Increased mitochondrial fatty acid oxidation is sufficient to protect skeletal muscle cells from palmitate-induced apoptosis.', *The Journal of biological chemistry*, 285(47), pp. 36818–27.

Herman, J. P., McKlveen, J. M., Ghosal, S., Kopp, B., Wulsin, A., … Myers, B. (2016). 'Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response.', *Comprehensive Physiology*, 6(2), pp. 603–21.

van Herpen, N. A. and Schrauwen-Hinderling, V. B. (2008). 'Lipid accumulation in non-adipose tissue and lipotoxicity', *Physiology and Behavior*, 94(2), pp. 231–241.

Hesselink, M. K. C., Schrauwen-Hinderling, V. and Schrauwen, P. (2016). 'Skeletal muscle mitochondria as a target to prevent or treat type 2 diabetes mellitus', *Nature Reviews Endocrinology*, 12(11), pp. 633–645.

Hession, M., Rolland, C., Kulkarni, U., Wise, A. and Broom, J. (2009). 'Systematic

review of randomized controlled trials of low-carbohydrate vs. low-fat/low-calorie diets in the management of obesity and its comorbidities', *Obesity Reviews*, 10(1), pp. 36–50.

Hickner, R. C., Racette, S. B., Binder, E. F., Fisher, J. S. and Kohrt, W. M. (1999). 'Suppression of Whole Body and Regional Lipolysis by Insulin: Effects of Obesity and Exercise', *The Journal of Clinical Endocrinology and Metabolism*, 84(11), pp. 3886–3895.

Hill, J. O., Wyatt, H. R. and Peters, J. C. (2012). 'Energy balance and obesity.', *Circulation*, 126(1), pp. 126–32.

Hill, R. J. and Davies, P. S. (2001). 'The validity of self-reported energy intake as determined using the doubly labelled water technique.', *The British journal of nutrition*, 85(4), pp. 415–30.

Hills, A. P., Mokhtar, N. and Byrne, N. M. (2014). 'Assessment of physical activity and energy expenditure: an overview of objective measures.', *Frontiers in nutrition*, 1, p. 5.

Hirano, A., Fu, Y.-H. and Ptáček, L. J. (2016). 'The intricate dance of post-translational modifications in the rhythm of life', *Nature Structural and Molecular Biology*, 23(12), pp. 1053–1060.

Hoddy, K. K., Gibbons, C., Kroeger, C. M., Trepanowski, J. F., Barnosky, A., … Varady, K. A. (2016). 'Changes in hunger and fullness in relation to gut peptides before and after 8 weeks of alternate day fasting', *Clinical Nutrition*, 35(6), pp. 1380–1385.

Hoddy, K. K., Kroeger, C. M., Trepanowski, J. F., Barnosky, A., Bhutani, S. and Varady, K. A. (2014). 'Meal timing during alternate day fasting: Impact on body weight and cardiovascular disease risk in obese adults', *Obesity*, 22(12), pp. 2524–2531.

Hoenig, J. M. and Heisey, D. M. (2001). 'The Abuse of Power', *The American Statistician*, 55(1), pp. 19–24.

Hopkins, M., Finlayson, G., Duarte, C., Whybrow, S., Ritz, P., … Stubbs, R. J. (2016). 'Modelling the associations between fat-free mass, resting metabolic rate and energy intake in the context of total energy balance', *International Journal of Obesity*, 40(2), pp. 312–318.

Horne, J. A. and Ostberg, O. (1976). 'A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms.', *International Journal of Chronobiology*, 4, pp. 97–110.

Horton, J. D., Goldstein, J. L. and Brown, M. S. (2002). 'SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver.', *The Journal of Clinical Investigation*, 109(9), pp. 1125–1131.

Horton, T. J., Drougas, H., Brachey, A., Reed, G. W., Peters, J. C. and Hill, J. O. (1995). 'Fat and carbohydrate overfeeding in humans: different effects on energy storage.', *The American Journal of Clinical Nutrition*, 62(1), pp. 19–29.

Hoyle, N. P., Seinkmane, E., Putker, M., Feeney, K. A., Krogager, T. P., … O'Neill, J. S. (2017). 'Circadian actin dynamics drive rhythmic fibroblast mobilization during wound healing.', *Science Translational Medicine*, 9(415), p. eaal2774.

Huang, L. O., Loos, R. J. F. and Kilpeläinen, T. O. (2018). 'Evidence of genetic predisposition for metabolically healthy obesity and metabolically obese normal weight', *Physiological Genomics*, 50(3), pp. 169–178.

Huo, L., Maeng, L., Bjørbæk, C. and Grill, H. J. (2007). 'Leptin and the Control of Food Intake: Neurons in the Nucleus of the Solitary Tract Are Activated by Both Gastric Distension and Leptin', *Endocrinology*, 148(5), pp. 2189–2197.

Il'yasova, D., Fontana, L., Bhapkar, M., Pieper, C. F., Spasojevic, I., … Kraus, W. E. (2018). 'Effects of 2 years of caloric restriction on oxidative status assessed by urinary F2-isoprostanes: The CALERIE 2 randomized clinical trial', *Aging Cell*, 17(2), p. e12719.

Illner, K., Brinkmann, G., Heller, M., Bosy-Westphal, A. and Müller, M. J. (2000). 'Metabolically active components of fat free mass and resting energy expenditure in nonobese adults', *American Journal of Physiology-Endocrinology and Metabolism*, 278(2), pp. E308–E315.

Janssen, I., Katzmarzyk, P. T. and Ross, R. (2004). 'Waist circumference and not body mass index explains obesity-related health risk', *The American Journal of Clinical Nutrition*, 79(3), pp. 379–384.

Jeffery, R. W., Wing, R. R., Thorson, C., Burton, L. R., Raether, C., … Mullen, M. (1993). 'Strengthening Behavioral Interventions for Weight Loss: A Randomized Trial of Food Provision and Monetary Incentives', *Journal of Consulting and Clinical Psychology*, 61(6), pp. 1038–1045.

Jensen, J., Rustad, P. I., Kolnes, A. J. and Lai, Y.-C. (2011). 'The role of skeletal muscle glycogen breakdown for regulation of insulin sensitivity by exercise.', *Frontiers in physiology*, 2, p. 112.

Jensen, M. D. and Heiling, V. J. (1991). 'Heated hand vein blood is satisfactory for measurements during free fatty acid kinetic studies', *Metabolism*, 40(4), pp. 406–409.

Jéquier, E. and Felber, J.-P. (1987). 'Indirect calorimetry', *Baillière's Clinical Endocrinology and Metabolism*, 1(4), pp. 911–935.

Jeran, S., Steinbrecher, A. and Pischon, T. (2016). 'Prediction of activity-related energy expenditure using accelerometer-derived physical activity under free-living conditions: a systematic review', *International Journal of Obesity*, 40(8), pp. 1187–1197.

Jesudason, D. R., Monteiro, M. P., McGowan, B. M. C., Neary, N. M., Park, A. J., … Bloom, S. R. (2007). 'Low-dose pancreatic polypeptide inhibits food intake in man', *British Journal of Nutrition*, 97(3), p. 426.

Johnson, J. B., Summer, W., Cutler, R. G., Martin, B., Hyun, D.-H., … Mattson, M. P. (2007). 'Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma.', *Free Radical Biology and Medicine*, 42(5), pp. 665–74.

Johnson, M. L., Distelmaier, K., Lanza, I. R., Irving, B. A., Robinson, M. M., … Nair, K. S. (2016). 'Mechanism by Which Caloric Restriction Improves Insulin Sensitivity in Sedentary Obese Adults.', *Diabetes*, 65(1), pp. 74–84.

Johnston, J. D. (2014). 'Physiological links between circadian rhythms, metabolism and nutrition', *Experimental Physiology*, 99(9), pp. 1133–1137.

Johnston, J. D., Ordovás, J. M., Scheer, F. A. and Turek, F. W. (2016). 'Circadian Rhythms, Metabolism, and Chrononutrition in Rodents and Humans.', *Advances in nutrition (Bethesda, Md.)*, 7(2).

Johnstone, A. (2015). 'Fasting for weight loss: an effective strategy or latest dieting trend?', *International Journal of Obesity*, 39, pp. 727–733.

Johnstone, A. M., Murison, S. D., Duncan, J. S., Rance, K. A. and Speakman, J. R. (2005). 'Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine', *The American Journal of Clinical Nutrition*, 82(5), pp. 941–948.

Jumpertz, R., Le, D. S., Turnbaugh, P. J., Trinidad, C., Bogardus, C., … Krakoff, J. (2011). 'Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans', *The American Journal of Clinical Nutrition*, 94(1), pp. 58–65.

Jung, U. J. and Choi, M.-S. (2014). 'Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease.', *International Journal of Molecular Sciences*, 15(4), pp. 6184–6223.

Kahn, B. B. and Flier, J. S. (2000). 'Obesity and insulin resistance', *Journal of Clinical Investigation*, 106(4), pp. 473–481.

Karhunen, L. J., Juvonen, K. R., Huotari, A., Purhonen, A. K. and Herzig, K. H. (2008). 'Effect of protein, fat, carbohydrate and fibre on gastrointestinal peptide release in humans', *Regulatory Peptides*, 149(1–3), pp. 70–78.

Karim, S., Adams, D. H. and Lalor, P. F. (2012). 'Hepatic expression and cellular distribution of the glucose transporter family.', *World journal of gastroenterology*, 18(46), pp. 6771–81.

Katsanos, C. S., Aarsland, A., Cree, M. G. and Wolfe, R. R. (2009). 'Muscle Protein Synthesis and Balance Responsiveness to Essential Amino Acids Ingestion in the Presence of Elevated Plasma Free Fatty Acid Concentrations', *The Journal of Clinical Endocrinology and Metabolism*, 94(8), pp. 2984–2990.

Katzmarzyk, P. T. (2010). 'Physical Activity, Sedentary Behavior, and Health: Paradigm Paralysis or Paradigm Shift?', *Diabetes*, 59(11), pp. 2717–2725.

Kenny, G. P., Notley, S. R. and Gagnon, D. (2017). 'Direct calorimetry: a brief historical review of its use in the study of human metabolism and thermoregulation', *European Journal of Applied Physiology*, 117(9), pp. 1765–1785.

Keys, A., Fidanza, F., Karvonen, M. J., Kimura, N. and Taylor, H. L. (1972). 'Indices of relative weight and obesity', *Journal of Chronic Diseases*, 25, pp. 329–343.

Kim, K.-S., Seeley, R. J. and Sandoval, D. A. (2018). 'Signalling from the periphery to the brain that regulates energy homeostasis', *Nature Reviews Neuroscience*, 19(4), pp. 185–196.

Kim, K. and Park, S. M. (2018). 'Association of muscle mass and fat mass with insulin resistance and the prevalence of metabolic syndrome in Korean adults: a cross-sectional study', *Scientific Reports*, 8(1), p. 2703.

Kissileff, H. R., Carretta, J. C., Geliebter, A. and Pi-Sunyer, F. X. (2003). 'Cholecystokinin and stomach distension combine to reduce food intake in humans', *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 285(5), pp. R992–R998.

Kissileff, H. R., Thornton, J. C., Torres, M. I., Pavlovich, K., Mayer, L. S., … Rosenbaum, M. (2012). 'Leptin reverses declines in satiation in weight-reduced obese humans', *American Journal of Clinical Nutrition*, 95, pp. 309–317.

Klempel, M. C., Bhutani, S., Fitzgibbon, M., Freels, S. and Varady, K. A. (2010). 'Dietary and physical activity adaptations to alternate day modified fasting: implications for optimal weight loss.', *Nutrition Journal*, 9, p. 35.

Klempel, M. C., Kroeger, C. M., Bhutani, S., Trepanowski, J. F. and Varady, K. A. (2012). 'Intermittent fasting combined with calorie restriction is effective for weight loss and cardio-protection in obese women', *Nutrition Journal*, 11(1), p. 98.

Klempel, M. C., Kroeger, C. M. and Varady, K. A. (2013). 'Alternate day fasting (ADF) with a high-fat diet produces similar weight loss and cardio-protection as ADF with a low-fat diet.', *Metabolism - Clinical and Experimental*, 62(1), pp. 137–43.

