Associations Among Free-Living Sedentary and Active Behaviours, Adiposity and Appetite Control Within an Energy Balance Framework

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

The following are publications from this thesis:

- **MYERS, A**., GIBBONS, C., BUTLER, E., DALTON, M., BUCKLAND, N., BLUNDELL, J. & FINLAYSON, G. 2017. A novel procedure for integrating three objectively measured dimensions of free-living sedentary time. *Obesity facts*, 10 (suppl. 1), 104.
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- **MYERS, A**., GIBBONS, C., DALTON, M., BUCKLAND, N., BLUNDELL, J. & FINLAYSON, G. Disentangling the relationship between sedentariness and obesity: Low activity intensity, but not posture, is associated with adiposity in overweight women. (In preparation)
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“Apply yourself both now and in the next life. Without effort, you cannot be prosperous. Though the land be good, you cannot have an abundant crop without cultivation.” (Plato)

Abstract

Background

It was proposed over 60 years ago that "*the differences between the intakes of food must originate in differences in the expenditure of energy*" (Edholm et al., 1955). It was also proposed that a 'U' shaped function described the relationship between physical activity (PA) energy expenditure (EE) and dietary intake (Mayer et al., 1956); this relationship also involved body mass. These relationships served as the basis for the studies conducted for this thesis. The main objective was to examine the associations among free-living sedentary and active behaviours, adiposity and appetite control. The investigation was conducted within an energy balance framework. The main focus of the thesis was to extend understanding of the interaction between PA, sedentary behaviour (SB), adiposity and appetite.

Methods

The methodology was based on measurements of body composition together with anthropometric, physiological, behavioural and psychological variables and involved a combination of cross-sectional and medium-term (12-weeks) intervention studies. The thesis used state-of-the-art methodology for measuring free-living activity and aimed to detect a measure of SB based on both posture and activity intensity.

Results

Study 1 - SB was positively associated with adiposity and moderate-to-vigorous physical activity (MVPA) was negatively associated with adiposity.

Study 2 - A procedure was developed to integrate data on two dimensions of free-living SB (posture and activity intensity) using two validated activity monitors.

Study 3 - Posture alone (as a marker of SB) is not a good indicator of the tendency to accumulate fat mass (FM).

Study 4 - Total EE and the metabolic contributors to total EE (fat-free mass (FFM) and resting metabolic rate (RMR)) were associated with subjective appetite sensations and EI, and provisionally can be regarded as drivers of appetite.

Study 5 - The 12-week exercise intervention resulted in a significant (compensatory) increase in EI, however, there was no change in non-exercise physical activity (NEPA).

Study 6 - Diet induced weight loss (mainly FM loss) did not lead to a compensatory reduction in PA or increase in SB.

Conclusions

These studies have extended the understanding of the associations among PA, SB, adiposity and appetite control. The outcomes of the studies have contributed to a theoretical framework for understanding the interactions between physiological and behavioural variables that contribute to energy balance and body mass (adiposity) regulation under realistic conditions. It could be deduced that a combination of increased EE (through exercise) and reduced EI are likely to produce greater weight loss and more favourable changes in body composition than either exercise or diet alone.

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Abbreviations

ANOVA: Analysis of variance

AP: activPAL

APMHR: Age-predicted maximum heart rate

AUC: Area under the curve

BES: Binge Eating Scale

BG: Blood glucose

BMI: Body mass index

BP: Blood pressure

CCK: Cholecystokinin

CNS: Central nervous system

CoEQ: Control of Eating Questionnaire

CR: Calorie restrictive diet

DLW: Doubly-labelled water

DIT: Diet induced thermogenesis

EARS: Electronic appetite rating system

EE: Energy expenditure

E_I: Energy intake

E_O: Energy expenditure

E_S: Energy storage

EI: Energy intake

EoDQ: End of Day Questionnaire

FFM: Fat-free mass

FM: Fat mass

GEM: Gas exchange measurement

GLP-1: Glucagon-like peptide-1

HARU: Human Appetite Research Unit

HED: High energy dense

HR: Heart rate

IC: Indirect calorimetry

ICC: Intraclass correlation

LED: Low energy dense diet

MET: Metabolic equivalent

MVPA: Moderate-to-vigorous physical activity

NEAT: Non-exercise activity thermogenesis

NEPA: Non-exercise physical activity

NHANES: National Health and Nutrition Examination Survey

PA: Physical activity

PFC: Prospective food consumption

RMR: Resting metabolic rate

SB: Sedentary behaviour

SD: Standard deviation

SE: Standard error

SED^{AP}: Sedentary behaviour measured with the activPAL

SED^{INT}: Sedentary behaviour measured with the integrated data

SED^{SWA}: Sedentary behaviour measured with the SenseWear Armband

SQ: Satiety Quotient

SWA: SenseWear Armband

TEF: Thermic effects of food

TFEQ: Three Factor Eating Questionnaire

TFEQ-D: Disinhibition

TFEQ-H: Hunger

TFEQ-R: Restraint

TEF: Thermic effects of food

VAS: Visual analogue scale

$\dot{V}O_{2max}$: Maximal aerobic capacity

WC: Waist circumference

WHO: World Health Organisation

Chapter 1
General Introduction

1.1 Recent trends in obesity

Obesity rates in the United Kingdom are among the highest in Europe (World Health Organization, 2014). Between 1993 and 2015 there has been a marked increase in the proportion of adults in England who are overweight, including obese, from 58% to 68% in men and from 49% to 58% in women (Health Survey for England, 2015). Obesity rates have remained stable since 2010 at around 27% for both men and women. However, research from the University of Glasgow concluded almost 40% of Scottish and English adults are now obese (Vlassopoulos et al., 2014).The Foresight report, published in 2007, suggested over half of the UK population would be obese by 2050 with a cost of £50 billion per year (Butland et al., 2007). Ten years on there appears to be contradictory information regarding obesity levels with the Health Survey for England suggesting a plateau in obesity levels, whilst independent studies suggest obesity levels are close to those predicted in the Foresight report. Never the less, the more consistent upward trends in the proportion of the population who are overweight represent a significant public health crisis.

There is substantial evidence to support a link between overweight and obesity and an increased risk of developing comorbidities. Guh et al. (2009) conducted a systematic review and meta-analysis and found evidence for 18 comorbidities including; type II diabetes, all cancers except oesophageal and prostate cancer, all cardiovascular diseases (except congestive heart failure), asthma, gallbladder disease, osteoarthritis and chronic back pain. Considering the prevalence of overweight and obesity and health consequences of excess weight, it is a public health priority to develop effective interventions to reverse the trends in overweight and obesity.

1.2 Causes of obesity - the energy balance wars

The obesity systems map illustrates the complexity of the obesity epidemic (see Figure 1.1) and it identifies over 100 variables directly or indirectly influencing energy balance (Butland et al., 2007). These are then grouped in to seven cross-cutting themes and two of the main themes are food consumption and individual activity. The contribution of under expenditure of energy and over consumption of food to obesity has been much debated in the literature. Some argue the increased prevalence of overweight and obesity is due to an increase in food availability (Swinburn et al., 2009), whilst others argue the decline in work-related physical activity (PA), and therefore energy

expenditure (EE), is to blame (Church et al., 2011). It is unlikely that either one is solely responsible for the continued rise in obesity seen globally. Rather, both have an effect on weight gain and arguing for one cause over the other only serves to delay scientific advancement in the field of obesity research.

There is evidence to support a change in both EE and energy intake (EI) patterns over the last few decades. For example, over the last 50 years there has been a significant reduction in occupation and household EE as well as a reduction in active transportation (Church et al., 2011, Archer et al., 2013b, Wen et al., 2006). Global PA levels have also declined and only one third of the global population are estimated to be achieving the recommended amount (Hallal et al., 2012). Simultaneously, our food environment has changed considerably. Termed the 'nutrition transition' there has been a shift from a diet consisting of nutritious home cooked foods to one characterized by easily available, highly processed, energy-dense foods which promote overconsumption (Crino et al., 2015). Under such pervasive environmental and behavioural conditions maintaining an energy balance can be a major challenge.

> *"Obesity is the result of people responding normally to the obesogenic environments they find themselves in." (Swinburn et al., 2011, p.804)*

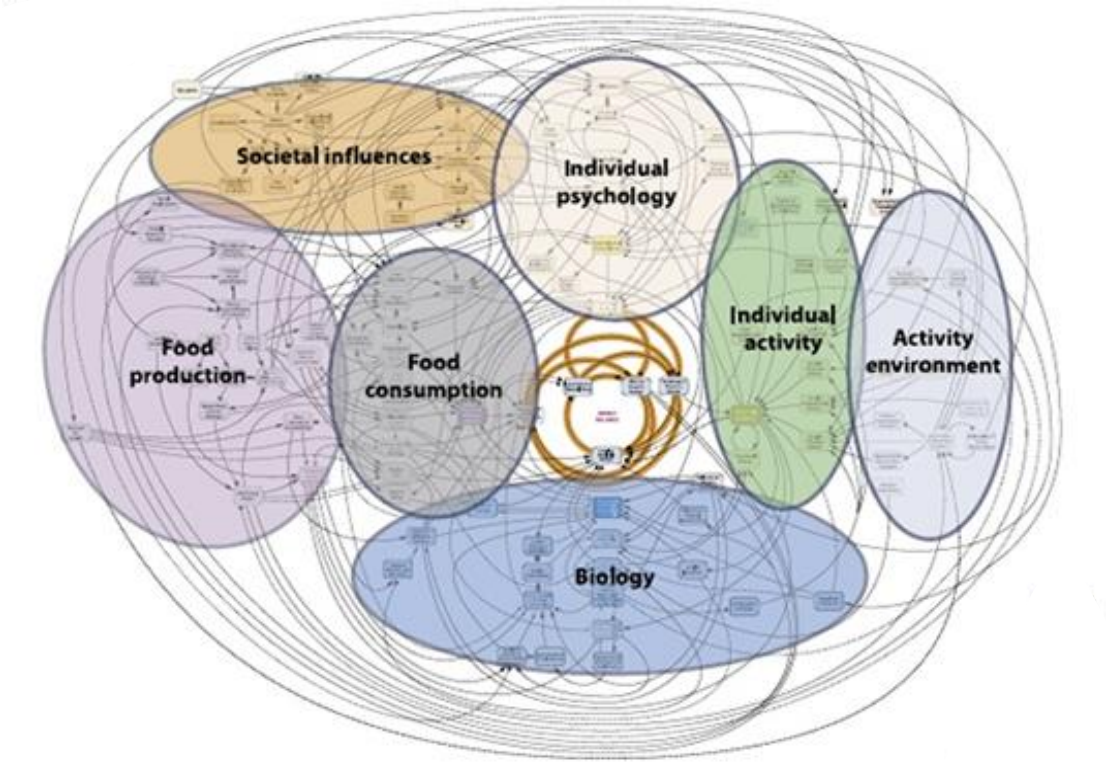


Figure 1.1 The Obesity Systems map identifies over 100 variables which influence energy balance and the obesity epidemic. These are then categorised into seven cross-cutting themes, source: Butland et al. (2007)

1.3 Energy balance

The development of obesity can be considered in terms of energy balance. Energy balance refers to the flow of energy into (food consumption) and out of (energy metabolism) the body. In simple terms, unhealthy weight gain results from an imbalance between EI and EE. The first law of thermodynamics states that energy can be transferred from one system to another but cannot be created or destroyed. Human physiology complies with this law and is formulated as follows: the rate of change in the body's macronutrient stores (E_S) is equal to the difference between the rate of chemical energy from the foods and drinks consumed (E_I) and the heat lost through radiation, conduction, convection and evaporation (E_O) (Hall et al., 2012). It follows that body mass change occurs if EI does not match EE. For example, if EI exceeds EE body mass will increase (positive energy balance), and if EE exceeds EI body mass will decrease (negative energy balance). The three basic components of energy balance are EI, EE, and energy storage. For the body's energy stores to remain stable, EI must match EE. This is called energy homeostasis.

EI is 100% behaviour and consists of three major macronutrient groups; carbohydrates (3.75 kcal/g), proteins (4 kcal/g), and fats (9 kcal/g), and to a lesser extent alcohol (7 kcal/g). Survey data suggests that carbohydrate accounts for 40-50% of EI, protein 15-20% and fat 30-40% (Austin et al., 2011). Not all energy consumed can be metabolised and used for biological processes. The bioavailability of ingested foods varies and faecal losses account for around 2-10% of energy consumed. Several factors affect the variability in absorptive efficiency between individuals including gut flora, food preparation, and diet composition (Hall et al., 2012).

Total daily EE consists of resting metabolic rate (RMR), which reflects the minimal daily energy requirements needed to perform key biological and behavioural processes at rest; the thermic effects of food (TEF), which is the energy used to digest foods; and the energy expended through PA, the most variable component of EE (see Figure 1.2) (Melanson, 2017). PA is the only behavioural element of EE and accounts for 25-35% of total daily EE, depending on individual PA levels. PA can be further divided into exercise EE (structured and planned PA) and non-exercise PA or NEPA (fidgeting, activities of daily living and ambulation) (Levine et al., 1999). RMR is the largest proportion of total daily EE and comprises approximately 50-70% of total EE (Goran, 2000, Shetty, 2005). RMR increases with increased body mass due to greater fat-free mass (FFM). FFM and fat mass (FM) explain 60-70% and 6% of the variance in RMR, respectively (Johnstone et al., 2005). TEF from a mixed diet consumed at energy balance contributes around 10% to total daily EE. Some macronutrients affect EE to a greater extent than others; reported TEF values for separate nutrients are 0-3% for fat, 5-10% for carbohydrate, 20-30% for protein (Westerterp, 2004).

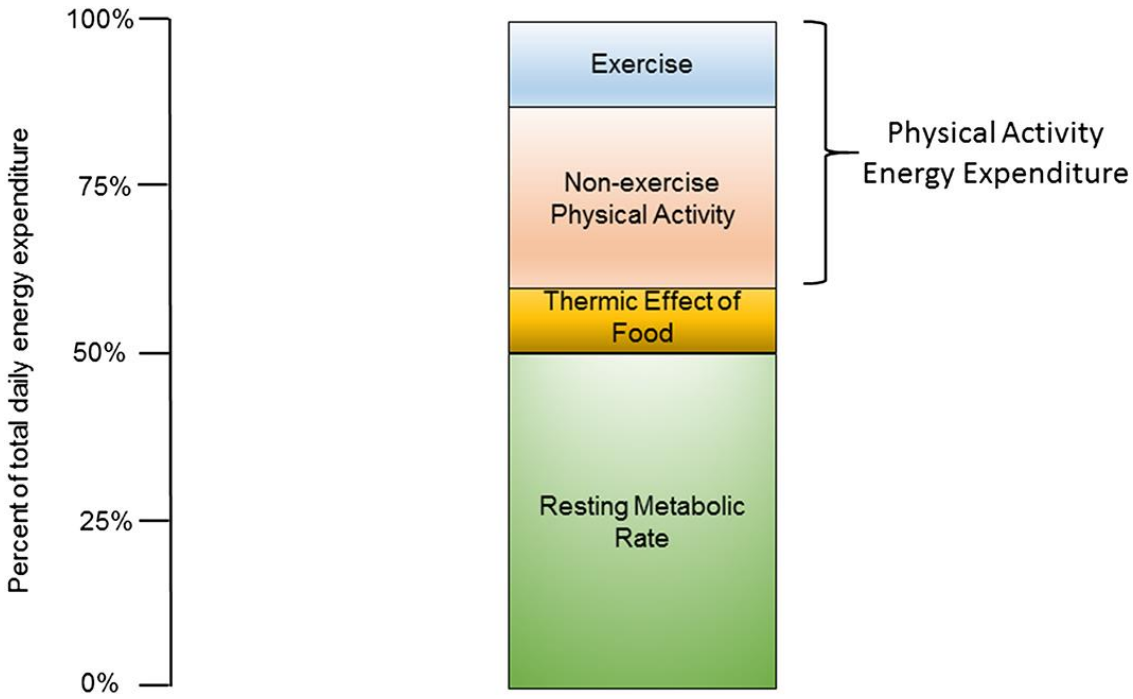


Figure 1.2 Components of total daily EE, source: Melanson (2017)

The mechanisms controlling the energy balance system are not fully understood, but it is clear that complicated physiological processes are involved. The energy balance equation is often depicted as a set of kitchen scales with EI on one side and EE on the other; this is inaccurate and overly simplistic. Energy balance is a dynamic process and there is a reciprocal relationship between food intake and EE. Furthermore, depicting energy balance as a simple mathematic formula ignores the potential for behavioural or metabolic adaptations to occur to restore energy homeostasis during times of energy surfeit of deficit (King et al., 2007). Changes in either side of the equation (EI or EE) do not have a simple additive or subtractive effect on the body's energy stores. Instead, perturbations in the energy balance system are subject to physiologically regulated processes. One important implication of the physiological regulation of energy balance is that the system operates asymmetrically; it defends against weight loss (negative energy balance) more vigorously than it does weight gain (positive energy balance) (Schwartz et al., 2003, Blundell and Gillett, 2001). This mechanism would have been useful when food was scarce, however, in our current environment where highly palatable energy dense foods are readily available, maintaining a healthy body mass is extremely difficult.

1.4 Appetite regulation

1.4.1 Early theories of homeostatic appetite control

EI is 100% behaviour; and in principal the amount of food and drink we consume is under our volitional control. Traditionally, the regulation of food intake has been viewed as a physiological system (Bernard, 1855, Cannon, 1932). Homeostatic control of feeding is concerned primarily with regulation of energy balance. Early theories of homeostatic appetite where based on signals arising from different body energy stores to relay information about the body's energy stores to the central nervous system. These included the aminostatic theory (Mellinkoff et al., 1956), the glucostatic theory (Mayer, 1953) and the lipostatic theory (Kennedy, 1953). The discovery of leptin provided support for the lipostatic theory (Zhang et al., 1994). Forty years prior to the discovery of leptin Kennedy described a circulating metabolite that acted on the hypothalamus to inhibit feeding and leptin provided support for this mechanism (Kennedy, 1953). However, the lipostatic theory cannot explain eating behaviours exhibited in the current obesogenic environment.

1.4.2 The Satiety Cascade

Whereas the theories above were related to an understanding of the total amount of energy consumed, the Satiety Cascade was developed to understand the pattern of eating throughout the day. Since the early theories of appetite control, a number of physiological and psychological processes have been identified that influence appetite control. The concept is that eating behaviour is stimulated and supressed by physiological signals thereby producing an episodic pattern of eating occasions throughout the day. The homeostatic control of appetite can be conceptualized through a series of psychobiological processes that initiate and terminate feeding episodes (satiation), and those which suppress inter-meal hunger (satiety). Over 25 years ago Blundell et al. (1987) proposed the Satiety Cascade to help explain the underlying processes controlling food intake; for example, what initiates an eating episode and what determines its termination. The satiety cascade has been updated several times since it was first described in order to incorporate new developments in the field of appetite control (Figure 1.3). It describes the events that occur before, during and after the consumption of food which help to regulate EI. The satiety cascade can be partitioned into two distinct processes; satiation and satiety. Satiation describes the processes that bring an eating episode to an end and therefore determines meal size; along with the macronutrient composition of the food, these determine the amount of energy consumed. Satiation occurs when the stomach feels full or when the individual is satisfied with the amount of food consumed. Satiation can be measured by accurately measuring food consumption during meals. Satiety is defined as the inhibition of further eating together with the continued suppression of

hunger and increase in fullness that occurs once eating has ceased. The feeling of satiety lasts until the recovery of hunger and readiness for the next meal. Satiety can be measured by assessing changes in subjective appetite sensation such as hunger and fullness (using visual analogue scales) which provide valid markers of the intensity and rate of change of satiety (Flint et al., 2000).

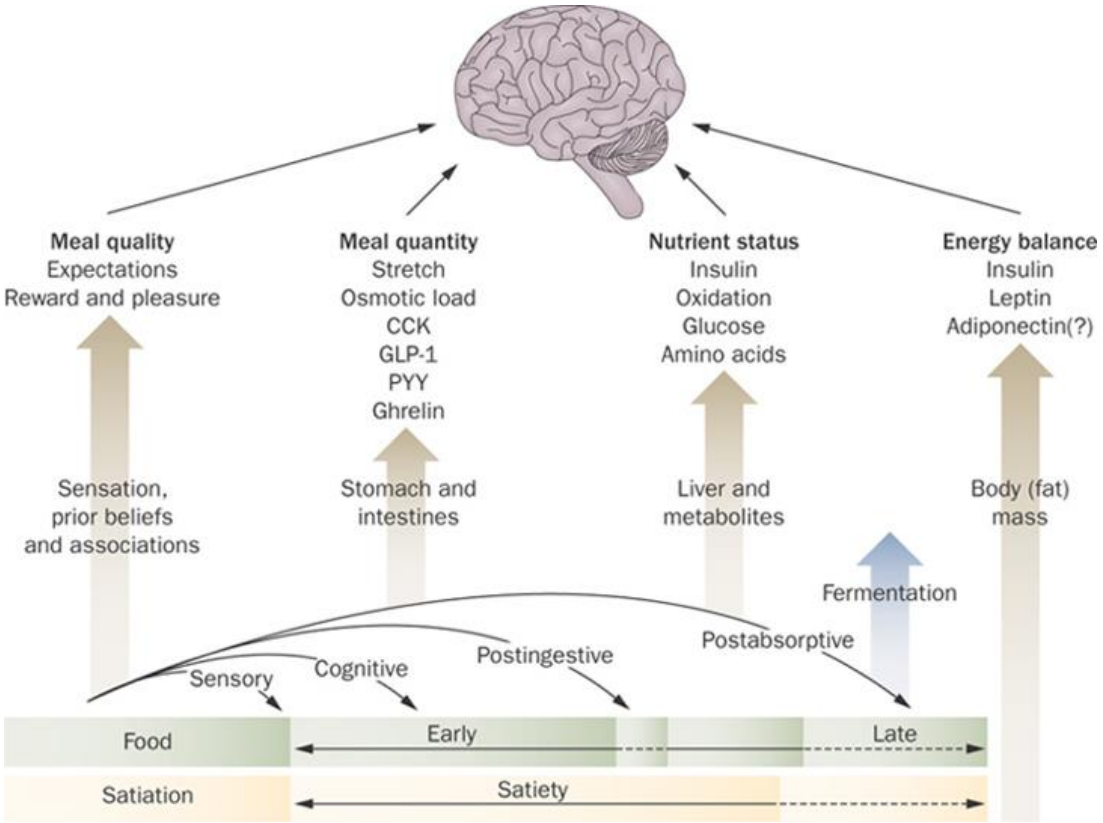


Figure 1.3 The Satiety Cascade illustrates how the pattern of eating is influenced by psychological and physiological processes arising from food consumption, source: Blundell (2010)

The processes of the satiety cascade are influenced by physiological actions of consumed foods in the stomach and the hormones released in the gastro-intestinal tract in response to the digestion and absorption of foods (Wang et al., 2008a). Neural and hormone signals communicate information to key regions of the brain (the hypothalamus and brainstem) about the current state of energy balance to either stimulate or supress hunger and subsequent eating behaviour. These hormones can be categorised as either tonic, which are important for energy storage over the long term, or episodic, which are released in response to feeding (Blundell, 2006). The hormone leptin, discovered in 1994, can be considered a tonic hormone (Zhang et al., 1994). It is secreted by adipose tissue and signals to the brain the size of the adipose tissue store in order to inhibit hunger, however, most obese individuals have high

leptin levels suggesting a leptin resistance (Heymsfield et al., 1999). Episodic hormones include ghrelin, peptide YY (PYY), cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1). Ghrelin, the only gut hormone known to enhance appetite, is an orexigenic (hunger) hormone thought to play a role in short-term meal initiation as circulating levels rise before a meal and decline once food has been consumed (Cummings et al., 2004). Ghrelin has been shown to increase with dietary-induced weight loss suggesting it may play a role in weight regain (Cummings et al., 2002). Anorexigenic (satiety) hormones are released from the gut in response to food ingestion and play a role in early suppression of appetite. Hormones such as PYY, CCK and GLP-1 all increase in response to food consumption and are thought to inhibit food intake (Badman and Flier, 2005). Recent research has shown that despite similar levels of satiety and satiation following a high fat versus a high carbohydrate meal, the peptide response was markedly different. This indicates there is no single peptide or peptide profile that is solely responsible for satiety and different peptide profiles can confer the same degree of satiety (Gibbons et al., 2013). For a detailed review of the molecular mechanisms which regulate appetite see Schwartz et al. (2000). It is important to note that the homeostatic mechanisms of appetite control described here may be modified at times by reward pathways relating to the pleasurable qualities of food and drink; a major concern in our obesogenic environment. Biological mechanisms which regulate appetite interact with environmental, psychological and social factors to influence food intake (Berthoud, 2006). Non-homeostatic pathways involved in the control of food intake can override homeostatic signals promoting eating in the absence of physiological hunger (Finlayson et al., 2007).

1.4.3 A new formulation of appetite control using an energy balance framework

The original lipostatic theory was only concerned with preventing excessive EI and fat gain and did not identify mechanisms driving ingestive behaviour to prevent loss of FM by maintaining a lower limit of EI. Recently, the role of FFM in appetite control has received attention. Accumulating evidence suggests FFM and RMR play an important role in the orexigenic drive to eat. Studies have demonstrated FFM and RMR are associated with hunger, self-selected meal size and EI (Blundell et al., 2012b, Caudwell et al., 2013a, Weise et al., 2014, Blundell et al., 2015a). RMR reflects the lower limit of the amount of energy required to maintain key biological and behavioural processes and it has been proposed that RMR produces a tonic drive to eat in order to maintain these processes (Blundell et al., 2012b). A new formulation of the major contributing factors to appetite control has been proposed in which both FM and FFM both influence eating behaviour (Hopkins and Blundell, 2016). This new formulation is depicted in Figure 1.4. Tonic signals (enduring, relatively stable over days) arise from

FM, FFM and metabolism. Signals arising from FM, such as leptin, inhibit EI whereas signals arising from FFM and RMR promote EI. The tonic appetite signals arising from FFM and RMR are, as yet, unidentified and represent a target for future research. Episodic signals arise following food consumption as previously described. The overall strength of the orexigenic drive for food depends on the interplay between tonic excitatory and inhibitory processes. There is evidence to suggest that the tonic inhibitory effect of adipose tissue becomes blunted as FM accumulates in the body due to leptin and insulin resistance. It follows that as people accumulate more FM, it becomes more difficult to control their appetite and further weight gain ensues.