Klok, M. D., Jakobsdottir, S. and Drent, M. L. (2007). 'The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review', *Obesity Reviews*, 8(1), pp. 21–34.

Kluge, M., Riedl, S., Uhr, M., Schmidt, D., Zhang, X., … Steiger, A. (2010). 'Ghrelin affects the hypothalamus–pituitary–thyroid axis in humans by increasing free thyroxine and decreasing TSH in plasma', *European Journal of Endocrinology*, 162(6), pp. 1059–1065.

Kolaczynski, J. W., Considine, R. V, Ohannesian, J., Marco, C., Opentanova, I., … Caro, J. F. (1996). 'Responses of leptin to short-term fasting and refeeding in humans: a link with ketogenesis but not ketones themselves.', *Diabetes*, 45(11), pp. 1511–5.

Kolditz, C.-I. and Langin, D. (2010). 'Adipose tissue lipolysis', *Current Opinion in Clinical Nutrition and Metabolic Care*, 13(4), pp. 377–381.

Konner, M. and Eaton, S. B. (2010). 'Paleolithic Nutrition: Twenty-Five Years Later', *Nutrition in Clinical Practice*, 25(6), pp. 594–602.

Krajmalnik-Brown, R., Ilhan, Z.-E., Kang, D.-W. and DiBaise, J. K. (2012). 'Effects of Gut Microbes on Nutrient Absorption and Energy Regulation', *Nutrition in Clinical Practice*, 27(2), pp. 201–214.

Kroeger, C. M., Trepanowski, J. F., Klempel, M. C., Barnosky, A., Bhutani, S., … Varady, K. A. (2018). 'Eating behavior traits of successful weight losers during 12

months of alternate-day fasting: An exploratory analysis of a randomized controlled trial', *Nutrition and Health*, 24(1), pp. 5–10.

Kurpad, A. V, Khan, K. and Elia, M. (1994). 'The effect of arterialization of blood by hand warming on the interpretation of forearm metabolic studies', *Physiological Measurement*, 15(2), pp. 139–145.

Kwon, H. and Pessin, J. E. (2013). 'Adipokines mediate inflammation and insulin resistance.', *Frontiers in Endocrinology*, 4, p. 71.

Kwon, I., Choe, H. K., Son, G. H. and Kim, K. (2011). 'Mammalian molecular clocks.', *Experimental neurobiology*, 20(1), pp. 18–28.

Kwon, I., Lee, J., Chang, S. H., Jung, N. C., Lee, B. J., … Lee, K. H. (2006). 'BMAL1 Shuttling Controls Transactivation and Degradation of the CLOCK/BMAL1 Heterodimer', *Molecular and Cellular Biology*, 26(19), pp. 7318–7330.

Laffel, L. (1999). 'Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes.', *Diabetes Metabolism Research and Reviews*, 15(6), pp. 412–26.

Lam, Y. Y. and Ravussin, E. (2016). 'Analysis of energy metabolism in humans: A review of methodologies.', *Molecular metabolism*, 5(11), pp. 1057–1071.

Lambert, J. E. and Parks, E. J. (2012). 'Postprandial metabolism of meal triglyceride in humans', *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, 1821(5), pp. 721–726.

Lanfray, D. and Richard, D. (2017). 'Emerging Signaling Pathway in Arcuate Feeding-Related Neurons: Role of the Acbd7', *Frontiers in Neuroscience*, 11, p. 328.

Larson-Meyer, D. E., Heilbronn, L. K., Redman, L. M., Newcomer, B. R., Frisard, M. I., … Ravussin, E. (2006). 'Effect of calorie restriction with or without exercise on insulin sensitivity, beta-cell function, fat cell size, and ectopic lipid in overweight subjects.', *Diabetes Care*, 29(6), pp. 1337–44.

De Lartigue, G. (2016). 'The Journal of Physiology Neuroscience Role of the vagus nerve in the development and treatment of diet-induced obesity', *Journal of Physiology*, 594, pp. 5791–5815.

Lean, M. E. J. and Malkova, D. (2016). 'Altered gut and adipose tissue hormones in overweight and obese individuals: cause or consequence?', *International journal of obesity (2005)*, 40(4), pp. 622–32.

Lecoultre, V., Ravussin, E. and Redman, L. M. (2011). 'The Fall in Leptin Concentration Is a Major Determinant of the Metabolic Adaptation Induced by Caloric Restriction Independently of the Changes in Leptin Circadian Rhythms', *The Journal of Clinical Endocrinology and Metabolism*, 96(9), pp. E1512–E1516.

Lee, B. and Shao, J. (2014). 'Adiponectin and energy homeostasis', *Reviews in Endocrine and Metabolic Disorders*, 15(2), pp. 149–156.

Lee, S. Y. and Gallagher, D. (2008). 'Assessment methods in human body composition.', *Current opinion in clinical nutrition and metabolic care*, 11(5), pp.

566–72.

Leech, R. M., Timperio, A., Livingstone, K. M., Worsley, A. and McNaughton, S. A. (2017). 'Temporal eating patterns: associations with nutrient intakes, diet quality, and measures of adiposity', *The American Journal of Clinical Nutrition*, 106(4), pp. 1121–1130.

LeGates, T. A., Fernandez, D. C. and Hattar, S. (2014). 'Light as a central modulator of circadian rhythms, sleep and affect', *Nature Reviews Neuroscience*, 15(7), pp. 443–454.

Leibel, R. L., Rosenbaum, M. and Hirsch, J. (1995). 'Changes in Energy Expenditure Resulting from Altered Body Weight', *New England Journal of Medicine*, 332(10), pp. 621–628.

Leidy, H. J., Armstrong, C. L. H., Tang, M., Mattes, R. D. and Campbell, W. W. (2010). 'The Influence of Higher Protein Intake and Greater Eating Frequency on Appetite Control in Overweight and Obese Men', *Obesity*, 18(9), pp. 1725–1732.

Leidy, H. J. and Campbell, W. W. (2011). 'The Effect of Eating Frequency on Appetite Control and Food Intake: Brief Synopsis of Controlled Feeding Studies', *The Journal of Nutrition*, 141(1), pp. 154–157.

Levine, J. A. (2005). 'Measurement of energy expenditure.', *Public health nutrition*, 8(7A), pp. 1123–32.

Lhuissier, A., Tichit, C., Caillavet, F., Cardon, P., Masullo, A., … Chauvin, P. (2013). 'Who still eats three meals a day? Findings from a quantitative survey in the Paris area', *Appetite*, 63, pp. 59–69.

Licinio, J., Caglayan, S., Ozata, M., Yildiz, B. O., de Miranda, P. B., … Wong, M.-L. (2004). 'Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults.', *Proceedings of the National Academy of Sciences of the United States of America*, 101(13), pp. 4531–6.

Liu, D., Moberg, E., Kollind, M., Lins, P. E., Adamson, U. and Macdonald, I. A. (1992). 'Arterial, arterialized venous, venous and capillary blood glucose measurements in normal man during hyperinsulinaemic euglycaemia and hypoglycaemia.', *Diabetologia*, 35(3), pp. 287–90.

Liu, M., Li, X.-C., Lu, L., Cao, Y., Sun, R.-R., … Zhang, P. (2014). 'Cardiovascular disease and its relationship with chronic kidney disease', *European Review for Medical and Pharmacological Sciences*, 18, pp. 2918–2926.

Livingstone, M. B. E. and Black, A. E. (2003). 'Markers of the validity of reported energy intake.', *Journal of Nutrition*, 133, p. 895S–920S.

Livingstone, M. B., Prentice, A. M., Coward, W. A., Ceesay, S. M., Strain, J. J., … Hickey, R. J. (1990). 'Simultaneous measurement of free-living energy expenditure by the doubly labeled water method and heart-rate monitoring', *The American Journal of Clinical Nutrition*, 52(1), pp. 59–65.

Livingstone, M. B., Prentice, A. M., Strain, J. J., Coward, W. A., Black, A. E., … Whitehead, R. G. (1990). 'Accuracy Of Weighed Dietary Records In Studies Of Diet',

British Medical Journal, 300(6726), pp. 708–712.

Lodwig, T. H. and Smeaton, W. A. (1974). 'The ice calorimeter of Lavoisier and Laplace and some of its critics', *Annals of Science*, 31(1), pp. 1–18.

Loizides-Mangold, U., Perrin, L., Vandereycken, B., Betts, J. A., Walhin, J.-P., … Dibner, C. (2017). 'Lipidomics reveals diurnal lipid oscillations in human skeletal muscle persisting in cellular myotubes cultured in vitro.', *Proceedings of the National Academy of Sciences of the United States of America*, 114(41), pp. E8565–E8574.

Longo, V. D. and Mattson, M. P. (2014). 'Fasting: Molecular Mechanisms and Clinical Applications', *Cell Metabolism*, 19(2), pp. 181–192.

Longo, V. D. and Panda, S. (2016). 'Fasting, Circadian Rhythms, and Time-Restricted Feeding in Healthy Lifespan', *Cell Metabolism*, 23(6), pp. 1048–1059.

van Loon, L. J. C. (2004). 'Use of intramuscular triacylglycerol as a substrate source during exercise in humans', *Journal of Applied Physiology*, 97(4), pp. 1170–1187.

van Loon, L. J., Saris, W. H., Verhagen, H. and Wagenmakers, A. J. (2000). 'Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate', *The American Journal of Clinical Nutrition*, 72(1), pp. 96–105.

López, M., Alvarez, C. V, Nogueiras, R. N. and Dié Guez, C. (2013). 'Energy balance regulation by thyroid hormones at central level', *Trends in Molecular Medicine*, 19, pp. 418–427.

Lorente Ramos, R. M., Azpeitia Armán, J., Arévalo Galeano, N., Muñoz Hernández, A., García Gómez, J. M. and Gredilla Molinero, J. (2012). 'Dual energy X-ray absorptimetry: fundamentals, methodology, and clinical applications', *Radiología*, 54(5), pp. 410–423.

Louie, S. M., Roberts, L. S. and Nomura, D. K. (2013). 'Mechanisms linking obesity and cancer', *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, 1831(10), pp. 1499–1508.

Ludbrook, J. (2000). 'Multiple inferences using confidence intervals.', *Clinical and Experimental Pharmacology and Physiology*, 27(3), pp. 212–5.

Luke, A., Maki, K. C., Barkey, N., Cooper, R. and McGee, D. (1997). 'Simultaneous monitoring of heart rate and motion to assess energy expenditure.', *Medicine and science in sports and exercise*, 29(1), pp. 144–8.

Luo, L. and Liu, M. (2016). 'Adipose tissue in control of metabolism', *Journal of Endocrinology*, 231(3), pp. R77–R99.

Lynch, M. and Marinov, G. K. (2015). 'The bioenergetic costs of a gene.', *Proceedings of the National Academy of Sciences of the United States of America*, 112(51), pp. 15690–5.

Maachi, M., Piéroni, L., Bruckert, E., Jardel, C., Fellahi, S., … Bastard, J.-P. (2004). 'Systemic low-grade inflammation is related to both circulating and adipose tissue TNFalpha, leptin and IL-6 levels in obese women.', *International Journal of Obesity*, 28, pp. 993–997.

Mäntele, S., Otway, D. T., Middleton, B., Bretschneider, S., Wright, J., … Johnston, J. D. (2012). 'Daily Rhythms of Plasma Melatonin, but Not Plasma Leptin or Leptin mRNA, Vary between Lean, Obese and Type 2 Diabetic Men', *PLoS ONE*. Edited by G. Tosini, 7(5), p. e37123.

Mantzoros, C. S., Magkos, F., Brinkoetter, M., Sienkiewicz, E., Dardeno, T. A., … Bornfeldt, K. (2011). 'Leptin in human physiology and pathophysiology.', *American Journal of Physiology - Endocrinology and Metabolism*, 301(4), pp. E567–E584.

Marlatt, K. L., Redman, L. M., Burton, J. H., Martin, C. K. and Ravussin, E. (2017). 'Persistence of weight loss and acquired behaviors 2 y after stopping a 2-y calorie restriction intervention', *The American Journal of Clinical Nutrition*, 105(4), pp. 928–935.