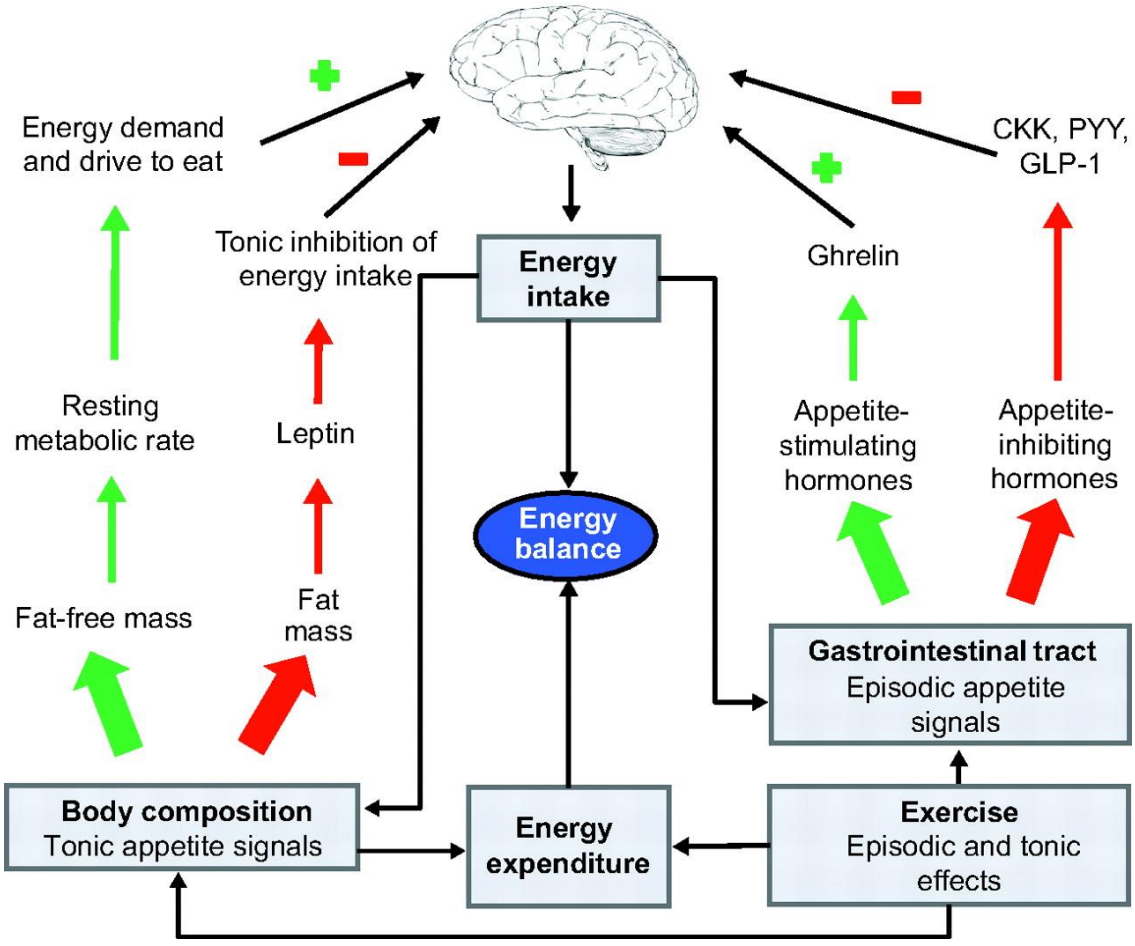


Figure 1.4 Factors that influence appetite control within an energy balance framework, source: Blundell et al. (2012a)

The rising number of adults who are overweight and obese highlights how difficult it is to maintain energy homeostasis in the current obesogenic environment. Theoretically, maintaining a stable body mass, and even reducing body mass, should be straight forward; consume less energy than is expended. However, energy balance is affected by complex biological and behavioural mechanisms that operate asymmetrically to

defend against weight loss whilst permitting weight gain. A new formulation of appetite control provides insight into the underlying mechanisms controlling eating behaviour using an energy balance framework. This approach enables the investigation of factors influencing appetite control that would otherwise be examined in isolation. Because this energy balance formulation provides a framework for considering the effect of PA and sedentary behaviour (SB) on appetite and body composition, it will be used as a framework for the studies in this thesis. It can be considered that exercise and PA could modify appetite directly (through a drive from EE) or indirectly by altering FM and FFM. This formulation therefore provides a way of linking PA, SB and appetite control within an energy balance framework.

Chapter 2 Appetite Control and Energy Balance; the Role of Physical Activity and Sedentary Behaviour

This review provides a background to the work that is going to be presented in this thesis. It will not attempt to be an exhaustive review. Additional information relevant to each study will be included in the introduction to each experimental chapter.

2.1 Physical activity and sedentary behaviour: definition of terms

There is an abundance of evidence to support the beneficial effects of moderate-to-vigorous physical activity (MVPA), as well as the detrimental effect of physical inactivity, on multiple health outcomes including coronary heart disease, cardiovascular disease, type II diabetes, metabolic syndrome, stroke, and some cancers (Lee et al., 2012, Warburton et al., 2006). As a result of such evidence, guidelines have been developed to encourage adequate levels of PA (Department of Health, 2011a). Over the course of a week, adults should aim to achieve at least 150 minutes of MVPA accumulated in bouts of at least 10 minutes. Although PA and exercise are terms that are often used interchangeably, a distinction can be made between the two. PA refers to 'any bodily movement produced by skeletal muscle that results in EE', whereas exercise 'is a subset of PA that is planned, structured, and repetitive and has as a final or an intermediate objective the improvement or maintenance of physical fitness' (Caspersen et al., 1985). All exercise can be referred to as PA, however, not all PA is exercise. A relatively new area of research has emerged focusing on the negative associations of SB with a number of health outcomes (Ekelund et al., 2016, Biswas et al., 2015, Chau et al., 2013, Thorp et al., 2011, Edwardson et al., 2012, Wilmot et al., 2012, Young et al., 2016). The health consequences of SB and physical inactivity are particularly worrying given that adults spend the majority (46% - 72%) of their waking day sedentary (Owen et al., 2014, Henson et al., 2013, Jefferis et al., 2015) and engage in very little PA (British Heart Foundation, 2017). Since the early 2000s SB research has proliferated and this has led to the development of clearer SB definitions. Until recently, SB was considered to be a lack of PA such that a person not achieving the recommended amount of PA was considered to be sedentary; however, individuals not achieving the PA recommendations should be referred to as inactive. There is an emerging consensus that SB (from the Latin sedere, which means 'to sit') is distinct from a lack of MVPA and a new and widely accepted definition has been developed. The Sedentary Behaviour Research Network (2012) propose that SB refers to 'any waking activity

characterized by an energy expenditure ≤1.5 metabolic equivalents while in a sitting or reclining posture'. One metabolic equivalent (MET) is defined as the amount of oxygen consumed while sitting at rest and is equal to 3.5 ml of oxygen per kg body mass per minute or 1 kcal per kg body mass per hour (Ainsworth et al., 2011). Therefore, SB is characterised by low EE and a seated or reclining posture. MVPA is defined as any PA that increases the metabolic rate to >3.0 METs. Developed countries have started to embed recommendations to minimise sitting within their recommendations to increase PA, however, these recommendations remain broad. The UK Start Active, Stay Active report states 'All adults should minimise the amount of time spent being sedentary (sitting) for extended periods' (Department of Health, 2011a). More specific guidance was issued in an expert statement on SB in the workplace. The authors recommended that prolonged SB should be minimised by replacing 2 hours of sitting with standing and light PA eventually progressing to 4 hours (Buckley et al., 2015). However, evidence for the recommended reduction in SB was lacking and was based on the fact that a number of occupations require employees to stand and move for more than 4 hours per day and, therefore, should not pose too many physical of cognitive challenges. These general guidelines and the lack of evidence to support more specific recommendations emphasise the infancy of the field of SB research. Evidence is still accumulating regarding the strength of associations, causality, mechanisms driving the observed associations and the support for dose-response relationships with multiple outcomes (Young et al., 2016). As yet, there is insufficient evidence to determine a threshold for how much SB is too much and a linear, dose response pattern with no identifiable threshold cannot be ruled out. As a result of the distinction between SB, physical inactivity and MVPA, researchers are now studying these behaviours as separate entities with differing health outcomes and determinants. It is important to note that although the associations between SB and poor health outcomes have been shown to be statistically independent of the amount of MVPA a person does (Biswas et al., 2015), research is emerging suggesting the negative health consequences of SB can be off-set if adequate amounts of PA are achieved (Bakrania et al., 2016, Ekelund et al., 2016). Furthermore, despite SB being considered as distinct from MVPA (it is possible for an individual to perform large amounts of SB and MVPA; the 'active couch potato') (Finni et al., 2014, Owen et al., 2010), research also shows that SB and PA are negatively correlated; the more sedentary time, the less PA (Mansoubi et al., 2014, Dempsey et al., 2014). The relationship between PA and SB makes it difficult to disentangle the independent effects of these behaviours on health.

2.2 Measuring free-living physical activity and sedentary behaviour

The gold standard for measuring free-living EE is doubly-labelled water (DLW) (Irwin et al., 2001). As well as total EE, PA level and PA EE can be obtained using this method in conjunction with (indirect calorimetry) IC derived measures of RMR. Although DLW is highly accurate and reliable when measuring average EE over the course of around two weeks (Melanson et al., 1996), it does not provide information on the intensity, duration or pattern of PA and SB performed under free-living conditions. This is an important limitation considering research suggests the way in which SB is accumulated may have different effects on risk biomarkers (Healy et al., 2008a, Healy et al., 2011b) and the importance of intensity of PA for health benefits (Powell et al., 2011). Furthermore, the cost of the DLW method as well as the methodological effort required limit its use mainly to small study groups. Cheaper, practical and accurate measures have been developed in recent years to provide information on frequency, duration, time and type of activities performed during daily life. These include questionnaire based self-report measures of PA and SB as well as objective measurement devices that measure movement using accelerometers (Sylvia et al., 2014).

2.2.1 Self-report questionnaire measures of physical activity and sedentary behaviour

Questionnaire based measures of PA and SB are the most widely used method to quantify free-living movement behaviours, particularly in large-scale epidemiological studies, because they are the most practical and cost-effective method (Dishman et al., 2001). However, a review by Prince et al. (2008) identified the potential for self-report measures of PA to be higher, as well as lower than direct measures of PA. This observation limits the ability to correct for differences in self-report and objective measures of PA. Furthermore, questionnaire measures of PA have limited reliability and validity (Shephard, 2003). Similarly, subjective measures of SB demonstrate moderate reliability and slight to moderate validity (Atkin et al., 2012).

2.2.2 Objective device-based measures of physical activity and sedentary behaviour

2.2.2.1 Validity of the SenseWear armband for estimating energy expenditure and classifying different intensities of activity

The SenseWear Armband (SWA) is a commercially available device that estimates EE, activity intensity, steps and sleep using sensors that detect motion (triaxial accelerometer) and other physiological information (galvanic skin response, skin temperature and heat flux). Using predefined activity intensity cut-points, it is also

possible to estimate the amount of time spent in different intensities of activity from sedentary to vigorous. There have been a number of different models of the SWA since it emerged on the market in the early 2000s (Liden et al., 2002), and there have been a number of updates to both the hardware and software to arrive at the latest model; the SWA mini. The device itself is smaller and contains a triaxial accelerometer as opposed to the biaxial accelerometer used in previous models. The software is periodically updated to improve EE estimates, however, details about the changes to the algorithm are not divulged due to the commercially sensitive nature of the information. A limited number of validation studies have been performed using the latest model and software algorithm in adults but there are a number of studies using previous versions. Therefore, studies included in this review were not limited to the most recent model and algorithm.

The accuracy of the SWA for measuring free-living EE has been examined. Johannsen et al. (2010) compared the SWA Pro 3 and the SWA mini against DLW for measuring total EE over two weeks. Both activity monitors showed good agreement with DLW measured total EE, the SWA Pro and mini under estimating total EE by 112 kcal/d and 22 kcal/d, respectively. The SWA mini provided estimates that were not significantly different to DLW and the two measures had an intraclass correlation (ICC) of 0.85. Both models showed a greater underestimation of EE at higher total EE. A number of other studies have also showed that the SWA provides a valid measure of total EE when compared with DLW, but there is evidence that the SWA overestimated total EE in those with low EE values and underestimated total EE in those with high EE values (St-Onge et al., 2007). When total and PA EE measures from the SWA and widely used predictive equations were compared with DLW, the SWA provided more accurate measures than the predictive equations. For example, the World Health Organisation (WHO) equation for estimating RMR was multiplied by a PA level of 1.6 to estimate total EE and this had an ICC of 0.64 with DLW measured total EE compared with the SWA Pro 2 ICC of 0.81 (St-Onge et al., 2007). Johannsen et al. (2010) assessed the ability of the SWA to measure EE associated with PA by calculating PA EE (total EE – (estimated RMR (WHO equation) + 10% of total EE for TEF)) and found that both models of the SWA tended to under estimate PA EE (123 kcal/d for the Pro 3 and 119 kcal/d for the mini). A similar degree of under estimation (225 kcal/d) was reported by St-Onge et al. (2007) using an earlier version of the SWA (Pro 2). St-Onge et al. (2007) also found that the SWA overestimated RMR compared with IC (ICC 0.77) and may explain why the SWA overestimated total EE in those with low total EE (St-Onge et al., 2007).

DLW can only measure average daily total and PA EE averaged over multiple days and cannot be used to determine the accuracy of the SWA for estimating different activity intensities and the resulting EE. Such studies are usually performed under

laboratory conditions using IC as the criterion measure of intensity and EE. Fruin and Rankin (2004) compared measures of EE from IC with the SWA Pro 2 at rest and during two modes of exercise (walking and cycling). The authors concluded the SWA provided a valid and reliable estimate of resting EE, however, the SWA was not as accurate when estimating EE during stationary cycling and treadmill walking at different speeds and inclines. The SWA performed poorly when estimating EE during stationary cycling with a correlation of r = .03 to .12 and there was a wide range of agreement (-3.8 to 3.5 kcal/min) in Bland-Altman analysis. However, compared with ActiGraph GT1M, the SWA performed favourably when estimating EE during stationary cycling (Herman Hansen et al., 2014). Furthermore, the SWA overestimated EE of walking on a horizontal treadmill (by 14% to 38%) and underestimated EE whilst walking on a 5% incline (by 22%). A strength of the multi-sensor SWA device compared with accelerometers alone is the ability to detect physiological parameters such as heat and sweating associated with PA. As a result the SWA was able to accurately estimate the EE in the recovery period shortly after treadmill walking. The SWA also provided accurate and reliable estimates of EE at rest (Malavolti et al., 2007, Fruin and Rankin, 2004).

The proprietary algorithm within the accompanying SenseWear software has undergone a number of iterations since the version used in the study by Fruin and Rankin (2004) in an attempt to improve estimates of EE during different activities. Recently, the validity of the latest SWA model (SWA mini) and algorithm (v5.2) to estimate EE and PA during different activity routines was assessed. Using IC as the criterion measure of EE, Bhammar et al. (2016) compared EE estimates from the SWA during structured and unstructured routines in a laboratory setting comprised of activities ranging from sedentary to vigorous. During the semi-structured routine (designed to mimic free-living activities) the SWA total EE estimate was not significantly different to IC. However, the SWA over and under estimated EE associated with the different activities. For example, the EE associated with cycling was underestimated whereas the EE associated with treadmill running was overestimated. This is contrary to a study by Drenowatz et al. (2011) who concluded the SWA (Pro 2) underestimated EE during treadmill running compared to IC, however, the intensity of running was higher in the latter study and the authors reported a ‘ceiling effect’ of EE estimates from the SWA at an intensity >10 METs. Bhammar et al. (2016) also reported the SWA misclassified activity intensity, generally underestimating time spent in light activities and overestimating time spent in moderate activities. This was due to the consistent overestimation of activities such as sweeping and loading/unloading boxes. The authors did not use measured values of resting EE to calculate MET values specific to each participant and instead assigned a general MET value for activities based on the compendium of physical activities.

Indeed, it is possible that if the MET value of activities was calculated relative to each participants measured RMR the activities in the routine might have been higher, as measured resting EE may produce a MET value closer to 2.6 ml/kg/min compared with the 3.5 ml/kg/min used in the compendium of physical activities estimate (Byrne et al., 2005). The difference in the energy cost of different activities when calculated using standardised MET equations compared with METs calculated from measured RMR have been examined (Mansoubi et al., 2015). The authors reported differences in the energy cost of activities when calculated using the standardised and individualized equations. For example, walking on a treadmill at 1.2 mph produced a MET value of 2.83 for the standardised equation and 3.03 METs for the individualised equation. The MET value from the standardised equation would classify the activity as light whereas the individualized equation would result in the activity being classified as moderate. A better agreement between the SWA and IC measures in the study by Bhammar et al. (2016) would have occurred if the true intensity classification of the activities had resulted in higher MET values as was observed by (Mansoubi et al., 2015). Although the SWA has limitations when assessing activity intensity and EE associated with different activities, it has been shown to provide more accurate estimates of EE during light to moderate intensity semi-structured activities compared with other activity monitors. Calabro et al. (2014) compared the SWA (mini and Pro 3), Actiheart, Actigraph (GT3X) and activPAL (uniaxial) estimates of EE during light intensity semi-structured activities with IC. The SWA mini provided the most accurate measure of total EE (within 1% of criterion measure) during the semi structured routine with a correlation with IC of r = .89. Absolute agreement between the SWA and IC when estimating EE during sedentary, light and moderate activities exceeded 85%. The SWA also performed better than the other activity monitors when classifying time spent in different activity intensities with a kappa value of 0.88 for SB, 0.69 for light PA and 0.74 for moderate PA . Berntsen et al. (2010) determined whether time spent in moderate-to-vigorous PA (MVPA) differed between the SWA (Pro 2) and IC whilst performing various activities lasting 120 minutes (lifestyle and sporting activities such as strength and conditioning exercises, ball games, home repair, occupational and home activities; the intensity of these activities was not limited). The SWA overestimated time in MVPA by 2.9% and the mean limits of agreement from Bland-Altman plots was 1.1 min (SD = 49.9). Although the estimation of time in MVPA is somewhat less precise than the estimate of total EE from the SWA, it presents a more accurate measure than questionnaire alternatives and performs better than other activity monitors.

Taken together, these studies demonstrate the SWA provides an accurate and valid measure of total EE under free-living conditions and EE at rest when compared with DLW and IC. The SWA EE measure appears to be more accurate in those with

moderate levels of total EE but is less accurate for extreme levels, either high or low. The SWA provides more accurate estimates of PA EE compared with widely used predictive equations, however, it tends to underestimate PA EE compared with DLW and IC. It is therefore appropriate to be cautious when interpreting SWA measures of PA EE. The estimation of EE during different intensities of activities is somewhat less precise than measures of total EE with evidence of underestimation of EE at high intensities and overestimation at low intensities. Similarly, the SWA has been shown to misclassify intensities of activities by underestimating time in light PA and overestimating time in moderate PA. Despite some small limitations, the SWA performs better than other activity monitors when measuring free-living PA. Although the SWA has been shown to accurately estimate time spent sedentary (Calabró et al., 2014) it may also classify standing motionless as sedentary as the device does not detect posture (Reece et al., 2015). The next section will examine the AP and its ability to detect posture.

2.2.2.2 Validity of the activPAL for measuring posture

Over the past 15 years, there has been an increased scientific interest in SB (Owen et al., 2010, Tremblay et al., 2010). A number of devices have been developed to objectively quantify SB and have helped improve our understanding of the impact of SB on health. The majority of studies have used accelerometer based devices which infer sedentary time from a lack of movement (Tremblay et al., 2010). However, this can lead to the misclassification of some light intensity activities as SB (Kozey-Keadle et al., 2012). For example, standing motionless may be categorised as sedentary using an accelerometer because of the lack of movement. To overcome this issue, activity monitors have been developed to directly measure posture. The activPAL (AP) is a device specifically designed to detect posture. Via proprietary algorithms, accelerometer derived information about thigh position and acceleration is used to determine posture (sitting/lying or standing), transitions between postures, stepping and stepping speed, from which EE is estimated. There are a number of different models of the AP (activPAL^{TM}, activPAL3^{TM}, and activPAL3^{TM} micro), the most notable difference being the upgrade from a uniaxial accelerometer to a triaxial accelerometer. A limited number of validation studies have been performed using the latest model (activPAL3^{TM} micro) but there are a number of validation studies using previous versions. Therefore, studies included in this review were not limited to the most recent model. As the AP was designed to assess posture and the SWA has been shown to provide more accurate estimates of EE (Calabró et al., 2014), the AP EE estimate will not be extensively reviewed as it will not be used as an output in this thesis. Briefly, the AP has been shown to significantly underestimate EE compared with IC by 22.2% during 60 minutes of semi-structured activities (Calabró et al., 2014). Furthermore, the

AP produced significantly different MET values compared with IC during different treadmill walking speeds with an ICC of 0.57 (Harrington et al., 2011).

The AP performs considerably better when classifying posture and has been used as the criterion measure to assess the validity of other activity monitors (Pavey et al., 2016, Rowlands et al., 2013). Validation studies are predominantly performed in a laboratory setting and involve participants performing a range of activities from sitting quietly to walking on a treadmill, as well as activities representative of active daily living tasks. Outputs such as postural allocation, number of steps and stepping speed from the AP are then compared with the criterion measure, either direct or video observation. Grant et al. (2006) evaluated the AP (activPAL^{TM}, uniaxial) as a measure of posture and motion during structured activities and randomly assigned everyday tasks incorporating sitting, standing and stepping. The AP measure of transitions was identical to direct observation and the percentage difference for time spent sitting and upright (standing and stepping) was less than 0.3% during the structured and active daily living activities. The percentage difference for total time spent standing and the total time spent walking were 1.4% and 2%, respectively. During active daily living tasks the agreement between AP and direct observation was excellent for sitting (0.3%) and upright postures (-0.6%), however, agreement was lower for standing (3.7%) and walking (-3.6%). The AP performed poorly when identifying breaks in walking which led to the overestimation of walking and underestimation of standing. A limitation of this study was the short observation period (34-47 minutes).

A more recent study by Kozey-Keadle et al. (2011) examined the accuracy of the AP (activPAL^{TM}, uniaxial) when identifying sedentary time (defined by a sitting/lying posture) compared with direct observation during a 6 hour period under free-living conditions in office workers. Compared with direct observation, the AP underestimated sedentary time by 7.7 minutes (2.8%) on average and was more accurate than the ActiGraph (GT3X) which underestimated sedentary time by 16.9 minutes (4.9%). Furthermore, the AP was sensitive to reductions in sedentary time resulting from advice to reduce sitting and increase standing, whereas the ActiGraph and multiple SB questionnaires were not (Kozey-Keadle et al., 2012, Kozey-Keadle et al., 2011). Kim et al. (2015) assessed the validity of the AP (activPAL3^{TM}, triaxial) for assessing SB under free-living conditions when compared with a proxy measure of direct observation (automated camera triggered by changes in behaviour from or to sedentary activities; SenseCam). During a 6 hour monitoring period the AP underestimated sedentary time by 10.7 minutes (3.5%) which is similar to the underestimation observed by Kozey-Keadle et al. (2011) and was more accurate than the ActiGraph (GT3X) evidenced by a mean absolute percentage error of 4.1% for the AP and 7.3% for the ActiGraph.

The accuracy of the AP (activPAL^{TM}) to estimate steps and cadence during treadmill and outdoor walking at different speeds compared with video observation was assessed by Ryan et al. (2006). There was excellent agreement between the AP and observation for all walking speeds both on the treadmill (0.90, 1.12, 1.33, 1.56, and 1.78 m/s) and outdoor (1.38 ± 0.12, 1.65 ± 0.12, and 1.84 ± 0.14 m/s) with an overall percent error of less than 1% for number of steps and less than 1.2% for cadence. This study demonstrates the AP provides an accurate estimate of steps and stepping speed during a range of walking speeds. However, when assessing the validity of the AP (activPAL3^{TM}) to detect steps at very low walking speeds, Stansfield et al. (2015) found that 90% of steps were detected at walking speeds of >0.5 m/s and a cadence of >69 steps/min. However, below these limits the percentage of steps detected reduced rapidly with zero steps detected at 0.1 m/s and at or below 24 steps/min.

These studies demonstrate the AP provides a more accurate estimate of sedentary time (defined by a sitting/lying posture) compared with questionnaire measures and other activity monitors. Furthermore, the AP provides an accurate measure of posture under free-living and laboratory conditions. Although the AP is unable to accurately detect steps at very low walking speeds, it has been shown to accurately detect steps and stepping speeds through a range of walking speeds more representative of those observed during free-living conditions in a healthy population (Öberg et al., 1993).

2.2.2.3 Sedentary time accumulation

In addition to total sedentary time, the way sedentary time is accumulated may impact on health outcomes. More breaks in sedentary time have been shown to be beneficially associated with metabolic biomarkers independent of total sedentary time, MVPA and mean intensity of activity. Healy et al. (2008a) found that a higher number of breaks in sedentary time (defined by a rise in accelerometer derived counts/min to >100) was beneficially associated with waist circumference, body mass index, triglycerides, and 2 hour plasma glucose. Therefore, it is important to determine not only total sedentary time but also how that time is accumulated. There are number of different methods reported within the literature to determine sedentary time accumulation. These include total number of breaks in sedentary time determined by a rise in counts per minute above a specific threshold, a ratio of the number of sedentary bouts divided by total sedentary time, and classification of bout lengths and summing the number of minutes accumulated within each bout (Healy et al., 2008a, Healy et al., 2011a, Chastin et al., 2012, Gabel et al., 2015, Saunders et al., 2013, Dowd et al., 2012).The AP proprietary software provides an indicator of sedentary time accumulation in the form of transitions; the number of sit-to-stand and stand-to-sit movements. However, the SWA proprietary software does not provide information on sedentary time accumulation. A Microsoft Excel template containing formulae was

developed to identify bouts of SB ranging from 1-5 minutes to >40 minutes using data from the SWA and the AP. Further information can be found in Chapter 6.

In order to assess the optimal procedure for measuring SB, Chapter 6Chapter 7 in this thesis will compare and integrate data from the SWA and AP. Accurate measurement of both PA and SB is central to the internal validity of the research reported in this thesis. The objective measurement of sedentary and active behaviours using the SWA and AP will shed light on the relationships among these behaviours and components of appetite control and energy balance.