Martin, C. K., Das, S. K., Lindblad, L., Racette, S. B., McCrory, M. A., … Ravussin, E. (2011). 'Effect of calorie restriction on the free-living physical activity levels of nonobese humans: results of three randomized trials.', *Journal of Applied Physiology*, 110(4), pp. 956–963.

Martins, C., Kulseng, B., King, N. A., Holst, J. J. and Blundell, J. E. (2010). 'The Effects of Exercise-Induced Weight Loss on Appetite-Related Peptides and Motivation to Eat', *Journal of Clinical Endocrinology and Metabolism*, 95, pp. 1609–1616.

Martire, S. I., Holmes, N., Westbrook, R. F. and Morris, M. J. (2013). 'Altered Feeding Patterns in Rats Exposed to a Palatable Cafeteria Diet: Increased Snacking and Its Implications for Development of Obesity', *PLoS ONE*. Edited by M. Covasa, 8(4), p. e60407.

Martyn, J. A. J., Kaneki, M. and Yasuhara, S. (2008). 'Obesity-induced insulin resistance and hyperglycemia: etiologic factors and molecular mechanisms.', *Anesthesiology*, 109(1), pp. 137–48.

Matsumoto, T., Miyawaki, C., Ue, H., Kanda, T., Yoshitake, Y. and Moritani, T. (2001). 'Comparison of Thermogenic Sympathetic Response to Food Intake between Obese and Non-obese Young Women', *Obesity Research*, 9(2), pp. 78–85.

Mattson, M. P., Allison, D. B., Fontana, L., Harvie, M., Longo, V. D., … Panda, S. (2014). 'Meal frequency and timing in health and disease.', *Proceedings of the National Academy of Sciences of the United States of America*, 111(47), pp. 16647–53.

Maukonen, M., Kanerva, N., Partonen, T., Kronholm, E., Tapanainen, H., … Männistö, S. (2017). 'Chronotype differences in timing of energy and macronutrient intakes: A population-based study in adults', *Obesity*, 25(3), pp. 608–615.

Maury, E., Ramsey, K. M. and Bass, J. (2010). 'Circadian rhythms and metabolic syndrome: from experimental genetics to human disease.', *Circulation research*, 106(3), pp. 447–62.

McArdle, M. A., Finucane, O. M., Connaughton, R. M., McMorrow, A. M. and Roche, H. M. (2013). 'Mechanisms of obesity-induced inflammation and insulin resistance: insights into the emerging role of nutritional strategies.', *Frontiers in Endocrinology*,

4, p. 52.

McArdle, W. D., Katch, F. I. and Katch, V. L. (2015). *Essentials of Exercise Physiology*. 5th edn. Philadelphia.

McGinnis, G. R. and Young, M. E. (2016). 'Circadian regulation of metabolic homeostasis: causes and consequences.', *Nature and science of sleep*, 8, pp. 163–80.

McGuire, E. A., Helderman, J. H., Tobin, J. D., Andres, R. and Berman, M. (1976). 'Effects of arterial versus venous sampling on analysis of glucose kinetics in man', *Journal of Applied Physiology*, 41(4), pp. 565–573.

McHill, A. W., Hull, J. T., McMullan, C. J. and Klerman, E. B. (2018). 'Chronic Insufficient Sleep Has a Limited Impact on Circadian Rhythmicity of Subjective Hunger and Awakening Fasted Metabolic Hormones', *Frontiers in Endocrinology*, 9, p. 319.

McKinsey Global Institute (2014). *Overcoming obesity: An initial economic analysis*. London: UK.

McMurray, R. G., Soares, J., Caspersen, C. J. and McCurdy, T. (2014). 'Examining variations of resting metabolic rate of adults: a public health perspective.', *Medicine and science in sports and exercise*, 46(7), pp. 1352–8.

McQuaid, S. E., Hodson, L., Neville, M. J., Dennis, A. L., Cheeseman, J., … Karpe, F. (2011). 'Downregulation of Adipose Tissue Fatty Acid Trafficking in Obesity: A Driver for Ectopic Fat Deposition?', *Diabetes*, 60(1), pp. 47–55.

Mead, J. R., Irvine, S. A. and Ramji, D. P. (2002). 'Lipoprotein lipase: structure, function, regulation, and role in disease.', *Journal of Molecular Medicine*, 80(12), pp. 753–769.

Medical Research Council (2017). *Workshop Report: Global Nutrition and Health Workshop*.

Menni, C., Jackson, M. A., Pallister, T., Steves, C. J., Spector, T. D. and Valdes, A. M. (2017). 'Gut microbiome diversity and high-fibre intake are related to lower long-term weight gain', *International Journal of Obesity*, 41(7), pp. 1099–1105.

Meyers, M. A., Chen, P.-Y., Lin, A. Y.-M. and Seki, Y. (2008). 'Biological materials: Structure and mechanical properties', *Progress in Materials Science*, 53(1), pp. 1–206.

Milan, G., Romanello, V., Pescatore, F., Armani, A., Paik, J.-H., … Sandri, M. (2015). 'Regulation of autophagy and the ubiquitin–proteasome system by the FoxO transcriptional network during muscle atrophy', *Nature Communications*, 6(1), p. 6670.

Minehira, K., Vega, N., Vidal, H., Acheson, K. and Tappy, L. (2004). 'Effect of carbohydrate overfeeding on whole body macronutrient metabolism and expression of lipogenic enzymes in adipose tissue of lean and overweight humans', *International Journal of Obesity*, 28(10), pp. 1291–1298.

Mohawk, J. A., Green, C. B. and Takahashi, J. S. (2012). 'Central and peripheral circadian clocks in mammals.', *Annual Review of Neuroscience*, 35, pp. 445–62.

Möhlig, M., Spranger, J., Otto, B., Ristow, M., Tschöp, M. and Pfeiffer, A. F. H. (2002). 'Euglycemic hyperinsulinemia, but not lipid infusion, decreases circulating ghrelin levels in humans', *Journal of Endocrinological Investigation*, 25(11), p. RC36-RC38.

Molfino, A., Cascino, A., Conte, C., Ramaccini, C., Fanelli, F. R. and Laviano, A. (2010). 'Caloric Restriction and L-Carnitine Administration Improves Insulin Sensitivity in Patients With Impaired Glucose Metabolism', *Journal of Parenteral and Enteral Nutrition*, 34(3), pp. 295–299.

Moore, D. R., Robinson, M. J., Fry, J. L., Tang, J. E., Glover, E. I., … Phillips, S. M. (2008). 'Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men', *American Journal of Clinical Nutrition*, 89(1), pp. 161–168.

van Moorsel, D., Hansen, J., Havekes, B., Scheer, F. A. J. L., Jörgensen, J. A., … Schrauwen, P. (2016). 'Demonstration of a day-night rhythm in human skeletal muscle oxidative capacity', *Molecular Metabolism*, 5(8), pp. 635–645.

Moro, T., Tinsley, G., Bianco, A., Marcolin, G., Pacelli, Q. F., … Paoli, A. (2016). 'Effects of eight weeks of time-restricted feeding (16/8) on basal metabolism, maximal strength, body composition, inflammation, and cardiovascular risk factors in resistance-trained males', *Journal of Translational Medicine*, 14(1), p. 290.

Morton, G. J., Blevins, J. E., Williams, D. L., Niswender, K. D., Gelling, R. W., … Schwartz, M. W. (2005). 'Leptin action in the forebrain regulates the hindbrain response to satiety signals.', *The Journal of clinical investigation*, 115(3), pp. 703–10.

Morton, G. J., Cummings, D. E., Baskin, D. G., Barsh, G. S. and Schwartz, M. W. (2006). 'Central nervous system control of food intake and body weight.', *Nature*, 443(7109), pp. 289–95.

Morton, G. J., Meek, T. H., Matsen, M. E. and Schwartz, M. W. (2015). 'Evidence against hypothalamic-pituitary-adrenal axis suppression in the antidiabetic action of leptin.', *The Journal of clinical investigation*, 125(12), pp. 4587–91.

Moshfegh, A. J., Rhodes, D. G., Baer, D. J., Murayi, T., Clemens, J. C., … Cleveland, L. E. (2008). 'The US Department of Agriculture Automated Multiple-Pass Method reduces bias in the collection of energy intakes', *The American Journal of Clinical Nutrition*, 88(2), pp. 324–332.

Most, J., Gilmore, L. A., Smith, S. R., Han, H., Ravussin, E. and Redman, L. M. (2018). 'Significant improvement in cardiometabolic health in healthy nonobese individuals during caloric restriction-induced weight loss and weight loss maintenance', *American Journal of Physiology-Endocrinology and Metabolism*, 314(4), pp. E396–E405.

Most, J., Tosti, V. and Redman, L. M. (2017). 'Calorie restriction in humans: An update', *Ageing Research Reviews*, 39, pp. 36–45.

Mota, M. C., Waterhouse, J., De-Souza, D. A., Rossato, L. T., Silva, C. M., … Crispim, C. A. (2016). 'Association between chronotype, food intake and physical activity in medical residents', *Chronobiology International*, 33(6), pp. 730–739.

Müller, M. J. and Bosy-Westphal, A. (2013). 'Adaptive thermogenesis with weight loss in humans', *Obesity*, 21(2), pp. 218–228.

Müller, M. J., Enderle, J. and Bosy-Westphal, A. (2016). 'Changes in Energy Expenditure with Weight Gain and Weight Loss in Humans.', *Current obesity reports*, 5(4), pp. 413–423.

Muller, M. J., Enderle, J., Pourhassan, M., Braun, W., Eggeling, B., … Bosy-Westphal, A. (2015). 'Metabolic adaptation to caloric restriction and subsequent refeeding: the Minnesota Starvation Experiment revisited', *American Journal of Clinical Nutrition*, 102(4), pp. 807–819.

Müller, M. J. and Geisler, C. (2017). 'From the past to future: from energy expenditure to energy intake to energy expenditure.', *European Journal of Clinical Nutrition*, 71(3), pp. 358–364.

Mullur, R., Liu, Y.-Y. and Brent, G. A. (2014). 'Thyroid hormone regulation of metabolism.', *Physiological Reviews*, 94(2), pp. 355–82.

Münzberg, H., Qualls-Creekmore, E., Berthoud, H.-R., Morrison, C. D. and Yu, S. (2016). 'Neural Control of Energy Expenditure.', *Handbook of experimental pharmacology*, 233, pp. 173–94.

Murakami, K. and Livingstone, M. B. E. (2015). 'Eating Frequency Is Positively Associated with Overweight and Central Obesity in US Adults', *The Journal of Nutrition*, 145(12), pp. 2715–2724.

Myers, M. G., Leibel, R. L., Seeley, R. J., Schwartz, M. W. and Schwartz, M. W. (2010). 'Obesity and leptin resistance: distinguishing cause from effect.', *Trends in Endocrinology and Metabolism*, 21(11), pp. 643–51.

Näslund, E., Barkeling, B., King, N., Gutniak, M., Blundell, J. E., … Hellström, P. M. (1999). 'Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men.', *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity*, 23(3), pp. 304–11.

Nass, R., Liu, J., Patrie, J., Pezzoli, S. S., Farhy, L. S., … Thorner, M. O. (2014). 'Four-hour infusion of hydrocortisone does not suppress the nocturnal increase of circulating acyl- or desacyl-ghrelin concentrations in healthy young adults.', *The Journal of clinical endocrinology and metabolism*, 99(9), pp. E1696-700.

Natalucci, G., Riedl, S., Gleiss, A., Zidek, T. and Frisch, H. (2005). 'Spontaneous 24-h ghrelin secretion pattern in fasting subjects: maintenance of a meal-related pattern', *European Journal of Endocrinology*, 152(6), pp. 845–850.

Nath, S. (2016). 'The thermodynamic efficiency of ATP synthesis in oxidative phosphorylation', *Biophysical Chemistry*, 219, pp. 69–74.

Nedelec, R., Jokelainen, J., Miettunen, J., Ruokonen, A., Herzig, K.-H., … Sebert, S. (2018). 'Early determinants of metabolically healthy obesity in young adults: study of the Northern Finland Birth Cohort 1966', *International Journal of Obesity*.

Neel, J. V (1962). 'Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"?', *American Journal of Human Genetics*, 14, pp. 353–62.

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., … Flegal, K. (2014). 'Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013', *The Lancet*, 384(9945), pp. 766–781.

NHS Digital (2017). *Health Survey for England, 2016*. Leeds: UK.

Nielsen, T. S., Jessen, N., Jørgensen, J. O. L., Møller, N. and Lund, S. (2014). 'Dissecting adipose tissue lipolysis: molecular regulation and implications for metabolic disease', *Journal of Molecular Endocrinology*, 52(3), pp. R199–R222.

Nieuwenhuizen, A. G. and Rutters, F. (2008). 'The hypothalamic-pituitary-adrenal-axis in the regulation of energy balance', *Physiology and Behavior*, 94(2), pp. 169–177.

Nightingale, T. E., Walhin, J.-P., Turner, J. E., Thompson, D. and J Bilzon, J. L. (2016). 'The influence of a home-based exercise intervention on human health indices in individuals with chronic spinal cord injury (HOMEX-SCI): study protocol for a randomised controlled trial', *Trials*, 17(1), p. 284.

Nordestgaard, B. G., Palmer, T. M., Benn, M., Zacho, J., Tybjærg-Hansen, A., … Timpson, N. J. (2012). 'The Effect of Elevated Body Mass Index on Ischemic Heart Disease Risk: Causal Estimates from a Mendelian Randomisation Approach', *PLoS Medicine*. Edited by C. Minelli, 9(5), p. e1001212.

Nye, C., Kim, J., Kalhan, S. C. and Hanson, R. W. (2008). 'Reassessing triglyceride synthesis in adipose tissue', *Trends in Endocrinology and Metabolism*, 19(10), pp. 356–361.

Oppliger, R. A., Magnes, S. A., Popowski, L. A. and Gisolfi, C. V (2005). 'Accuracy of urine specific gravity and osmolality as indicators of hydration status.', *International journal of sport nutrition and exercise metabolism*, 15(3), pp. 236–51.

Ortega, R. M., Pérez-Rodrigo, C. and López-Sobaler, A. M. (2015). 'Dietary assessment methods: dietary records.', *Nutricion Hospitalaria*, 31(Suppl 3), pp. 38–45.

Ørtenblad, N., Westerblad, H. and Nielsen, J. (2013). 'Muscle glycogen stores and fatigue.', *The Journal of physiology*, 591(18), pp. 4405–13.

Ottaway, N., Mahbod, P., Rivero, B., Norman, L. A., Gertler, A., … Perez-Tilve, D. (2015). 'Diet-Induced Obese Mice Retain Endogenous Leptin Action', *Cell Metabolism*, 21(6), pp. 877–882.

Otway, D. T., Mäntele, S., Bretschneider, S., Wright, J., Trayhurn, P., … Johnston, J. D. (2011). 'Rhythmic diurnal gene expression in human adipose tissue from individuals who are lean, overweight, and type 2 diabetic.', *Diabetes*, 60(5), pp. 1577–81.

Owen, O. E., Reichard, G. A., Patel, M. S. and Boden, G. (1979). 'Energy metabolism in feasting and fasting.', *Advances in Experimental Medicine and Biology*, 111, pp. 169–88.

Pagani, L., Semenova, E. A., Moriggi, E., Revell, V. L., Hack, L. M., … Brown, S. A.

(2010). ‘The Physiological Period Length of the Human Circadian Clock In Vivo Is Directly Proportional to Period in Human Fibroblasts’, *PLoS ONE*. Edited by A. Reif, 5(10), p. e13376.

Palou, M., Priego, T., Sánchez, J., Villegas, E., Rodríguez, A. M., … Picó, C. (2008). ‘Sequential changes in the expression of genes involved in lipid metabolism in adipose tissue and liver in response to fasting’, *European Journal of Physiology*, 456(5), pp. 825–836.

Pan, A., Schernhammer, E. S., Sun, Q. and Hu, F. B. (2011). ‘Rotating night shift work and risk of type 2 diabetes: two prospective cohort studies in women.’, *PLoS Medicine*, 8(12), p. e1001141.

Pan, W. W. and Myers, M. G. (2018). ‘Leptin and the maintenance of elevated body weight’, *Nature Reviews Neuroscience*, 19(2), pp. 95–105.

Panda, S., Hogenesch, J. B. and Kay, S. A. (2002). ‘Circadian rhythms from flies to human’, *Nature*, 417(6886), pp. 329–335.

Patterson, R. E. and Sears, D. D. (2017). ‘Metabolic Effects of Intermittent Fasting’, *Annual Review of Nutrition*, 37(1), pp. 371–393.

Pekkarinen, T., Kaukua, J. and Mustajoki, P. (2015). ‘Long-term weight maintenance after a 17-week weight loss intervention with or without a one-year maintenance program: a randomized controlled trial.’, *Journal of Obesity*, 2015, p. 651460.

Pennings, B., Groen, B. B., van Dijk, J.-W., de Lange, A., Kiskini, A., … van Loon, L. J. (2013). ‘Minced beef is more rapidly digested and absorbed than beef steak, resulting in greater postprandial protein retention in older men’, *The American Journal of Clinical Nutrition*, 98(1), pp. 121–128.

Perrin, L., Loizides-Mangold, U., Chanon, S., Gobet, C., Hulo, N., … Dibner, C. (2018). ‘Transcriptomic analyses reveal rhythmic and CLOCK-driven pathways in human skeletal muscle’, *eLife*, 7.

Perrin, L., Loizides-Mangold, U., Skarupelova, S., Pulimeno, P., Chanon, S., … Dibner, C. (2015). ‘Human skeletal myotubes display a cell-autonomous circadian clock implicated in basal myokine secretion.’, *Molecular Metabolism*, 4(11), pp. 834–45.

Petersen, M. C. and Shulman, G. I. (2018). ‘Mechanisms of Insulin Action and Insulin Resistance’, *Physiological Reviews*, 98(4), pp. 2133–2223.

Pi-Sunyer, X., Blackburn, G., Brancati, F. L., Bray, G. A., Bright, R., … Yanovski, S. Z. (2007). ‘Reduction in weight and cardiovascular disease risk factors in individuals with type 2 diabetes: one-year results of the look AHEAD trial.’, *Diabetes Care*, 30(6), pp. 1374–83.

Pietrobelli, A., Formica, C., Wang, Z. and Heymsfield, S. B. (1996). ‘Dual-energy X-ray absorptiometry body composition model: review of physical concepts’, *American Journal of Physiology-Endocrinology and Metabolism*, 271(6), pp. E941–E951.

Plasqui, G. and Westerterp, K. R. (2007). ‘Physical Activity Assessment With Accelerometers: An Evaluation Against Doubly Labeled Water’, *Obesity*, 15(10), pp.

2371–2379.

Plomgaard, P., Bouzakri, K., Krogh-Madsen, R., Mittendorfer, B., Zierath, J. R. and Pedersen, B. K. (2005). 'Tumor necrosis factor-alpha induces skeletal muscle insulin resistance in healthy human subjects via inhibition of Akt substrate 160 phosphorylation.', *Diabetes*, 54(10), pp. 2939–45.

Poggiogalle, E., Jamshed, H. and Peterson, C. M. (2018). 'Circadian regulation of glucose, lipid, and energy metabolism in humans', *Metabolism*, 84, pp. 11–27.

Pontiroli, A. E. and Morabito, A. (2011). 'Long-term Prevention of Mortality in Morbid Obesity Through Bariatric Surgery. A Systematic Review and Meta-analysis of Trials Performed With Gastric Banding and Gastric Bypass', *Annals of Surgery*, 253(3), pp. 484–487.

Poppitt, S., Swann, D., Black, A. and Prentice, A. (1998). 'Assessment of selective under-reporting of food intake by both obese and non-obese women in a metabolic facility', *International Journal of Obesity*, 22, pp. 303–311.

Prentice, A., Hennig, B. and Fulford, A. (2008). 'Evolutionary origins of the obesity epidemic: natural selection of thrifty genes or genetic drift following predation release?', *International Journal of Obesity*, 32, pp. 1607–1610.

Prentice, R. L., Mossavar-Rahmani, Y., Huang, Y., Van Horn, L., Beresford, S. A. A., … Neuhouser, M. L. (2011). 'Evaluation and Comparison of Food Records, Recalls, and Frequencies for Energy and Protein Assessment by Using Recovery Biomarkers', *American Journal of Epidemiology*, 174(5), pp. 591–603.

Public Health England (2015). *Composition of foods integrated dataset (CoFID)*.

Pulimeno, P., Mannic, T., Sage, D., Giovannoni, L., Salmon, P., … Dibner, C. (2013). 'Autonomous and self-sustained circadian oscillators displayed in human islet cells', *Diabetologia*, 56(3), pp. 497–507.

Qi, Q., Strizich, G., Hanna, D. B., Giacinto, R. E., Castañeda, S. F., … Kaplan, R. C. (2015). 'Comparing measures of overall and central obesity in relation to cardiometabolic risk factors among US Hispanic/Latino adults.', *Obesity (Silver Spring, Md.)*, 23(9), pp. 1920–8.

Rabasa, C. and Dickson, S. L. (2016). 'Impact of stress on metabolism and energy balance', *Current Opinion in Behavioral Sciences*, 9, pp. 71–77.

Racette, S. B., Weiss, E. P., Villareal, D. T., Arif, H., Steger-May, K., … Washington University School of Medicine CALERIE Group (2006). 'One year of caloric restriction in humans: feasibility and effects on body composition and abdominal adipose tissue.', *The Journal of Gerontology. Series A.*, 61(9), pp. 943–50.

Ranasinghe, C., Gamage, P., Katulanda, P., Andraweera, N., Thilakarathne, S. and Tharanga, P. (2013). 'Relationship between Body mass index (BMI) and body fat percentage, estimated by bioelectrical impedance, in a group of Sri Lankan adults: a cross sectional study', *BMC Public Health*, 13(1), p. 797.

Ravussin, E., Redman, L. M., Rochon, J., Das, S. K., Fontana, L., … CALERIE Study Group, for the C. S. (2015). 'A 2-Year Randomized Controlled Trial of Human

Caloric Restriction: Feasibility and Effects on Predictors of Health Span and Longevity.', *The Journals of Gerontology. Series A - Biological Sciences and Medical Sciences*, 70(9), pp. 1097–104.

Raynor, H. A., Goff, M. R., Poole, S. A. and Chen, G. (2015). 'Eating Frequency, Food Intake, and Weight: A Systematic Review of Human and Animal Experimental Studies.', *Frontiers in nutrition*, 2, p. 38.

Redman, L. M., Heilbronn, L. K., Martin, C. K., Alfonso, A., Smith, S. R., … Pennington CALERIE Team (2007). 'Effect of Calorie Restriction with or without Exercise on Body Composition and Fat Distribution', *The Journal of Clinical Endocrinology and Metabolism*, 92(3), pp. 865–872.

Redman, L. M., Heilbronn, L. K., Martin, C. K., de Jonge, L., Williamson, D. A., … Ravussin, E. (2009). 'Metabolic and behavioral compensations in response to caloric restriction: implications for the maintenance of weight loss.', *PloS ONE*, 4(2), p. E4377.

Redman, L. M. and Ravussin, E. (2011). 'Caloric restriction in humans: impact on physiological, psychological, and behavioral outcomes.', *Antioxidants and Redox Signaling*, 14(2), pp. 275–87.

Redman, L. M., Smith, S. R., Burton, J. H., Martin, C. K., Il'yasova, D. and Ravussin, E. (2018). 'Metabolic Slowing and Reduced Oxidative Damage with Sustained Caloric Restriction Support the Rate of Living and Oxidative Damage Theories of Aging.', *Cell Metabolism*, 27(4), pp. 805–815.

Refinetti, R., Lissen, G. C. and Halberg, F. (2007). 'Procedures for numerical analysis of circadian rhythms.', *Biological Rhythm Research*, 38(4), pp. 275–325.

Reiter, R. J. (1991). 'Neuroendocrine effects of light.', *International journal of biometeorology*, 35(3), pp. 169–75.