2.3 The myth of physical inactivity and obesity?

2.3.1 Structured exercise and weight loss

There is considerable ambiguity regarding the effectiveness of exercise for weight management. This is not surprising considering the unhelpful messages portrayed in the media with eye catching headlines such as 'Why exercise won't make you thin' (Time Magazine, 2009) and 'How exercise can make you pile on the pounds' (Daily Mail, 2015). The latter headline emanated from the damaging editorial by Malhotra et al. (2015) who refer to 'the myth of physical inactivity and obesity'. These unhelpful messages from the main stream media and academics alike wrongly reinforce the public's preference to avoid exercise by suggesting exercise is futile for weight loss. They condone the largely sedentary lifestyle which is prevalent in most technologically developed countries. This is particularly damaging as there is good evidence that exercise, when carried out over long periods of time, does in fact produce weight loss (Donnelly et al., 2003, Jakicic et al., 2008). There is a dose-response effect; the more exercise carried out, the greater the weight loss. Furthermore, several reviews (Ballor and Keesey, 1991, Catenacci and Wyatt, 2007, Swift et al., 2014), including a Cochrane review by Shaw et al. (2006), also support the beneficial effect of exercise on weight independent of diet.

2.3.2 Impact of free-living physical activity and sedentary behaviour on adiposity

The relationship between free-living sedentary and active behaviours and weight status has received greater attention particularly since the development of objective PA measurement devices. PA impacts on energy balance through multiple pathways, including increased total EE (Plasqui et al., 2013), improved appetite control (Hopkins and Blundell, 2016, Shook et al., 2015), and there is also evidence to suggest PA has a positive influence on RMR, perhaps due to greater FFM (Speakman and Selman, 2003). On the other hand, a negative association between SB and weight has been reported, however, this relationship is less consistent and questionnaires are often

used to quantify sedentary time (Biddle et al., 2010). Furthermore, TV viewing is often used as a proxy of SB, but TV viewing has been shown to only correlate weakly with overall sedentary time when measured using accelerometers. It has been suggested that SB impacts on weight status by displacing MVPA (Mansoubi et al., 2014) and by altering EI, for example, TV viewing has been associated with increased EI and snacking (Bowman, 2006). However, this association may not be due to SB *per se* and could be a result of exposure to food related advertisements (Scully et al., 2009).

Since the development of objective measurement devices, large scale observational and prospective studies have begun to quantify PA and SB using accelerometer based activity monitors. One such study is the National Health and Nutrition Examination Survey (NHANES) in America, which examined the independent and combined associations of PA and SB with obesity. Between 2003 and 2006 Maher et al. (2013) collected PA and SB data for 5,546 adults using accelerometers (ActiGraph 7164) and TV viewing time was assessed with a questionnaire. Stature and weight were measured by trained health technicians during a physical examination using standardised procedures and BMI was calculated from stature and weight. All analyses were controlled for potential confounders such as age, ethnicity, EI, alcohol intake and smoking status. Low MVPA was consistently associated with higher risk of obesity regardless of the amount of SB (determined by both accelerometry and TV viewing questionnaire). A similar relationship has been reported when PA and SB were measured using questionnaires (Sugiyama et al., 2008). The relationship between SB and obesity varied depending on the way in which SB was measured. In men, higher TV viewing was associated with greater risk of obesity but there was no relationship in women. A positive association between TV viewing and adiposity (BMI and waist circumference) has previously been reported in another large scale national survey (Heinonen et al., 2013). Accelerometer derived SB was not associated with obesity in men or women. There was a greater risk of obesity when low MVPA was combined with high TV time compared with risk of obesity associated with low MVPA or high TV time alone. Interestingly, Healy et al. (2011b) reported a positive association between accelerometer measured SB and obesity in the same sample of participants. However, Healy et al. (2012b) used waist circumference (WC) as a measure of adiposity compared to the use of BMI in the study by Maher et al. (2013). These studies demonstrate the impact that measurement method (for both SB and adiposity) can have on the reported relationship between SB and obesity. In the same sample of participants, two dissimilar conclusions were drawn; one supporting a positive association between SB and obesity and the other showing no relationship.

In another study, 878 participants from two diabetes prevention programmes in the UK had their PA and SB measured objectively (ActiGraph GT3X) (Henson et al., 2013). There was a positive relationship between total sedentary time and indices of adiposity

and the opposite was true for MVPA and adiposity. After statistically controlling for time spent in MVPA, the relationship between SB and adiposity was no longer significant. However, the relationship between MVPA and adiposity remained after controlling for SB. Similar relationships were reported in breast cancer survivors using data from the NHANES survey (Lynch et al., 2010) and in the International Physical activity and the Environment Network (IPEN) study (Van Dyck et al., 2015). Interestingly, Healy et al. (2008c) reported the opposite in a sub-sample of the AusDiab 2005 cohort. The relationship between SB and WC was independent of MVPA, but the relationship between MVPA and adiposity was no longer significant after controlling for SB. A possible explanation could be the very low levels of MVPA in the study by Healy et al. compared to the other two studies.

MVPA is consistently beneficially associated with indices of adiposity (Healy et al., 2008c, Lynch et al., 2010, Murabito et al., 2015, Van Dyck et al., 2015). However, the relationship between SB and indices of adiposity is less consistent with some studies reporting a positive association with adiposity (Healy et al., 2008c, Lynch et al., 2010) and others reporting no relationship (McGuire and Ross, 2012, Smith et al., 2014, Van Dyck et al., 2015, Murabito et al., 2015). The inconsistent relationship between adiposity and SB could be due to the way in which SB is operationally defined and measured. For example, when SB is defined by posture (AP) there is no association with either BMI or total adiposity (Smith et al., 2014). However, when SB is defined by activity intensity (SWA) there is a relationship with both BMI and adiposity (Scheers et al., 2012, Shook et al., 2015). Whether the relationships between SB and adiposity depends on the way SB is defined and measured requires further investigation. Furthermore, whether the relationships among PA, SB and adiposity remain after statistically controlling for other intensities of activity remains equivocal and requires further examination.

Prospective cohort studies have examined the change in PA, SB and adiposity over time, using statistical models to examine whether change in sedentary and active behaviours predicts change in adiposity and whether change in adiposity predicts change in behaviour. Shook et al. (2015) found that those with low levels of PA at baseline gained the most FM over 12 months. Golubic et al. (2014) showed that MVPA and sedentary time both significantly predicted weight gain over 1 and 7 years. For example, a 1.5 hour reduction in sedentary time and a 16 minute increase in MVPA per day were associated with a 1.4 kg and 0.5 kg reduction in body mass over 1 year, respectively. Furthermore, when MVPA and SB were modelled as the outcome variable and indices of adiposity the exposure variable there was a three times greater inverse association between adiposity and MVPA compared with when MVPA was the exposure variable. The magnitude of the relationship between adiposity and SB remained the same as when SB was the exposure. These findings suggest adiposity is

an important determinant of decreased PA as well as increased adiposity being a consequence of decreased MVPA. Interestingly, Ekelund et al. (2008) found that sedentary time was not a significant predictor of indices of adiposity, but rather indices of adiposity predicted sedentary time over a period of 5.6 years.

These cross-sectional and prospective cohort studies suggest in general that SB is associated with higher adiposity and MVPA is associated with lower adiposity, although the former relationship is less consistent. Furthermore, the relationship between MVPA and weight status appears to be independent of SB, whereas controlling for MVPA in the relationship between SB and adiposity often nullifies the association. Prospective cohort studies suggest a bidirectional relationship can account for the link between sedentary and active behaviours and weight status; low levels of PA and high SB will favour weight gain. In turn, greater adiposity will lead to lower MVPA and higher SB. However, the evidence for this bidirectional relationship is speculative and is based on observational studies. Controlled trials provide evidence for the beneficial effects of exercise on weight, however, the same level of evidence does not exist for SB. In order to address the issue of causality, randomised controlled trials would need to be undertaken where SB is manipulated and change in adiposity is measured. However, such trials are unlikely due to the ethical issues related to an enforced increase in SB for long durations.

2.4 Relationship between energy intake and energy expenditure

Researchers have been interested in the relationship between EI and EE for over 60 years. Studies examining this relationship were noted as early as the 1950s. Interest was sparked in this area due to '*a desire to find out more about the mechanisms which relate intake to expenditure-what regulates appetite, in fact'* (Edholm et al., 1955, p.286). Edholm and colleagues measured the EI from meals and snacks and the EE from various physical activities including military duties and sports in a series of studies carried out on army cadets (Edholm et al., 1955, Edholm et al., 1970, Edholm, 1977). The authors found no relationship between EI and EE over the course of a single day but there was a strong relationship when daily EI and EE were averaged over the course of a week. The authors also observed a positive relationship between daily body mass change and daily EI and a negative relationship with EE demonstrating the importance of energy balance for body mass regulation (Edholm et al., 1970). Subsequent studies have also found a positive relationship between EI and EE (Caudwell et al., 2013a, Weise et al., 2014). In keeping with the work of Edholm et al., in a landmark study by Mayer et al. (1956) jute mill workers were categorised based on the physical demand of their jobs and the dietary intake of individual workers

was calculated. There was a positive linear relationship between EI and EE in those with physically active occupations. At moderate and high levels of occupation related PA, EI matches EE, however, at lower levels of occupation related PA, EI exceeds EE. Furthermore, those with inactive occupations, involving large volumes of sedentary time, exhibited a greater body mass compared to those with more active occupations. However, the lack of body composition measures precludes any conclusions regarded the relationship between FM, FFM and EI. These data suggest the reciprocal relationship between EI and EE only operates above a certain level of PA (see Figure 2.1). Below that critical level it appears appetite control is lost and EI no longer operates in the interest of energy balance. The jobs on the left hand side of the x axis in Figure 2.1 would have involved low levels of MVPA and large amounts of SB resulting in low EE. This observation has considerable implications for our current sedentary lifestyle.

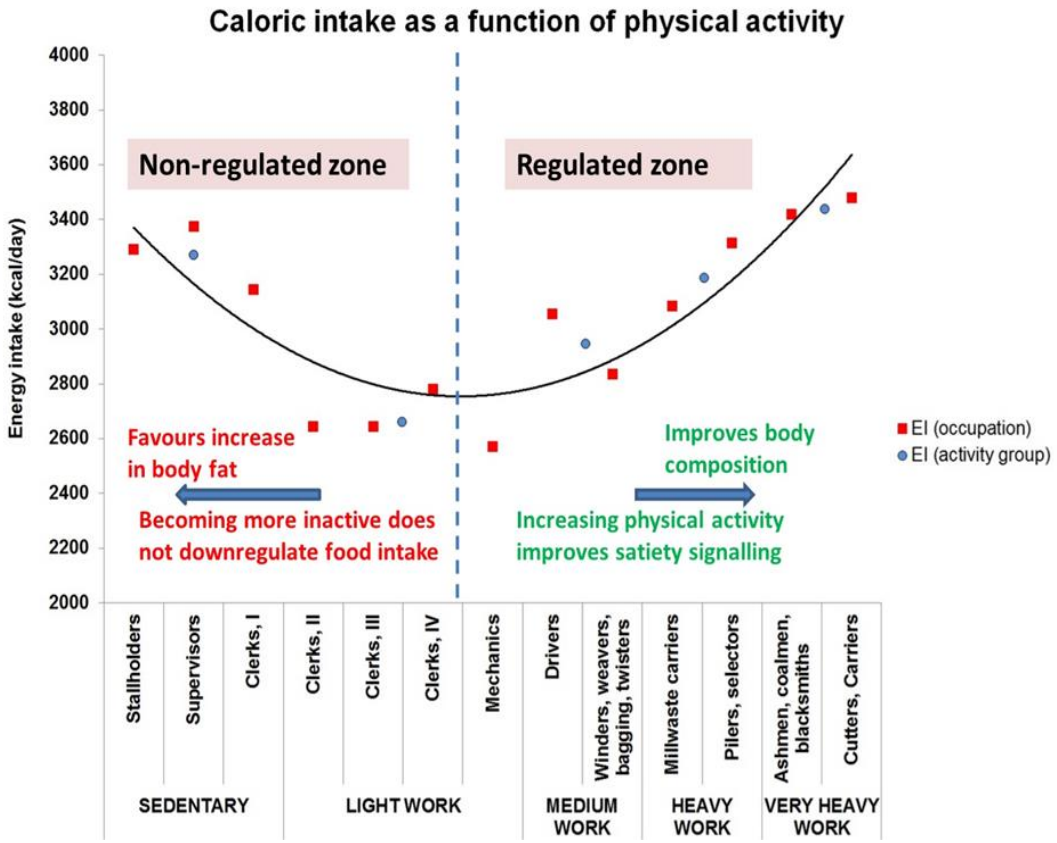


Figure 2.1 The 'Mayer curve' (1956) adapted by Blundell (2011) which illustrates the proposed relationship between EI and EE of jute mill workers. The figure has been adapted to include further interpretation based on more contemporary research

When examining the relationship between EI and EE it is important to make the distinction between behavioural (discretionary PA) and metabolic (FFM and RMR) components of total daily EE. It has been postulated that whilst metabolic components

of EE are related to the tonic drive to eat, the behavioural components of EE act on the satiety element of appetite control, modulating the orexigenic drive to eat (Blundell et al., 2015b).