Rennie, K., Rowsell, T., Jebb, S. A., Holburn, D. and Wareham, N. J. (2000). 'A combined heart rate and movement sensor: proof of concept and preliminary testing study.', *European journal of clinical nutrition*, 54(5), pp. 409–14.

Reseland, J. E., Anderssen, S. A., Solvoll, K., Hjermann, I., Urdal, P., … Drevon, C. A. (2001). 'Effect of long-term changes in diet and exercise on plasma leptin concentrations.', *American Journal of Clinical Nutrition*, 73(2), pp. 240–245.

Reuter, S., Gupta, S. C., Chaturvedi, M. M. and Aggarwal, B. B. (2010). 'Oxidative stress, inflammation, and cancer: how are they linked?', *Free Radical Biology and Medicine*, 49(11), pp. 1603–16.

Richards, J. and Gumz, M. L. (2012). 'Advances in understanding the peripheral circadian clocks', *The FASEB Journal*, 26(9), pp. 3602–3613.

Rickman, A. D., Williamson, D. A., Martin, C. K., Gilhooly, C. H., Stein, R. I., … Das, S. K. (2011). 'The CALERIE Study: design and methods of an innovative 25% caloric restriction intervention.', *Contemporary Clinical Trials*, 32(6), pp. 874–81.

Robertson, M. D. (2012). 'Dietary-resistant starch and glucose metabolism', *Current Opinion in Clinical Nutrition and Metabolic Care*, 15(4), pp. 362–367.

Robinson, E., Hardman, C. A., Halford, J. C. and Jones, A. (2015). 'Eating under observation: a systematic review and meta-analysis of the effect that heightened awareness of observation has on laboratory measured energy intake', *The American Journal of Clinical Nutrition*, 102(2), pp. 324–337.

Robinson, E., Proctor, M., Oldham, M. and Masic, U. (2016). 'The effect of heightened awareness of observation on consumption of a multi-item laboratory test meal in females', *Physiology and Behavior*, 163, pp. 129–135.

Robinson, M. M., Soop, M., Sohn, T. S., Morse, D. M., Schimke, J. M., … Nair, K. S. (2014). 'High insulin combined with essential amino acids stimulates skeletal muscle mitochondrial protein synthesis while decreasing insulin sensitivity in healthy humans.', *The Journal of clinical endocrinology and metabolism*, 99(12), pp. E2574-83.

Rocha, V. Z. and Libby, P. (2009). 'Obesity, inflammation, and atherosclerosis.', *Nature Reviews - Cardiology*, 6(6), pp. 399–409.

Rochon, J., Bales, C. W., Ravussin, E., Redman, L. M., Holloszy, J. O., … CALERIE Study Group (2011). 'Design and Conduct of the CALERIE Study: Comprehensive Assessment of the Long-term Effects of Reducing Intake of Energy', *The Journals of Gerontology: Series A*, 66A(1), pp. 97–108.

Röder, P. V, Wu, B., Liu, Y. and Han, W. (2016). 'Pancreatic regulation of glucose homeostasis.', *Experimental and Molecular Medicine*, 48(3), p. e219.

Rodríguez, E. M., Blázquez, J. L. and Guerra, M. (2010). 'The design of barriers in the hypothalamus allows the median eminence and the arcuate nucleus to enjoy private milieus: The former opens to the portal blood and the latter to the cerebrospinal fluid', *Peptides*, 31(4), pp. 757–776.

Roe, M., Pinchen, H., Church, S. and Finglas, P. (2015). 'McCance and Widdowson's The Composition of Foods Seventh Summary Edition and updated Composition of Foods Integrated Dataset', *Nutrition Bulletin*, 40(1), pp. 36–39.

Roenneberg, T., Wirz-Justice, A. and Merrow, M. (2003). 'Life between Clocks: Daily Temporal Patterns of Human Chronotypes', *Journal of Biological Rhythms*, 18(1), pp. 80–90.

Roepstorff, C., Vistisen, B. and Kiens, B. (2005). 'Intramuscular triacylglycerol in energy metabolism during exercise in humans.', *Exercise and sport sciences reviews*, 33(4), pp. 182–8.

Rohan, T. E., Heo, M., Choi, L., Datta, M., Freudenheim, J. L., … Kabat, G. C. (2013). 'Body fat and breast cancer risk in postmenopausal women: a longitudinal study.', *Journal of cancer epidemiology*, 2013, p. 754815.

Rolls, B. J. (2014). 'What is the role of portion control in weight management?', *International Journal of Obesity*, 38(Suppl 1), pp. S1-8.

Romero-Corral, A., Somers, V. K., Sierra-Johnson, J., Thomas, R. J., Collazo-Clavell, M. L., … Lopez-Jimenez, F. (2008). 'Accuracy of body mass index in diagnosing obesity in the adult general population.', *International Journal of Obesity*, 32(6), pp. 959–66.

Romieu, I., Dossus, L., Barquera, S., Blottière, H. M., Franks, P. W., … IARC working group on Energy Balance and Obesity (2017). 'Energy balance and obesity: what are the main drivers?', *Cancer Causes and Control*, 28(3), pp. 247–258.

Rosen, E. D. and Spiegelman, B. M. (2006). 'Adipocytes as regulators of energy balance and glucose homeostasis.', *Nature*, 444(7121), pp. 847–853.

Rosenbaum, M., Goldsmith, R., Bloomfield, D., Magnano, A., Weimer, L., … Leibel, R. L. (2005). 'Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight.', *The Journal of Clinical Investigation*, 115(12), pp. 3579–86.

Rosenbaum, M., Hirsch, J., Gallagher, D. A. and Leibel, R. L. (2008). 'Long-term persistence of adaptive thermogenesis in subjects who have maintained a reduced body weight.', *The American Journal of Clinical Nutrition*, 88(4), pp. 906–912.

Rosenbaum, M. and Leibel, R. L. (2010). 'Adaptive thermogenesis in humans.', *International Journal of Obesity*, 34, pp. S47–S55.

Rosenbaum, M. and Leibel, R. L. (2014). '20 years of leptin: role of leptin in energy homeostasis in humans.', *The Journal of endocrinology*, 223(1), pp. T83-96.

Rosenbaum, M., Sy, M., Pavlovich, K., Leibel, R. L. and Hirsch, J. (2008). 'Leptin reverses weight loss-induced changes in regional neural activity responses to visual food stimuli.', *The Journal of Clinical Investigation*, 118(7), pp. 2583–91.

Rostom, H. and Shine, B. (2018). 'Basic metabolism: proteins', *Surgery (Oxford)*, 36(4), pp. 153–158.

Ruan, H. and Dong, L. Q. (2016). 'Adiponectin signaling and function in insulin target tissues', *Journal of Molecular Cell Biology*, 8(2), pp. 101–109.

Ruge, T., Hodson, L., Cheeseman, J., Dennis, A. L., Fielding, B. A., … Karpe, F. (2009). 'Fasted to Fed Trafficking of Fatty Acids in Human Adipose Tissue Reveals a Novel Regulatory Step for Enhanced Fat Storage', *The Journal of Clinical Endocrinology and Metabolism*, 94(5), pp. 1781–1788.

Rui, L. (2014). 'Energy metabolism in the liver.', *Comprehensive Physiology*, 4(1), pp. 177–97.

Rutkowski, J. M., Stern, J. H. and Scherer, P. E. (2015). 'The cell biology of fat expansion', *The Journal of Cell Biology*, 208(5), pp. 501–512.

Sacks, F. M., Bray, G. A., Carey, V. J., Smith, S. R., Ryan, D. H., … Williamson, D. A. (2009). 'Comparison of Weight-Loss Diets with Different Compositions of Fat, Protein, and Carbohydrates', *New England Journal of Medicine*, 360(9), pp. 859–873.

Sahar, S. and Sassone-Corsi, P. (2012). 'Regulation of metabolism: the circadian clock dictates the time', *Trends in Endocrinology and Metabolism*, 23(1), pp. 1–8.

Saini, C., Morf, J., Stratmann, M., Gos, P. and Schibler, U. (2012). 'Simulated body temperature rhythms reveal the phase-shifting behavior and plasticity of mammalian circadian oscillators.', *Genes and Development*, 26(6), pp. 567–80.

Saini, C., Petrenko, V., Pulimeno, P., Giovannoni, L., Berney, T., … Dibner, C. (2016). 'A functional circadian clock is required for proper insulin secretion by human pancreatic islet cells', *Diabetes, Obesity and Metabolism*, 18(4), pp. 355–365.

Saltiel, A. R. and Kahn, C. R. (2001). 'Insulin signalling and the regulation of glucose and lipid metabolism.', *Nature*, 414(6865), pp. 799–806.

Saltiel, A. R. and Olefsky, J. M. (2017). 'Inflammatory mechanisms linking obesity and metabolic disease', *Journal of Clinical Investigation*, 127(1), pp. 1–4.

Samra, J., Giles, S., Summers, L., Evans, R., Arner, P., … Frayn, K. (1998). 'Peripheral fat metabolism during infusion of an exogeneous triacylglycerol emulsion', *International Journal of Obesity*, 22(8), pp. 806–812.

Sanchez, A. M. J., Candau, R. B. and Bernardi, H. (2014). 'FoxO transcription factors: their roles in the maintenance of skeletal muscle homeostasis', *Cellular and Molecular Life Sciences*, 71(9), pp. 1657–1671.

Santarpia, L., Contaldo, F. and Pasanisi, F. (2017). 'Dietary protein content for an optimal diet: a clinical view.', *Journal of Cachexia, Sarcopenia and Muscle*, 8(3), pp. 345–348.

Sargent, C., Zhou, X., Matthews, R., Darwent, D., Roach, G., … Roach, G. D. (2016). 'Daily Rhythms of Hunger and Satiety in Healthy Men during One Week of Sleep Restriction and Circadian Misalignment', *International Journal of Environmental Research and Public Health*, 13(2), p. 170.

Sato, T., Nakamura, Y., Shiimura, Y., Ohgusu, H., Kangawa, K. and Kojima, M. (2012). 'Structure, regulation and function of ghrelin', *Journal of Biochemistry*, 151(2), pp. 119–128.

Schaeffer, M., Langlet, F., Lafont, C., Molino, F., Hodson, D. J., … Tomas M Hökfelt, by G. (2013). 'Rapid sensing of circulating ghrelin by hypothalamic appetite-modifying neurons', *Proceedings of the National Academy of Sciences of the United States of America*, 110(4), pp. 1512–1517.

Scheer, F. A. J. L., Hilton, M. F., Mantzoros, C. S. and Shea, S. A. (2009). 'Adverse metabolic and cardiovascular consequences of circadian misalignment.', *Proceedings of the National Academy of Sciences of the United States of America*, 106(11), pp. 4453–8.

Scheer, F. A. J. L., Morris, C. J. and Shea, S. A. (2013). 'The internal circadian clock increases hunger and appetite in the evening independent of food intake and other behaviors.', *Obesity*, 21(3), pp. 421–3.

Schmidt, S. L., Kealey, E. H., Horton, T. J., VonKaenel, S. and Bessesen, D. H. (2013). 'The effects of short-term overfeeding on energy expenditure and nutrient oxidation in obesity-prone and obesity-resistant individuals.', *International journal of obesity*, 37(9), pp. 1192–7.

Schmutz, I., Ripperger, J. A., Baeriswyl-Aebischer, S. and Albrecht, U. (2010). 'The mammalian clock component PERIOD2 coordinates circadian output by interaction with nuclear receptors', *Genes and Development*, 24(4), pp. 345–357.

Schoeller, D. A., Cella, L. K., Sinha, M. K. and Caro, J. F. (1997). 'Entrainment of the diurnal rhythm of plasma leptin to meal timing.', *Journal of Clinical Investigation*, 100(7), pp. 1882–1887.

Schoeller, D. A. and van Santen, E. (1982). 'Measurement of energy expenditure in humans by doubly labeled water method.', *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, 53(4), pp. 955–9.

Schoffelen, P. F. M. and Plasqui, G. (2018). 'Classical experiments in whole-body metabolism: open-circuit respirometry-diluted flow chamber, hood, or facemask systems.', *European Journal of Applied Physiology*, 118(1), pp. 33–49.

Schreurs, M., Kuipers, F. and van der Leij, F. R. (2010). 'Regulatory enzymes of mitochondrial β-oxidation as targets for treatment of the metabolic syndrome', *Obesity Reviews*, 11(5), pp. 380–388.

Schulz, K. F. and Grimes, D. A. (2002). 'Allocation concealment in randomised trials: defending against deciphering.', *Lancet*, 359(9306), pp. 614–8.

Schulz, K. F., Grimes, D. A., Keirse, M., Cates, W., Grimes, D., … McPeek, B. (2002). 'Unequal group sizes in randomised trials: guarding against guessing.', *Lancet*, 359(9310), pp. 966–70.

Schutz, Y. (1995). 'The basis of direct and indirect calorimetry and their potentials', *Diabetes / Metabolism Reviews*, 11(4), pp. 383–408.

SCHWARTZ, M. W. and SEELEY, R. J. (1997). 'The New Biology of Body Weight Regulation', *Journal of the American Dietetic Association*, 97(1), pp. 54–58.

Schwartz, M. W., Woods, S. C., Seeley, R. J., Barsh, G. S., Baskin, D. G. and Leibel, R. L. (2003). 'Is the energy homeostasis system inherently biased toward weight gain?', *Diabetes*, 52(2), pp. 232–8.

Seufert, J. (2004). 'Leptin effects on pancreatic beta-cell gene expression and function.', *Diabetes*, 53, pp. S152–S158.

Shani, M., Zevin-Sonkin, D., Saxel, O., Carmon, Y., Katcoff, D., … Yaffe, D. (1981). 'The correlation between the synthesis of skeletal muscle actin, myosin heavy chain, and myosin light chain and the accumulation of corresponding mRNA sequences during myogenesis', *Developmental Biology*, 86(2), pp. 483–492.

Shea, S. A., Hilton, M. F., Orlova, C., Ayers, R. T. and Mantzoros, C. S. (2005). 'Independent circadian and sleep/wake regulation of adipokines and glucose in humans.', *The Journal of Clinical Endocrinology and Metabolism*, 90(5), pp. 2537–44.

Shelmet, J. J., Reichard, G. A., Skutches, C. L., Hoeldtke, R. D., Owen, O. E. and Boden, G. (1988). 'Ethanol causes acute inhibition of carbohydrate, fat, and protein oxidation and insulin resistance.', *The Journal of Clinical Investigation*, 81(4), pp. 1137–45.

Shepherd, P. R. and Kahn, B. B. (1999). 'Glucose Transporters and Insulin Action — Implications for Insulin Resistance and Diabetes Mellitus', *New England Journal of Medicine*. Edited by F. H. Epstein, 341(4), pp. 248–257.

Shim, J.-S., Oh, K. and Kim, H. C. (2014). 'Dietary assessment methods in epidemiologic studies.', *Epidemiology and Health*, 36, p. e2014009.

Shook, R. P., Hand, G. A., O'Connor, D. P., Thomas, D. M., Hurley, T. G., … Blair, S. N. (2018). 'Energy Intake Derived from an Energy Balance Equation, Validated Activity Monitors, and Dual X-Ray Absorptiometry Can Provide Acceptable Caloric Intake Data among Young Adults', *The Journal of Nutrition*, 148(3), pp. 490–496.

Shulman, G. I., Rothman, D. L., Jue, T., Stein, P., DeFronzo, R. A. and Shulman, R. G. (1990). 'Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy.', *The New England Journal of Medicine*, 322(4), pp. 223–8.

Siler, S. Q., Neese, R. A. and Hellerstein, M. K. (1999). 'De novo lipogenesis, lipid kinetics, and whole-body lipid balances in humans after acute alcohol consumption', *The American Journal of Clinical Nutrition*, 70(5), pp. 928–936.

Silva, K. D. R. R., Wright, J. W., Williams, C. M. and Lovegrove, J. A. (2005). 'Meal ingestion provokes entry of lipoproteins containing fat from the previous meal: possible metabolic implications', *European Journal of Nutrition*, 44(6), pp. 377–383.

Simon, C., Gronfier, C., Schlienger, J. L. and Brandenberger, G. (1998). 'Circadian and Ultradian Variations of Leptin in Normal Man under Continuous Enteral Nutrition: Relationship to Sleep and Body Temperature', *The Journal of Clinical Endocrinology and Metabolism*, 83(6), pp. 1893–1899.

Singh, R., Martin, B. R., Hickey, Y., Teegarden, D., Campbell, W. W., … Weaver, C. M. (2009). 'Comparison of self-reported, measured, metabolizable energy intake with total energy expenditure in overweight teens.', *The American Journal of Clinical Nutrition*, 89(6), pp. 1744–50.

Skene, D. J., Skornyakov, E., Chowdhury, N. R., Gajula, R. P., Middleton, B., … Gaddameedhi, S. (2018). 'Separation of circadian- and behavior-driven metabolite rhythms in humans provides a window on peripheral oscillators and metabolism.', *Proceedings of the National Academy of Sciences of the United States of America*, 115(30), pp. 7825–7830.

Skogerboe, K. J., Labbé, R. F., Rettmer, R. L., Sundquist, J. P. and Gargett, A. M. (1990). 'Chemiluminescent measurement of total urinary nitrogen for accurate calculation of nitrogen balance.', *Clinical Chemistry*, 36(5), pp. 752–5.

Sletten, T. L., Revell, V. L., Middleton, B., Lederle, K. A. and Skene, D. J. (2009). 'Age-Related Changes in Acute and Phase-Advancing Responses to Monochromatic Light', *Journal of Biological Rhythms*, 24(1), pp. 73–84.

Slominski, R. M., Reiter, R. J., Schlabritz-Loutsevitch, N., Ostrom, R. S. and Slominski, A. T. (2012). 'Melatonin membrane receptors in peripheral tissues: Distribution and functions', *Molecular and Cellular Endocrinology*, 351(2), pp. 152–166.

Smith, R. L., Soeters, M. R., Wüst, R. C. I. and Houtkooper, R. H. (2018). 'Metabolic flexibility as an adaptation to energy resources and requirements in health and disease', *Endocrine Reviews*, 39(4), pp. 489–517.

Snellen, J. W., Chang, K. S. and Smith, W. (1983). 'Technical description and performance characteristics of a human whole-body calorimeter', *Medical and Biological Engineering and Computing*, 21(1), pp. 9–20.

Soares, M. J., Sheela, M. L., Kurpad, A. V, Kulkarni, R. N. and Shetty, P. S. (1989). 'The influence of different methods on basal metabolic rate measurements in human subjects.', *The American Journal of Clinical Nutrition*, 50(4), pp. 731–6.

Soeters, M. R., Lammers, N. M., Dubbelhuis, P. F., Ackermans, M., Jonkers-Schuitema, C. F., … Serlie, M. J. (2009). 'Intermittent fasting does not affect whole-body glucose, lipid, or protein metabolism', *American Journal of Clinical Nutrition*, 90(5), pp. 1244–1251.

Soeters, M. R., Soeters, P. B., Schooneman, M. G., Houten, S. M. and Romijn, J. A. (2012). 'Adaptive reciprocity of lipid and glucose metabolism in human short-term starvation', *American Journal of Physiology-Endocrinology and Metabolism*, 303(12), pp. E1397–E1407.

Solinas, G., Borén, J. and Dulloo, A. G. (2015). 'De novo lipogenesis in metabolic homeostasis: More friend than foe?', *Molecular Metabolism*, 4(5), pp. 367–77.

Solomon, T. P. J., Chambers, E. S., Jeukendrup, A. E., Toogood, A. A. and Blannin, A. K. (2008). 'The effect of feeding frequency on insulin and ghrelin responses in human subjects', *British Journal of Nutrition*, 100(4), pp. 810–819.

Song, X., Kestin, M., Schwarz, Y., Yang, P., Hu, X., … Kratz, M. (2016). 'A low-fat high-carbohydrate diet reduces plasma total adiponectin concentrations compared to a moderate-fat diet with no impact on biomarkers of systemic inflammation in a randomized controlled feeding study', *European Journal of Nutrition*, 55(1), pp. 237–246.

Southgate, D. A. T. and Durnin, J. V. G. A. (1970). 'Calorie conversion factors. An experimental reassessment of the factors used in the calculation of the energy value of human diets', *British Journal of Nutrition*, 24(2), pp. 517–535.

de Souza, R. J., Bray, G. A., Carey, V. J., Hall, K. D., LeBoff, M. S., … Smith, S. R. (2012). 'Effects of 4 weight-loss diets differing in fat, protein, and carbohydrate on fat mass, lean mass, visceral adipose tissue, and hepatic fat: results from the POUNDS LOST trial', *The American Journal of Clinical Nutrition*, 95(3), pp. 614–625.

Spalding, K. L., Bernard, S., Näslund, E., Salehpour, M., Possnert, G., … Arner, P. (2017). 'Impact of fat mass and distribution on lipid turnover in human adipose tissue', *Nature Communications*, 8, p. 15253.

Sparks, L. M., Redman, L. M., Conley, K. E., Harper, M.-E., Yi, F., … Smith, S. R. (2017). 'Effects of 12 Months of Caloric Restriction on Muscle Mitochondrial Function in Healthy Individuals.', *The Journal of Clinical Endocrinology and Metabolism*, 102(1), pp. 111–121.

Speakman, J. R. (1990). 'Principles, problems and a paradox with the measurement of energy expenditure of free-living subjects using doubly-labelled water.', *Statistics in Medicine*, 9(11), pp. 1365–80.

Speakman, J. R. and Hambly, C. (2016). 'Using doubly-labelled water to measure free-

living energy expenditure: Some old things to remember and some new things to consider', *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 202, pp. 3–9.

Spengler, C. M., Czeisler, C. A. and Shea, S. A. (2000). 'An endogenous circadian rhythm of respiratory control in humans.', *The Journal of Physiology*, 526(3), pp. 683–94.

Spiegel, K., Tasali, E., Leproult, R., Scherberg, N. and Van Cauter, E. (2011). 'Twenty-Four-Hour Profiles of Acylated and Total Ghrelin: Relationship with Glucose Levels and Impact of Time of Day and Sleep', *The Journal of Clinical Endocrinology and Metabolism*, 96(2), pp. 486–493.

Spiegelman, B. M. and Flier, J. S. (2001). 'Obesity and the Regulation of Energy Balance', *Cell*, 104(4), pp. 531–543.

St-Onge, M.-P., Ard, J., Baskin, M. L., Chiuve, S. E., Johnson, H. M., … Varady, K. (2017). 'Meal Timing and Frequency: Implications for Cardiovascular Disease Prevention: A Scientific Statement From the American Heart Association', *Circulation*, 135(9), pp. e96–e121.

St John, P. C., Hirota, T., Kay, S. A. and Doyle, F. J. (2014). 'Spatiotemporal separation of PER and CRY posttranslational regulation in the mammalian circadian clock.', *Proceedings of the National Academy of Sciences of the United States of America*, 111(5), pp. 2040–5.

Stefan, N., Häring, H.-U. and Schulze, M. B. (2018). 'Metabolically healthy obesity: the low-hanging fruit in obesity treatment?', *Lancet - Diabetes and Endocrinology*, 6(3), pp. 249–258.

Stefanowicz, M., Nikołajuk, A., Matulewicz, N. and Karczewska-Kupczewska, M. (2018). 'Adipose tissue, but not skeletal muscle, sirtuin 1 expression is decreased in obesity and related to insulin sensitivity', *Endocrine*, 60(2), pp. 263–271.

Steinert, R. E., Schirra, J., Meyer-Gerspach, A. C., Kienle, P., Fischer, H., … Beglinger, C. (2014). 'Effect of glucagon-like peptide-1 receptor antagonism on appetite and food intake in healthy men', *The American Journal of Clinical Nutrition*, 100(2), pp. 514–523.

Stote, K. S., Baer, D. J., Spears, K., Paul, D. R., Harris, G. K., … Mattson, M. P. (2007). 'A controlled trial of reduced meal frequency without caloric restriction in healthy, normal-weight, middle-aged adults', *The American Journal of Clinical Nutrition*, 85(4), pp. 981–988.

Stratton, R. J. and Elia, M. (1999). 'The effects of enteral tube feeding and parenteral nutrition on appetite sensations and food intake in health and disease.', *Clinical Nutrition*, 18(2), pp. 63–70.