2.5 Fat-free mass as a driver of energy intake

The role of adipose tissue in the regulation of EI has dominated the field of appetite control, particularly since the discovery of leptin and its relationship with FM (Zhang et al., 1994). Until recently, the extent to which FFM influences EI has been overlooked, despite Lissner et al. (1989) reporting a positive association between FFM, but not FM, and laboratory measured EI in normal weight and overweight women more than 25 years ago. The relationship between FFM and EI has gained attention since Blundell et al. (2012b) found FFM, but not FM or BMI, was positively associated with self-selected meal size and EI in overweight and obese participants. Subsequent studies have also demonstrated a positive association between FFM, but not FM, and EI and between RMR and EI (Caudwell et al., 2013a, Weise et al., 2014). Furthermore, studies have reported RMR and FFM are positively associated and FM negatively associated with fasting hunger (King et al., 2017, Blundell et al., 2015a). FFM is comprised of metabolically active organs and tissues and contributes substantially to RMR (60-70%) (Johnstone et al., 2005) and RMR comprises the majority of total daily EE (50-70%) (Shetty, 2005, Goran, 2000). It has been suggested that RMR acts as a mediating variable in the relationship between FFM and EI and this was recently demonstrated using path analysis whereby the effects of FFM on EI were fully mediated through its effect on RMR (Hopkins et al., 2016).

The relationship between FFM and EI provides one possible explanation as to why overweight and obese individuals tend to eat more than their lean counterparts. The development of obesity is accompanied, not only by an increase in FM (70-80%), but also an increase in FFM (20-30%) (Webster et al., 1984). This increase in FFM will lead to an increase in EI to match the higher energy requirement associated with greater FFM until a new and higher energy balance is achieved. This has been referred to as the passive role of FFM on EI (Dulloo et al., 2016). On the other hand, Dulloo et al. (2016) have also proposed a more active role of FFM in the regulation of EI. Dulloo et al. (1997) reported that both FM and FFM losses independently predicted the post-starvation hyperphagic response. Importantly, despite the full restoration of body mass and FM, the hyperphagiac response continued until pre-starvation FFM levels were fully restored. FFM loss may also occur in response to physical inactivity due to muscle disuse. This assertion is supported by studies demonstrating relatively short periods of bed rest (<7 days) can lead to loss of skeletal muscle and lean body

mass (Dirks et al., 2016). It is possible that the uncoupling of EI to EE at low levels of PA described previously could be attributed to a compensatory increase in food intake to defend against further FFM loss and promote the restoration of FFM (with a concomitant increase in FM). A second plausible explanation for the dysregulated appetite control in inactive individuals is the difference in body composition and sensitivity to tonic inhibitory appetite signals (Hopkins and Blundell, 2016). As FM accumulates, the tonic inhibitory effect of fat on EI becomes weaker due to insulin and leptin resistance. This results in a mismatch between the tonic inhibition of food intake arising from FM and the excitatory drive to eat arising from FFM further promoting over consumption and appetite dysregulation.

There is evidence to support the existence of a feedback signal that informs the brain about the state of the body's FFM to bring about a compensatory increase in EI to defend against FFM loss or to restore depleted FFM levels. These non-adipostatic signals may interact with feedback signals arising from adipose tissue (such as leptin) to regulate EI and body mass. The molecular signals and mechanisms linking energy requirements (FFM and RMR) with appetite and EI are not well understood and represent a target for further investigation.

2.6 Physical activity and appetite control

Not only does PA impact on energy balance directly by increasing EE, there is accumulating evidence that PA also impacts on appetite control and EI (Blundell et al., 2015b). Acute studies (single day) demonstrate that exercise has a transient effect on appetite and there is no compensatory increase in EI to compensate for the energy expended through exercise. Broom et al. (2007) demonstrated that a single 60 minute bout of treadmill running at 72% ± 2.0 of maximal oxygen uptake suppressed acylated ghrelin and hunger during the exercise but did not differ significantly post exercise compared with the resting control condition. Similarly, King et al. (2010a) found that 90 minutes of treadmill running at 68.8% ± 0.3 of maximal oxygen uptake suppressed acylated ghrelin and hunger during and immediately after the exercise but did not differ compared with the resting control condition during the 22.5 hours after exercise. The suppression of hunger during and immediately after (15 minutes) acute exercise was reported in the early 1990s and was referred to as 'exercise-induced anorexia' (King et al., 1994). EI did not differ between conditions at any of the four ad libitum meals despite an energy deficit of 1273 ± 45 kcal. Brisk walking has also been shown to result in an energy deficit as there was no increase in EI to compensate for the energy expended through walking (King et al., 2010b). Pooled analysis of 17 studies also demonstrated acute exercise transiently supresses hunger and acylated ghrelin during exercise and has no effect on EI (King et al., 2017). There is some evidence that the

suppression of hunger and acylated ghrelin remains significant immediately post-exercise and persists for several hours post-exercise, but this requires further investigation (Broom et al., 2017, King et al., 2017, Broom et al., 2009). When the exercise is continued over several days, EI begins to rise to account for approximately 30% (on average) of the energy expended through exercise (Whybrow et al., 2008, Stubbs et al., 2002b).

Interestingly, medium-term exercise interventions have demonstrated large inter-individual differences in weight loss in response to increased exercise (King et al., 2008). Participants performed supervised exercise at 70% heart rate (HR) maximum, individually prescribed to expend 500 kcal per session five times per week for 12 weeks. Those who lost less weight than expected (compensators) showed an increase in EI (268.2 kcal/d ± 455) and hunger during post-intervention probe days, whereas those who achieved the expected weight loss (non compensators) showed a decrease in EI (130.0 kcal/d ± 485) and no change in subjective appetite sensations. In a similar study, King et al. (2009a) found that in response to the same 12 week exercise regimen previously described, fasting hunger increased in those who experienced modest weight loss, but not in those who achieved expected weight loss. In addition the effect of a personalised fixed-breakfast on satiety was improved in both groups suggesting an enhancement of satiety signalling. This has been termed the 'duel-process' action of exercise on appetite control and is characterised by an increased overall drive to eat and a concomitant increase in the satiating efficiency of a fixed meal. These studies demonstrate the effect of exercise on weight loss varies substantially between individuals. This variability is, in part, due to changes in the drive to eat and subsequent EI.

Observational studies have also examined the effects of habitual PA on appetite control. In line with the early work of Mayer et al. (1956) who demonstrated a curvilinear relationship between EI and EE, more recent research has also identified an apparent uncoupling of EI to EE at low levels of PA. Harrington et al. (2013) reported differences in ad libitum EI at a buffet style meal for men (but not women) across tertiles of DLW measured PA EE. Men in the high, middle and low PA EE tertile consumed 1365 kcal ± 101, 866 kcal ± 104 and 1090 kcal ± 101, respectively. Only the difference between middle and high tertiles reached statistical significance, however, there was a trend towards significance between the middle and low tertiles. Furthermore, men in the low PA EE tertile exhibited a significantly greater drive to eat in the fasted state (appetite sensations) compared to the high tertile, an effect which could be driving the uncoupling of EI and EE at low levels of PA. In a more recent study, Shook et al. (2015) grouped individuals by PA level on the basis of quintiles of MVPA measured using the SWA (mini). Twenty-four hour dietary recalls were administered on three random occasion during a two week period, however, this data

was not used in analyses due to potential under reporting and instead EI was estimated using an equation based on change in body composition over a three month period. The authors observed a 'j-shaped' relationship between PA group and EI; EI increased with increased PA with the exception of the least active group. The least active group had a higher EI than group two and three, however, these differences were not statistically significant. The Disinhibition factor of the Three Factor Eating Questionnaire (TFEQ) was significantly higher in the lowest activity group compared to all other groups. Furthermore, there was a negative linear relationship between activity category and body mass and FM with those in the lowest activity category exhibiting the highest body mass and FM. The greater FM in the lowest activity category could explain the significantly higher Disinhibition score compared with other activity categories since FM is positively associated with Disinhibition (Lawson et al., 1995, Hays et al., 2002). Finally, Long et al. (2002) examined the effects of PA status (self-report) on appetite sensations and EI following a dietary pre load. Physically active individuals (two or more >40 minute exercise sessions per week) had lower subjective hunger sensations compared with inactive males and were shown to have more sensitive appetite control. Following a high energy dense preload (600.2 kcal) physically inactive individuals (one or less >40 minute exercise session) failed to compensate by reducing their EI at a subsequent ad libitum buffet meal whereas those who were physically active reduced their EI to account for 90% of the preload. These studies suggest exercise and PA play an important role in appetite control and energy balance.

2.7 Individual variability in weight loss and compensatory responses to perturbation in energy balance

Body mass change is related to an imbalance between EI and EE. If EI exceeds EE, weight gain will occur and if EE exceeds EI, weight loss will occur. This equations appears very simple but is in fact complex (Hall et al., 2011). The depiction of energy balance as a set of kitchen scales is inaccurate and misleading. Perturbations in energy balance can be induced through dietary restriction (reduced EI) or an increase in PA (increased EE). Large individual variability and less than expected weight loss has been reported in response to both exercise (Thomas et al., 2012, King et al., 2008) and diet interventions (Camps et al., 2013). The effectiveness of a weight loss intervention is largely dependent on adherence to the diet or exercise regime. However, even when compliance is accounted for weight loss is less than expected and highly variable between individuals. For example, King et al. (2008) reported weight change ranged from -14.7 to +1.7 kg in response to a 12 week supervised and monitored exercise intervention. This variability can be attributed to metabolic and behavioural compensatory responses that act to restore energy balance. It has been

noted that body mass regulation is asymmetrical; a positive energy balance and weight gain are permitted whilst a negative energy balance and weight loss are strongly defended against (Blundell and Gillett, 2001). The current obesity epidemic supports this notion. Compensatory responses that defend against a negative energy balance include increased EI and reduced NEPA (behavioural) and reduced FFM and RMR (metabolic) (King et al., 2007, Stiegler and Cunliffe, 2006). Behavioural adaptations can be further categorised as either automatic (occur passively, without any deliberate intent; e.g. reduced spontaneous activity/increased sitting) or volitional (overt behaviour over which the individual can exert a choice; e.g. increased EI). Together, the metabolic and behavioural compensatory responses compromise the effectiveness of weight loss interventions. The intensity of these compensatory responses vary between individuals and go some way to explaining why some individuals experience less than expected weight loss. Individualised interventions targeting these compensatory adaptations could lead to more successful weight loss outcomes.

2.8 Summary

As noted previously, this review section has described research related to the main focus of studies in this thesis. Advances in motion sensing technology have made the objective measurement of free-living PA and SB more affordable and accessible. In turn, this has shed light on the relationship between MVPA, SB and energy balance; there is consistent evidence for a negative relationship between free-living MVPA and adiposity, however, the relationship between SB and weight status is less consistent and requires further investigation (and will be examined in this thesis). Objective activity monitors provide an opportunity to further explore the relationship between EI and EE. Good evidence exists to support the role of FFM and RMR (metabolic) as drivers of EI, but the relationship between other components of total EE, such as PA EE (behavioural), and EI are not well characterised. Observational studies suggest that those who are more physically active are better able to match EI to EE, perhaps due to increased sensitivity of the appetite control system. On the other hand, those who are less physically active consume calories in excess of their energy needs and it has been suggested that appetite signals go awry in inactive individuals. Physical inactivity and SB are risk factors for overconsumption and further weight gain. This issue will be investigated later. Furthermore, the greater adiposity associated with inactivity and SB could also be contributing to the mismatch between EI and EE as a result of insulin and leptin resistance. Prescribed and monitored supervised exercise brings about significant improvements in body composition (increased FFM, decreased FM), however, there is large individual variability in weight loss response reflecting individual differences in behavioural and metabolic compensation. Becoming

more physically active through structured exercise increases the drive to eat but also improves post-prandial satiety signalling. The strength of these processes are not the same between individuals who take up exercise and this may contribute to whether individuals lose or maintain weight through energy compensation. A key issue is whether or not free-living PA and SB is adjusted to compensate for loss of body mass induced by either obligatory exercise or a diet regime. It may be surmised that reduced NEPA is another compensatory mechanism that closes the energy balance gap generated by both exercise and diet interventions. Objective PA monitors make it possible to explore the effects of perturbations in energy balance on NEPA. The studies discussed in this review highlight the complexity of the relationship between EI and EE. This thesis will examine the associations among free-living PA and SB, adiposity and appetite control within an energy balance framework.

Chapter 3
Aims and Objectives

3.1 General aims:

A fundamental component of this thesis is to develop a research platform to quantify free-living sedentary and active behaviours in order to investigate the associations among free-living sedentary and active behaviours, appetite control and body composition. The primary aim is to establish the relationship of SB and MVPA to adiposity. The secondary aim is to evaluate how PA and SB may change after exercise induced or diet induced weight loss. To assess the role of SB and PA in weight loss and weight maintenance the research platform was embedded within medium term intervention studies investigating the effects of i) supervised exercise and ii) dietary manipulation on energy balance, appetite control and free-living sedentary and active behaviours. This thesis will examine the relationship between objectively measured sedentary and active behaviours and appetite control within an energy balance framework. This work will inform the conceptualisation of a theoretical framework to describe the relationship between free-living sedentary and active behaviours and appetite control.