Stratton, R. J., Stubbs, R. J. and Elia, M. (2003). 'Short-Term Continuous Enteral Tube Feeding Schedules Did Not Suppress Appetite and Food Intake in Healthy Men in a Placebo-Controlled Trial', *The Journal of Nutrition*, 133(8), pp. 2570–2576.

Stubbs, R. J., Harbron, C. G., Murgatroyd, P. R. and Prentice, A. M. (1995). 'Covert manipulation of dietary fat and energy density: effect on substrate flux and food intake

in men eating ad libitum', *The American Journal of Clinical Nutrition*, 62(2), pp. 316–329.

Stubbs, R. J., Hughes, D. A., Johnstone, A. M., Rowley, E., Reid, C., … Blundell, J. E. (2000). 'The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings', *British Journal of Nutrition*, 84, pp. 405–415.

Stubbs, R. J., Johnstone, A. M., O'Reilly, L. M., Barton, K. and Reid, C. (1998). 'The effect of covertly manipulating the energy density of mixed diets on ad libitum food intake in "pseudo free-living" humans.', *International Journal of Obesity and Related Metabolic Disorders*, 22(10), pp. 980–7.

Stubbs, R. J., O'Reilly, L. M., Whybrow, S., Fuller, Z., Johnstone, A. M., … Horgan, G. W. (2014). 'Measuring the difference between actual and reported food intakes in the context of energy balance under laboratory conditions', *British Journal of Nutrition*, 111(11), pp. 2032–2043.

Stumvoll, M., Jacob, S., Wahl, H. G., Hauer, B., Löblein, K., … Häring, H. (2000). 'Suppression of Systemic, Intramuscular, and Subcutaneous Adipose Tissue Lipolysis by Insulin in Humans', *The Journal of Clinical Endocrinology and Metabolism*, 85(10), pp. 3740–3745.

Sullivan, W. R., Hughes, J. G., Cockman, R. W. and Small, D. M. (2017). 'The effects of temperature on the crystalline properties and resistant starch during storage of white bread', *Food Chemistry*, 228, pp. 57–61.

Sumithran, P., Prendergast, L. A., Delbridge, E., Purcell, K., Shulkes, A., … Proietto, J. (2011). 'Long-Term Persistence of Hormonal Adaptations to Weight Loss', *New England Journal of Medicine*, 365(17), pp. 1597–1604.

Sun, K., Kusminski, C. M. and Scherer, P. E. (2011). 'Adipose tissue remodeling and obesity.', *The Journal of Clinical Investigation*, 121(6), pp. 2094–101.

Sundfør, T. M., Svendsen, M. and Tonstad, S. (2018). 'Effect of intermittent versus continuous energy restriction on weight loss, maintenance and cardiometabolic risk: a randomized 1-year trial', *Nutrition, Metabolism and Cardiovascular Diseases*.

Suter, P. M., Schutz, Y. and Jequier, E. (1992). 'The Effect of Ethanol on Fat Storage in Healthy Subjects', *New England Journal of Medicine*, 326(15), pp. 983–987.

Sutton, E. F., Beyl, R., Early, K. S., Cefalu, W. T., Ravussin, E. and Peterson, C. M. (2018). 'Early Time-Restricted Feeding Improves Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even without Weight Loss in Men with Prediabetes', *Cell Metabolism*, 27(6), pp. 1212–1221.

Swinburn, B. A., Sacks, G., Hall, K. D., McPherson, K., Finegood, D. T., … Obesity, W. H. T. F. on C. (2011). 'The global obesity pandemic: shaped by global drivers and local environments', *Lancet*, 378(9793), pp. 804–814.

Tan, T., Behary, P., Tharakan, G., Minnion, J., Al-Najim, W., … Bloom, S. R. (2017). 'The Effect of a Subcutaneous Infusion of GLP-1, OXM, and PYY on Energy Intake and Expenditure in Obese Volunteers', *The Journal of Clinical Endocrinology and*

Metabolism, 102(7), pp. 2364–2372.

Tappy, L. (1996). 'Thermic effect of food and sympathetic nervous system activity in humans.', *Reproduction, Nutrition, Development*, 36(4), pp. 391–7.

Tarnopolsky, M. A., Pearce, E., Smith, K. and Lach, B. (2011). 'Suction-modified Bergström muscle biopsy technique: Experience with 13,500 procedures', *Muscle and Nerve*, 43(5), pp. 716–725.

The Look AHEAD Research Group (2013). 'Cardiovascular Effects of Intensive Lifestyle Intervention in Type 2 Diabetes', *New England Journal of Medicine*, 369(2), pp. 145–154.

The Look AHEAD Research Group (2014). 'Eight-year weight losses with an intensive lifestyle intervention: The look AHEAD study', *Obesity*, 22(1), pp. 5–13.

Thomas, J. E. (1957). 'Mechanics and Regulation of Gastric Emptying', *Physiological Reviews*, 37(4), pp. 453–474.

Thomas, J. G., Bond, D. S., Phelan, S., Hill, J. O. and Wing, R. R. (2014). 'Weight-Loss Maintenance for 10 Years in the National Weight Control Registry', *American Journal of Preventive Medicine*, 46(1), pp. 17–23.

Thompson, D., Karpe, F., Lafontan, M. and Frayn, K. (2012). 'Physical Activity and Exercise in the Regulation of Human Adipose Tissue Physiology', *Physiological Reviews*, 92(1), pp. 157–191.

Thompson, D., Peacock, O. J. and Betts, J. A. (2014). 'Substitution and Compensation Erode the Energy Deficit from Exercise Interventions', *Medicine and Science in Sports and Exercise*, 46(2), p. 423.

Thompson, F. E., Subar, A. F., Loria, C. M., Reedy, J. L. and Baranowski, T. (2010). 'Need for technological innovation in dietary assessment.', *Journal of the American Dietetic Association*, 110(1), pp. 48–51.

Thorens, B. (2015). 'GLUT2, glucose sensing and glucose homeostasis', *Diabetologia*, 58(2), pp. 221–232.

Timper, K. and Brüning, J. C. (2017). 'Hypothalamic circuits regulating appetite and energy homeostasis: pathways to obesity', *Disease Models and Mechanisms*, 10(6), pp. 679–689.

Tinsley, G. M., Forsse, J. S., Butler, N. K., Paoli, A., Bane, A. A., … Grandjean, P. W. (2017). 'Time-restricted feeding in young men performing resistance training: A randomized controlled trial', *European Journal of Sport Science*, 17(2), pp. 200–207.

Tipton, K. D., Hamilton, D. L. and Gallagher, I. J. (2018). 'Assessing the Role of Muscle Protein Breakdown in Response to Nutrition and Exercise in Humans.', *Sports Medicine*, 48(Suppl 1), pp. 53–64.

Townsend, N., Williams, J., Bhatnagar, P., Wickramasinghe, K. and Rayner, M. (2014). *Cardiovascular Disease Statistics 2014*. Edited by E. Dicks. London.

Trabulsi, J. and Schoeller, D. A. (2001). 'Evaluation of dietary assessment instruments

against doubly labeled water, a biomarker of habitual energy intake', *American Journal of Physiology-Endocrinology and Metabolism*, 281(5), pp. E891–E899.

Travers, R. L., Motta, A. C., Betts, J. A. and Thompson, D. (2017). 'Adipose tissue metabolic and inflammatory responses to a mixed meal in lean, overweight and obese men', *European Journal of Nutrition*, 56(1), pp. 375–385.

Trepanowski, J., Canale, R. E., Marshall, K. E., Kabir, M. M. and Bloomer, R. J. (2011). 'Impact of caloric and dietary restriction regimens on markers of health and longevity in humans and animals: a summary of available findings', *Nutrition Journal*, 10, p. 107.

Trepanowski, J., Kroeger, C., Barnosky, A., Klempel, M., Bhutani, S., … Varady, K. (2017). 'Effect of Alternate-Day Fasting on Weight Loss, Weight Maintenance, and Cardioprotection Among Metabolically Healthy Obese Adults', *JAMA - Internal Medicine*, 177(7), pp. 930–938.

Trepanowski, J., Kroeger, C., Barnosky, A., Klempel, M., Bhutani, S., … Varady, K. (2017). 'Effects of alternate-day fasting or daily calorie restriction on body composition, fat distribution, and circulating adipokines: Secondary analysis of a randomized controlled trial', *Clinical Nutrition*, 37(6), pp. 1871-1878.

Tripathi, Y. B. and Pandey, V. (2012). 'Obesity and endoplasmic reticulum (ER) stresses', *Frontiers in Immunology*, 3, p. 240.

Troiano, R. P., McClain, J. J., Brychta, R. J. and Chen, K. Y. (2014). 'Evolution of accelerometer methods for physical activity research', *British Journal of Sports Medicine*, 48(13), pp. 1019–1023.

Trujillo, M. E. and Scherer, P. E. (2006). 'Adipose tissue-derived factors: impact on health and disease.', *Endocrine Reviews*, 27(7), pp. 762–78.

Trujillo, M. E., Sullivan, S., Harten, I., Schneider, S. H., Greenberg, A. S. and Fried, S. K. (2004). 'Interleukin-6 Regulates Human Adipose Tissue Lipid Metabolism and Leptin Production in Vitro', *The Journal of Clinical Endocrinology and Metabolism*, 89(11), pp. 5577–5582.

Tsukui, S., Kanda, T., Nara, M., Nishino, M., Kondo, T. and Kobayashi, I. (2000). 'Moderate-intensity regular exercise decreases serum tumor necrosis factor-α and HbA1c levels in healthy women', *International Journal of Obesity*, 24, pp. 1207–1211.

Tucker, K. L. (2007). 'Assessment of usual dietary intake in population studies of gene–diet interaction', *Nutrition, Metabolism and Cardiovascular Diseases*, 17(2), pp. 74–81.

Tudor-Locke, C., Williams, J. E., Reis, J. P. and Pluto, D. (2002). 'Utility of Pedometers for Assessing Physical Activity', *Sports Medicine*, 32(12), pp. 795–808.

Tuomainen, T. and Tavi, P. (2017). 'The role of cardiac energy metabolism in cardiac hypertrophy and failure', *Experimental Cell Research*, 360(1), pp. 12–18.

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R. and Gordon, J. I. (2006). 'An obesity-associated gut microbiome with increased capacity for energy

harvest', *Nature*, 444(7122), pp. 1027–1031.

Turner, J. E., Markovitch, D., Betts, J. A. and Thompson, D. (2010). 'Nonprescribed physical activity energy expenditure is maintained with structured exercise and implicates a compensatory increase in energy intake', *American Journal of Clinical Nutrition*, 92(5), pp. 1009–1016.

Turner, J. R. and Carroll, D. (1985). 'Heart rate and oxygen consumption during mental arithmetic, a video game, and graded exercise: further evidence of metabolically-exaggerated cardiac adjustments?', *Psychophysiology*, 22(3), pp. 261–7.

Uhlen, M., Fagerberg, L., Hallstrom, B. M., Lindskog, C., Oksvold, P., … Ponten, F. (2015). 'Tissue-based map of the human proteome', *Science*, 347(6220), pp. 1260419–1260419.

Uhlen, M., Oksvold, P., Fagerberg, L., Lundberg, E., Jonasson, K., … Ponten, F. (2010). 'Towards a knowledge-based Human Protein Atlas', *Nature Biotechnology*, 28(12), pp. 1248–1250.

Varady, K. A. (2011). 'Intermittent versus daily calorie restriction: which diet regimen is more effective for weight loss?', *Obesity Reviews*, 12(7), pp. E593–E601.

Varady, K. A., Bhutani, S., Church, E. C. and Klempel, M. C. (2009). 'Short-term modified alternate-day fasting: a novel dietary strategy for weight loss and cardioprotection in obese adults', *American Journal of Clinical Nutrition*, 90(5), pp. 1138–1143.

Varady, K. A., Bhutani, S., Klempel, M. C., Kroeger, C. M., Trepanowski, J. F., … Calvo, Y. (2013). 'Alternate day fasting for weight loss in normal weight and overweight subjects: a randomized controlled trial.', *Nutrition Journal*, 12(1), p. 146.