3.2 Specific objectives:

- To determine the relationship of objectively measured free-living PA and SB to body composition and eating behaviour traits **(Study 1)**
- To develop a novel integrative procedure to combine information from two validated activity monitors to obtain a measure of SB based on activity intensity (SWA) and posture (AP) **(Study 2)**
- To examine the relationship of different measures of SB (EE or posture) to adiposity **(Study 3)**
- To determine the relationship of objectively measured free-living PA and SB to measures of homeostatic appetite control **(Study 4)**
- To determine the nature, if any, of compensatory mechanisms of overweight and obese individuals undergoing an exercise regime that causes increased EE **(Study 5)** and a weight loss diet that causes reduced EI **(Study 6)**
- Specifically, this will measure the change in free-living PA and SB of overweight and obese individuals following a 12-week supervised exercise intervention **(Study 5)** and a 12-week weight loss diet **(Study 6)**

Chapter 4
General Methods

4.1 Overview of projects

There are six experimental studies included in this thesis, each dealing with a specific issue relating to the associations among free-living sedentary and activity behaviours, adiposity and appetite control within an energy balance framework. The data for the six experimental studies is taken from three large scale projects (Table 4.1):

1. **DAPHNE:** a collaborative European research project with the objective to develop an innovative ICT platform for reducing sedentariness and unhealthy lifestyle habits
2. **SATIN:** a 12-week exercise intervention study in overweight and obese women with a focus on weight change and measurement of homeostatic components of EI
3. **DINE:** a 12-week dietary intervention study in overweight and obese women with a focus on weight change and measurement of homeostatic components of EI

Table 4.1 Data for the six experimental studies was collected as part of three large scale projects

	Project	Participants
Study 1	DAPHNE and DINE	Normal weight/overweight/obese
Study 2	DAPHNE, SATIN and DINE	Normal weight/overweight/obese
Study 3	DAPHNE, SATIN and DINE	Normal weight/overweight/obese
Study 4	SATIN	Overweight/obese
Study 5	SATIN	Overweight/obese
Study 6	DINE	Overweight/obese

4.2 Ethical considerations and participant recruitment

Ethical approval was obtained from the Board of Ethics at the School of Psychology prior to the start of each study. With regards to the SATIN study, ethical approval was obtained from Leeds West National Health Service (NHS) Research Ethics Committee. The DINE study was registered on ClinicalTrials.gov (Identifier: NCT02012426) from December 2013. If changes to the study protocol were

necessary, ethical amendments were submitted and approved by the appropriate ethics board before they were implemented.

Participants were recruited from the University of Leeds, UK and surrounding areas using poster and leaflet advertisements as well as recruitment emails to relevant mailing lists. Recruitment strategies specific to each study are detailed in the relevant experimental chapter along with ethical approval numbers. Potential participants who expressed an interest in the studies were screened for eligibility. Eligible participants were fully informed about the study procedures before agreeing to take part in the research. The specific objectives of each study were not disclosed until completion of the study in order to avoid any undue bias. All participants signed informed consent prior to commencement of the study and they were informed of their right to withdraw from the study at any time with no obligation to disclose a reason for doing so. In the medium term exercise (SATIN) and diet (DINE) intervention studies participants received payment of £240 and £250, respectively, on completion of the study to reimburse them for their time and expenses.

4.2.1 Inclusion and exclusion criteria

Inclusion and exclusion criteria differed slightly across the empirical studies included in this thesis. Below are general inclusion and exclusion criteria that were applied across all studies. Study specific inclusion and exclusion criteria will be detailed in the relevant study chapter.

In order to be considered for inclusion in a study participants were required to be:

- Aged 18-75 years
- BMI 18.5-45 kg/m^2
- Non smokers
- Not taking any medication known to affect metabolism or appetite

The following criteria were used to exclude potential participants from a study:

- Recent change in PA or dietary habits
- Insufficient language skills to complete study questionnaires
- Contraindications to exercise
- Pregnant, planning to become pregnant or breastfeeding

4.3 Methodological procedures

The work conducted over the last 25 years in the Human Appetite Research Unit (HARU) has led to the development of the Leeds multi-level platform to investigate appetite control, see Figure 4.1 (Caudwell et al., 2011). This approach to the study of appetite control was adopted throughout this thesis. In each of the six studies included

in this thesis, there are a number of common physiological, behavioural and psychological measures. The procedures used to obtain these common measures are described in detail in this chapter. Additionally, details of study specific measures and procedures will be described in the relevant experimental chapters.

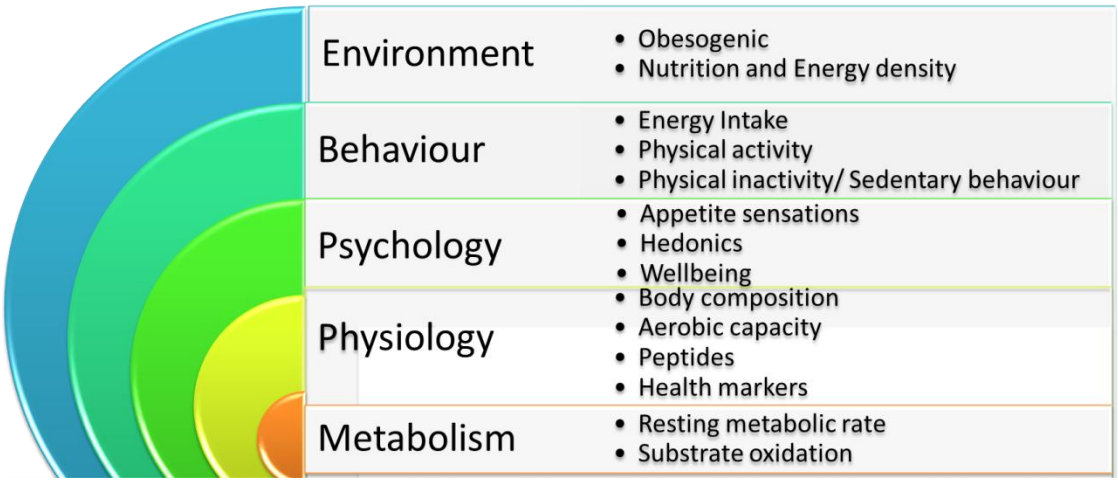


Figure 4.1 The Leeds multi-level systems approach to the study of appetite control

4.3.1 Physiological measurements

4.3.1.1 Body mass and body composition

Body mass and body composition were measured using the BOD POD (Body Composition Tracking System, Life Measurement, Inc., Concord, USA) which uses air displacement plethysmography to estimate body density (see Figure 4.2). This method is favoured over water displacement techniques as it is a simpler and more convenient method. The ability of the BOD POD to estimate body density and body fat % has previously been validated against several criterion methods including dual energy x-ray absorptiometry (Bentzur et al., 2008, Ballard et al., 2004) and hydrostatic weighing (Wagner et al., 2000, Fields et al., 2000). Furthermore, the BOD POD has been shown to accurately estimate body density and body fat % in lean, overweight and obese individuals (Ginde et al., 2005, Vescovi et al., 2001).

A full description of the BOD POD can be found in Fields et al. (2002). In brief, prior to the measurement of body composition, the BOD POD was calibrated according to the manufacturer's instructions using a two-point calibration process and a calibration cylinder of known volume (50.03 L). The scale was also calibrated weekly using two 10 kg weights. Before entering the BOD POD, weight was taken using the electronic scales. The participant was instructed to sit as still as possible whilst breathing normally in the sealed chamber wearing tight clothing and a swim cap to allow for an

accurate measure of body volume. Body volume is assessed indirectly by measuring the volume of air a person displaces inside the enclosed chamber. Two measurement were initially performed and the mean displacement value was used to calculate body composition if the two measures did not differ by more than 150 ml. However, if the difference between the two measurements of air displacement exceeded 150 ml a third measurement was taken and the mean of the three values was calculated. Thoracic gas volumes were estimated using the manufacturer's software. Body volume was calculated using Boyles law, which states 'For a fixed mass of ideal gas at fixed temperature, the product of pressure and volume is a constant'. Body density was then calculated as follows:

$$\text{Body Density} = \text{Body Mass} / \text{Body Volume}$$

Once body density was calculated, it was then applied to the body fat % formula developed by Siri (1961). The equation is based on the two compartment model; the body is made up of two distinct tissues, fat and FFM. The equation below was used to determine % FM using body density:

$$\%\ \text{Fat Mass} = (495 / \text{Body Density}) - 450$$

To determine FFM the following equation was used:

$$\%\ \text{Fat-free Mass} = 100 - \%\ \text{Body Fat}$$

Figure 4.2 BOD POD and related equipment

4.3.1.2 Anthropometrics

Stature was measured to the nearest 0.1 cm whilst participants were standing with their heels, buttocks and back against the stadiometer (Seca Ltd., Birmingham, UK), with their head erect and in the Frankfort horizontal plane. Waist circumference (WC) was measured horizontally in line with the umbilicus and hip circumference was measured horizontally at the maximum circumference of the hip, just below the gluteal fold. Three measurements were taken for each and averaged. Where possible, the same researcher completed all measurements. Measurements were taken to the nearest 0.1 cm.

4.3.1.3 Body mass index

Body mass index (BMI) is a proxy for body fatness and is calculated based on stature and weight using the following equation:

$$\text{BMI} = \text{Weight in kg} / \text{Stature in m}^2$$

The underlying assumption of using BMI to define obesity is that at a given stature, greater weight is associated with increased fatness (Benn, 1971). However, in some populations BMI can be an inaccurate proxy of body fatness (Roche et al., 1981, Wellens et al., 1996), largely because it does not distinguish between FM and FFM. Although BMI is an inaccurate estimate of individual body fatness, overall it corresponds fairly well within groups and categories of body fatness measured using DXA (Flegal et al., 2009). BMI is used to categorise individuals as underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese (≥30 kg/m^2) (WHO Expert Consultation, 1995).

4.3.1.4 Resting metabolic rate

RMR was measured in a fasted state (10-12 hours) in the morning (7:00 am to 9:30 am) using an indirect calorimeter fitted with a ventilated hood (GEM, NutrEn Technology Ltd, Cheshire, UK; see Figure 4.3). IC is a technique that provides accurate estimates of EE from measures of carbon dioxide production and oxygen consumption. Firstly, the gas exchange measurement (GEM) equipment was calibrated using two cylinders of reference gas; a 100% N_2 gas cylinder and a 1% CO_2, 21% O_2 and balance N_2 cylinder. The participant was instructed to lie supine on a bed and remain awake and motionless for 40 minutes during which expired air was collected using a ventilated hood system with a one-way valve. VO_2 and VCO_2 values were sampled every 30 seconds. RMR was determined automatically from VO_2 and VCO_2 values using the modified Weir Equation (Weir, 1949):

$$\text{Energy Expenditure} = (3.94 * VO_2) + (1.11 * VCO_2)$$

This equation does not use a protein factor because the effect is so small (less than 5%) and the likely error in measuring protein is greater than that. The average of the final 30 minute values (with outliers removed) was deemed to be the RMR expressed as kcal/d. Respiratory Quotient (RQ) was also calculated from VO_2 and VCO_2 values. This was the ratio of CO_2 production to the volume of O_2 consumed. Values range from 0.7 indicating pure fat oxidation to 1.0 which indicates pure carbohydrate oxidation. The procedure used to measure RMR followed the guidelines set out by The American Dietetic Association (Compher et al., 2006), the validity and reliability of this measurement technique has previously been established (Bassett et al., 2001, Cooper et al., 2009).

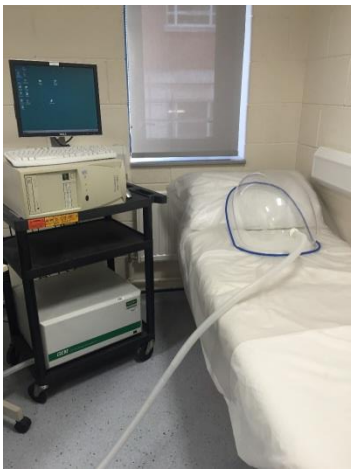


Figure 4.3 GEM equipment for measuring RMR

4.3.1.5 Blood pressure and resting heart rate

Resting heart rate (HR) and blood pressure (BP) were measured using an automated digital BP monitor (Omron M10-IT, Omron Healthcare Europe, Netherlands) after lying in a supine position for 40 minutes (following the measurement of RMR). The cuff was firmly wrapped around the upper arm aligned with the brachial artery and the arm was supported at heart level. As recommended by The American Heart Association, two measurements were taken, and a mean of these was recorded (Pickering et al., 2005).

4.3.1.6 Blood glucose

Participants provided a finger prick blood sample after an overnight fast with the exception of participants in the SATIN study who had intravenous blood samples taken from a cannula inserted in the antecubital vein while the participant was in a semi-supine position. Samples were analyzed using the blood glucose (BG) analyzer (YSI 2300 STAT PLUS Glucose and Lactate Analyzer, YSI Incorporated, Ohio, USA).

4.3.1.7 Maximal aerobic capacity

Maximal aerobic capacity ($\dot{V}O_{2max}$) was assessed using a maximal incremental treadmill test. $\dot{V}O_{2max}$ is the maximum volume of oxygen an individual can inhale and utilise to produce energy and is an indicator of cardiorespiratory fitness. O_2 consumption and CO_2 production was measured using an indirect calorimeter (SensorMedics Vmax 29, California, USA) and HR was recorded (Polar RS400, Polar, Kempele, Finland). The treadmill test was incremental until exhaustion using both speed and incline according to a validated Fatmax test protocol (Jeukendrup 2003). The treadmill gradient began at 1%, with a speed of 3.5 km/h. Every three minutes, the speed increased by 1.0 km/h until a speed of 6.5km/h was reached. Incline was then increased every three minutes by 2% until a gradient of 7% was reached. If the participant was able to continue, the protocol reverted back to increases in speed every three minutes. Expired air samples were taken constantly and HR recordings were taken every 15 seconds during the last minute of each three minute stage. Participants were instructed to indicate when they felt they could only continue for a further minute. Strong verbal encouragement was given to the participant to ensure they reached exhaustion.