Varady, K. A., Hoddy, K. K., Kroeger, C. M., Trepanowski, J. F., Klempel, M. C., … Bhutani, S. (2016). 'Determinants of weight loss success with alternate day fasting', *Obesity Research and Clinical Practice*, 10, pp. 476–480.

Varady, K. A., Hudak, C. S. and Hellerstein, M. K. (2009). 'Modified alternate-day fasting and cardioprotection: relation to adipose tissue dynamics and dietary fat intake', *Metabolism*, 58(6), pp. 803–811.

Varady, K. A., Tussing, L., Bhutani, S. and Braunschweig, C. L. (2009). 'Degree of weight loss required to improve adipokine concentrations and decrease fat cell size in severely obese women', *Metabolism*, 58(8), pp. 1096–1101.

Vedala, A., Wang, W., Neese, R. A., Christiansen, M. P. and Hellerstein, M. K. (2006). 'Delayed secretory pathway contributions to VLDL-triglycerides from plasma NEFA, diet, and de novo lipogenesis in humans', *Journal of Lipid Research*, 47(11), pp. 2562–2574.

Vendelbo, M. H., Møller, A. B., Christensen, B., Nellemann, B., Clasen, B. F. F., … Møller, N. (2014). 'Fasting Increases Human Skeletal Muscle Net Phenylalanine Release and This Is Associated with Decreased mTOR Signaling', *PLoS ONE*. Edited by V. Trajkovic, 9(7), p. e102031.

Vilberg, T. R. and Keesey, R. E. (1984). 'Reduced energy expenditure after ventromedial hypothalamic lesions in female rats', *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 247(1), pp. 183–188.

Vukovic, R., Milenkovic, T., Mitrovic, K., Todorovic, S., Plavsic, L., … Zdravkovic, D. (2015). 'Preserved insulin sensitivity predicts metabolically healthy obese phenotype in children and adolescents', *European Journal of Pediatrics*, 174(12), pp. 1649–1655.

Wadden, T. A., Butryn, M. L. and Byrne, K. J. (2004). 'Efficacy of Lifestyle Modification for Long-Term Weight Control', *Obesity Research*, 12(S12), p. 151S–162S.

Walhin, J.-P., Richardson, J. D., Betts, J. A. and Thompson, D. (2013). 'Exercise counteracts the effects of short-term overfeeding and reduced physical activity independent of energy imbalance in healthy young men.', *The Journal of Physiology*, 591(24), pp. 6231–43.

Wang, Y. C., McPherson, K., Marsh, T., Gortmaker, S. L., Brown, M., … Chisholm, D. (2011). 'Health and economic burden of the projected obesity trends in the USA and the UK', *Lancet*, 378(9793), pp. 815–825.

Wang, Z., Ying, Z., Bosy-Westphal, A., Zhang, J., Schautz, B., … Müller, M. J. (2010). 'Specific metabolic rates of major organs and tissues across adulthood: evaluation by mechanistic model of resting energy expenditure.', *The American Journal of Clinical Nutrition*, 92(6), pp. 1369–1377.

Wasserman, D. H. (2009). 'Four grams of glucose', *American Journal of Physiology-Endocrinology and Metabolism*, 296(1), pp. E11–E21.

Waterson, M. J. and Horvath, T. L. (2015). 'Neuronal Regulation of Energy Homeostasis: Beyond the Hypothalamus and Feeding', *Cell Metabolism*, 22(6), pp. 962–970.

Webster, J. D., Welsh, G., Pacy, P. and Garrow, J. S. (1986). 'Description of a human direct calorimeter, with a note on the energy cost of clerical work', *British Journal of Nutrition*, 55(1), pp. 1–6.

Wehrens, S. M. T., Christou, S., Isherwood, C., Middleton, B., Gibbs, M. A., … Johnston, J. D. (2017). 'Meal Timing Regulates the Human Circadian System.', *Current Biology*, 27(12), p. 1768–1775.e3.

Weigle, D. S., Duell, P. B., Connor, W. E., Steiner, R. A., Soules, M. R. and Kuijper, J. L. (1997). 'Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels.', *The Journal of Clinical Endocrinology and Metabolism*, 82(2), pp. 561–5.

Weir, J. B. (1949). 'New methods for calculating metabolic rate with special reference to protein metabolism.', *Journal of Physiology*, 109(1–2), pp. 1–9.

Weiss, E. P., Racette, S. B., Villareal, D. T., Fontana, L., Steger-May, K., … Holloszy, J. O. (2006). 'Improvements in glucose tolerance and insulin action induced by increasing energy expenditure or decreasing energy intake: a randomized controlled trial.', *The American Journal of Clinical Nutrition*, 84(5), pp. 1033–42.

Welbourn, R., le Roux, C. W., Owen-Smith, A., Wordsworth, S. and Blazeby, J. M. (2016). 'Why the NHS should do more bariatric surgery; how much should we do?', *British Medical Journal (Clinical Research Edition)*, 353, p. i1472.

Wells, J. C. K. and Fewtrell, M. S. (2006). 'Measuring body composition.', *Archives of Disease in Childhood*, 91(7), pp. 612–7.

Wells, J. C. K. and Siervo, M. (2011). 'Obesity and energy balance: is the tail wagging the dog?', *European Journal of Clinical Nutrition*, 65(11), pp. 1173–1189.

Wendel, A. A., Lewin, T. M. and Coleman, R. A. (2009). 'Glycerol-3-phosphate acyltransferases: rate limiting enzymes of triacylglycerol biosynthesis.', *Biochimica et Biophysica Acta*, 1791(6), pp. 501–506.

Wensveen, F. M., Valentić, S., Šestan, M., Turk Wensveen, T. and Polić, B. (2015). 'The "Big Bang" in obese fat: Events initiating obesity-induced adipose tissue inflammation', *European Journal of Immunology*, 45(9), pp. 2446–2456.

Westerterp-Plantenga, M., Luscombe-Marsh, N., Lejeune, M., Diepvens, K., Nieuwenhuizen, A., … Westerterp, K. (2006). 'Dietary protein, metabolism, and body-weight regulation: dose–response effects', *International Journal of Obesity*, 30(Suppl. 3), pp. 16–23.

Westerterp-Plantenga, M. S., Adamantidis, A., de Lecea, L., Froy, O., Wolk, R., … Garaulet, M. (2016). 'Sleep, circadian rhythm and body weight: parallel developments', *Proceedings of the Nutrition Society*, 75(4), pp. 431–439.

Westerterp, K. R. (2004). 'Diet induced thermogenesis', *Nutrition and Metabolism*, 1(1), p. 5.

Westerterp, K. R. (2017). 'Doubly labelled water assessment of energy expenditure: principle, practice, and promise.', *European Journal of Applied Physiology*, 117(7), pp. 1277–1285.

Weyer, C., Walford, R. L., Harper, I. T., Milner, M., MacCallum, T., … Ravussin, E. (2000). 'Energy metabolism after 2 y of energy restriction: the Biosphere 2 experiment', *The American Journal of Clinical Nutrition*, 72(4), pp. 946–953.

Whybrow, S., Horgan, G. and Stubbs, R. J. (2008). 'Low-energy reporting and duration of recording period', *European Journal of Clinical Nutrition*, 62(9), pp. 1148–1150.

Whybrow, S., Stubbs, R. J., Johnstone, A. M., O'Reilly, L. M., Fuller, Z., … Horgan, G. W. (2016). 'Plausible self-reported dietary intakes in a residential facility are not necessarily reliable', *European Journal of Clinical Nutrition*, 70(1), pp. 130–135.

Williams, J. E., Wells, J. C., Wilson, C. M., Haroun, D., Lucas, A. and Fewtrell, M. S. (2006). 'Evaluation of Lunar Prodigy dual-energy X-ray absorptiometry for assessing body composition in healthy persons and patients by comparison with the criterion 4-component model', *The American Journal of Clinical Nutrition*, 83(5), pp. 1047–1054.

Williamson, D. A., Allen, H. R., Martin, P. D., Alfonso, A. J., Gerald, B. and Hunt, A. (2003). 'Comparison of digital photography to weighed and visual estimation of portion sizes.', *Journal of the American Dietetic Association*, 103(9), pp. 1139–45.

Williamson, D. A., Bray, G. A. and Ryan, D. H. (2015). 'Is 5% weight loss a satisfactory criterion to define clinically significant weight loss?', *Obesity*, 23(12), pp. 2319–2320.

Wilson, J. L. and Enriori, P. J. (2015). 'A talk between fat tissue, gut, pancreas and brain to control body weight', *Molecular and Cellular Endocrinology*, 418, pp. 108–119.

Wing, R. R., Lang, W., Wadden, T. A., Safford, M., Knowler, W. C., … Wagenknecht, L. (2011). 'Benefits of modest weight loss in improving cardiovascular risk factors in overweight and obese individuals with type 2 diabetes.', *Diabetes Care*, 34(7), pp. 1481–6.

Wing, R. R. and Phelan, S. (2005). 'Long-term weight loss maintenance.', *The American Journal of Clinical Nutrition*, 82(Suppl 1), pp. S222–S225.

World Health Organization (2000). *Obesity: preventing and managing the global epidemic*. Geneva: Switzerland.

World Health Organization (2009). *Global health risks: mortality and burden of disease attributable to selected major risks*. Geneva: Switzerland.

World Health Organization (2013). *Global action plan for the prevention and control of noncommunicable diseases 2013-2020*. Geneva: Switzerland.

World Medical Association (2013). 'World Medical Association Declaration of Helsinki', *JAMA*, 310(20), p. 2191.

Wren, A. M., Seal, L. J., Cohen, M. A., Brynes, A. E., Frost, G. S., … Bloom, S. R. (2001). 'Ghrelin enhances appetite and increases food intake in humans.', *The Journal of Clinical Endocrinology and Metabolism*, 86(12), pp. 5992–5995.

Wyatt, J. K., Ritz-De Cecco, A., Czeisler, C. A. and Dijk, D. J. (1999). 'Circadian temperature and melatonin rhythms, sleep, and neurobehavioral function in humans living on a 20-h day.', *The American Journal of Physiology*, 277(4 Pt 2), pp. R1152-63.

Yates, L. and Warde, A. (2015). 'The evolving content of meals in Great Britain. Results of a survey in 2012 in comparison with the 1950s', *Appetite*, 84, pp. 299–308.

Ye, R., Selby, C. P., Chiou, Y.-Y., Ozkan-Dagliyan, I., Gaddameedhi, S. and Sancar, A. (2014). 'Dual modes of CLOCK:BMAL1 inhibition mediated by Cryptochrome and Period proteins in the mammalian circadian clock.', *Genes and Development*, 28(18), pp. 1989–98.

Yin, X., Gibbons, H., Rundle, M., Frost, G., McNulty, B. A., … Brennan, L. (2017). 'Estimation of Chicken Intake by Adults Using Metabolomics-Derived Markers', *The Journal of Nutrition*, 147(10), pp. 1850–1857.

Zello, G. A., Smith, J. M., Pencharz, P. B. and Ball, R. O. (1990). 'Development of a heating device for sampling arterialized venous blood from a hand vein', *Annals of Clinical Biochemistry*, 27, pp. 366–372.

Zera, A. J. and Harshman, L. G. (2001). 'The Physiology of Life History Trade-Offs

in Animals', *Annual Review of Ecology and Systematics*, 32(1), pp. 95–126.

Zhang, D., Tong, X., Arthurs, B., Guha, A., Rui, L., … Yin, L. (2014). 'Liver clock protein BMAL1 promotes de novo lipogenesis through insulin-mTORC2-AKT signaling.', *The Journal of Biological Chemistry*, 289(37), pp. 25925–35.

Zhang, J., Matheny, M. K., Tümer, N., Mitchell, M. K. and Scarpace, P. J. (2007). 'Leptin antagonist reveals that the normalization of caloric intake and the thermic effect of food after high-fat feeding are leptin dependent', *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 292(2), pp. R868–R874.

Zurlo, F., Larson, K., Bogardus, C. and Ravussin, E. (1990). 'Skeletal muscle metabolism is a major determinant of resting energy expenditure.', *The Journal of Clinical Investigation*, 86(5), pp. 1423–7.