After the test, oxygen consumption and carbon dioxide production was analysed, with the average value of oxygen consumption during the final minute of the test being accepted as the maximum oxygen uptake. In addition, the following criteria were assessed to confirm the result was a true maximum value:

a) A plateau in oxygen consumption with an increase in work load

b) HR within 20 beats of age-predicted maximum HR

c) A respiratory exchange ratio (RER) of ≥1.1

4.3.2 Psychological control of appetite

4.3.2.1 Three Factor Eating Questionnaire

The Three Factor Eating Questionnaire (TFEQ) (Stunkard and Messick, 1985) is a 51 item questionnaire that assesses three aspects of eating behaviour; cognitive control of restraint (TFEQ-R; 21 items; example question: *'When I have eaten my quota of calories I am usually very good about not eating anymore'*), disinhibition of eating (TFEQ-D; 16 items; example question: *'When I smell a sizzling steak or see a juicy piece of meat I find it very difficult to keep from eating, even if I have just finished a meal'*) and susceptibility to hunger (TFEQ-H; 14 items; example question: '*I am usually so hungry that I eat more than 3 times a day'*). TFEQ-R refers to the tendency of an individual to restrict their food intake in order to control their weight; TFEQ-D explores the tendency of the individual to overeat and to eat opportunistically in the obesogenic environment; and TFEQ-H measures the extent to which feelings of hunger are

experienced. The first 36 items of the scale require true or false responses, whereas the remaining items give a choice of four set responses varying in agreement with the specific statement (see Appendix A.1). The TFEQ has been widely used in appetite research and can potentially function as a tool to identify individuals susceptible or resistant to weight gain and obesity (Dykes et al., 2004).

4.3.2.2 Binge Eating Scale

The Binge Eating Scale (BES) measures binge eating behaviour and cognitions indicative of eating disorders (Gormally et al., 1982). It was developed to measure the severity of binge eating; defined as the uncontrolled consumption of a large amount of food. The BES has been widely used in research to determine whether potential research participants meet the inclusion criteria for binge eating and to measure severity of binge eating in the non-purge binge eating population. The BES consists of 16 items, eight describing the behavioural manifestations of binge eating and eight describing feelings and cognitions associated with a binge eating episode. Each item consists of four statements that reflect a range of severity (e.g. '*I don't have any difficulty eating slowly in the proper manner'* to *'I have the habit of bolting down my food without really chewing it. When this happens I usually feel uncomfortably stuffed because I've eaten too much'*). Subjects are required to choose the statement that best describes their perceptions and feelings about their eating behaviour (see Appendix A.2). The BES is scored by summing each individual score for the 16 items with a possible range of scores from 0 to 46 (Timmerman, 1999).

4.3.2.3 Control of Eating Questionnaire

The Control of Eating Questionnaire (CoEQ) is comprised of 21 items designed to assess the severity and type of food cravings experienced during the previous 7 days (Hill et al., 1991). The questionnaire is divided in to five sections. The first two sections measure general appetite and mood. The third sections assess the frequency and intensity of food cravings in general – with food cravings being defined as 'a strong urge to eat a particular food or drink'. The fourth section assesses cravings for specific foods (e.g. dairy, sweet or savoury foods). Finally, the fifth section assesses an individual's perceived level of control over eating a craved food. Twenty items are assessed by 100 mm visual analogue scales (VAS) and one item requires participants to enter their own response ('Which one food makes it most difficult for you to control eating?') (see Appendix A.3). The CoEQ has four subscales; Craving Control (5 items), Craving for Sweet (3 items), and Craving for Savoury (3 items) and Positive Mood (3 items). The CoEQ subscales have been shown to have good internal consistency (Dalton et al., 2015).

4.3.2.4 Subjective appetite sensations

Subjective feelings of appetite sensations were measured using VAS presented on a hand-held electronic appetite rating system (EARS-II; see Figure 4.4). The EARS-II system is based on a previously validated electronic rating system (EARS) (Stubbs et al., 2000). The EARS-II system (©Queensland University of Technology) uses a small hand-held computer (HP iPAQ 214) to measure subjective appetite ratings electronically. Advantages of this method are it is easy to use, portable, lightweight, uses a stylus which simulates pen and paper and provides date and timestamped data which can be directly downloaded to a computer eliminating the need to measure responses with a ruler as with the traditional pen and paper method. The VAS scale is anchored at both ends of an 80 mm horizontal line with the most negative (e.g. 'not at all') and the most positive (e.g. 'extremely') responses to each question. The horizontal line is 100 pixels in length, and therefore the VAS responses can be 0-100 units. The EARS-II system has been validated against the traditional pen and paper method as well as the previous EARS system (Gibbons et al., 2011).

VAS are a long established method to measure sensations of appetite (Rogers and Blundell, 1979) and have generally been found to have satisfactory test-retest reliability (Porrini et al., 1995, Barkeling et al., 1995) and reproducibility (Flint et al., 2000). In a review, Stubbs et al. (2000) concluded that appetitive VAS were sensitive to experimental manipulations and were reasonably good at predicting amount eaten. Their reliability and validity were found to increase under controlled laboratory conditions compared to natural free-living eating situations.

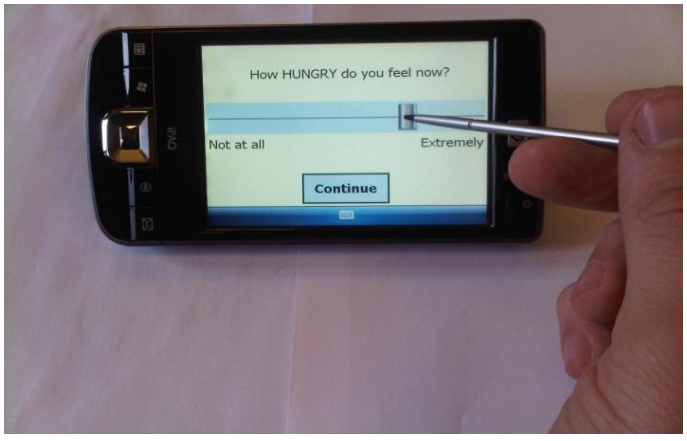


Figure 4.4 EARS-II device for measuring subjective appetite sensations

4.3.2.5 The Satiety Quotient

The satiety quotient (SQ) represents the satiating effects of an eating episode over time (Green et al., 1997). The advantage of the SQ is it provides a temporal measure

of the satiating power of a given food or meal. It is calculated using pre and post-prandial VAS ratings of the motivation to eat (hunger, fullness, desire to eat and prospective food consumption) relative to the energy content of a meal. As such, it reflects the capacity of a meal to modulate the strength of post-prandial satiety signals. Because the relationship between energy consumed and the consequent suppression of hunger is non-linear, the SQ works best when a fixed meal is used. The SQ was calculated using the following formula:

$$SQ(mm/kcal) = \frac{\text{(rating before eating episode – rating after eating episode)}}{\text{Energy intake of eating episode}} * 100$$

The SQ has previously been shown to be associated with ad libitum EI in men and women and weight loss in men only (Drapeau et al., 2007, Drapeau et al., 2005). It is also sensitive to changes in exercise-induced improvements in post-prandial satiety signalling (King et al., 2009a).

4.3.2.6 Area under the curve

The area under the curve (AUC) provides an aggregate for each subjective appetite sensation. The AUC represents the area spanned by the appetite sensation profile and is computed by the trapezoid method (see Figure 4.5). The AUC can be calculated for the whole of the measurement period, or parts of the measurement period. It is usual to exclude baseline or fasting values when calculating the AUC. The rationale for doing this is to remove any bias or differences at baseline which might artificially alter the mean AUC, and introduce more random variation into the measured response.

$$AUC_{1-n} = \sum \left\{ \frac{Cp_1 + Cp_2}{2} \bullet (t_2 - t_1) \right\} + \left\{ \frac{Cp_2 + Cp_3}{2} \bullet (t_3 - t_2) \right\} + \ldots$$

Figure 4.5 Formula for calculating AUC using the trapezoid method. Cp represents VAS rating and t represents time point

4.3.3 Behavioural measurements

4.3.3.1 Probe day energy intake

To examine features associated with appetite and eating behaviour, EI was objectively measured under controlled laboratory conditions in two of the three projects which form the basis of this thesis (SATIN and DINE). Laboratory based measures of eating behaviour provide greater precision and accuracy than the free-living alternative but lack ecological relevance (Blundell et al., 2009). Indeed, it should be noted that the

measurement of appetite in the laboratory is not an attempt to replicate the free-living environment. Rather, laboratory based measurements provide the opportunity to study the expression of appetite (e.g. hunger or fullness) in response to a specific stimuli (e.g. exercise or diet) whilst minimising contamination from extraneous input (e.g. social stimuli or distraction).

Standard probe day procedures will be described here, however, details of study specific foods and procedures will be provided in the respective experimental chapters. Prior to commencing the study, participants were screened to establish whether they liked the study foods. If participants strongly disliked any foods included in the test meals they were excluded from the study. Participants were instructed to be fasted from 10:00 pm the previous night and to abstain from exercise (apart from post-intervention probe days in the medium-term exercise intervention) and alcohol for at least 24 hours. Probe days began between 7:30 am and 9:30 am. All foods were prepared in the laboratory kitchens in the HARU at the University of Leeds by trained research staff. All test meals were consumed in isolation in specifically designed cubicles in the HARU except for foods provided to be eaten (not obligatory) in the evening at home if the participant became hungry. For ad libitum meals, participants were instructed to eat as much or as little as they wanted and to eat until they were comfortably full. Participants opened the cubicle door to indicate they had finished eating. Food was provided in excess of the anticipated amount a participant might consume for ad libitum meals. Alternatively, during fixed meal consumption, participants were instructed to eat all of the food and drink provided. Participants were instructed to refrain from using mobile phones/computers, listening to music and reading books/magazines during each eating episode. Foods and drinks were weighed before and after consumption to the nearest 0.1 g, and the energy value and macronutrient composition of foods were taken from the packaging of foods to establish energy and macronutrient intake. The energy content of a gram of fat, carbohydrate and protein was 9 kcal, 3.75 kcal and 4 kcal, respectively. VAS ratings were completed before and after each meal and at hourly intervals between meals using the method described in section 4.3.2.4 to quantify changes in subjective appetite sensations throughout the probe day.

4.3.3.2 Free-living physical activity and sedentary behaviour

The platform for measuring free-living PA and SB developed at the outset of this PhD is described in detail in Chapter 6. Each of the studies included in this thesis measured free-living PA and SB according to the protocol described in Chapter 6. Any study specific procedures are described in the relevant experimental chapter.

4.4 General statistical approach

Data are reported as mean ± SD throughout this thesis unless otherwise stated. Statistical analysis was performed using IBM SPSS for Windows (Chicago, Illinois, Version 21). All variables were checked for outliers and normality was assessed using the Shapiro-Wilk test prior to any analysis. Characteristics of the study population were summarised using descriptive statistics. Study specific statistical procedures are detailed in the statistical analysis section of each experimental chapter.

4.5 A note on statistical power

Power refers to the probability that a statistical test will detect a true effect (reject the null hypothesis (H_0) when the alternative hypothesis (H_1) is true). The studies in this thesis were based on secondary analyses of data sets from larger projects. A priori power calculations were performed specifically for the primary study outcomes within those projects. Change in body mass was a primary outcome in the SATIN and DINE projects. Both studies resulted in a significant reduction in body mass following the 12-weeks exercise and 12-weeks diet intervention indicating sufficient power.

The statistical power of a completed study becomes most relevant if there are null findings. The majority of studies throughout this thesis have significant findings and therefore had sufficient power to detect an effect. However, in Study 6, there was no effect of diet-induced weight loss on free-living sedentary and active behaviour. Post hoc power calculations were performed using G*Power (Faul et al., 2009) to assess the statistical power of the study given the sample size attained and the observed effect size, and a priori tests were calculated to identify the required sample size needed to detect an effect size of the same magnitude as that reported in the study (0.10 to 0.20) with 80% and 90% power ($1-\beta = 0.8$ and $1-\beta = 0.9$) with an $\alpha = .05$. The results are displayed in Table 4.2. A sample size of 206 to 866 would be needed to obtain statistical power at $1-\beta = 0.8$ and a sample size of 274 to 1158 would be needed to obtain statistical power of $1-\beta = 0.9$. It can therefore be argued that any real effects of diet-induced weight loss on free-living sedentary and active behaviours may be too small to hold any practical importance or clinical relevance.

Table 4.2 Post hoc and a priori sample size calculations for PA and SB outcome measures in Study 6. Data are the mean of the difference (SD) between baseline and post intervention measurements

n=64	Mean Δ (min/d)	d	Power	Sample # 1-β=0.8	Sample # 1-β=0.9
SB	8.27 (86.73)	0.10	0.1167	866	1158
Moderate PA	3.95 (32.91)	0.12	0.1570	547	732
Vigorous PA	0.91 (4.63)	0.20	0.3406	206	274
MVPA	4.85 (34.99)	0.14	0.1939	411	549

Chapter 5
Study 1 - Associations Among Free-Living Physical Activity and Sedentary Behaviour, Body Composition, Markers of Appetite Dysregulation and Health Markers

This chapter will examine the associations among free-living physical activity and sedentary behaviour, body composition, markers of appetite dysregulation and health markers. Advanced motion sensing technology was used to continuously track sedentary and active behaviours under free-living conditions for six to seven days. Energy expenditure and time spent in different categories of activity, from sedentary to vigorous, were used in correlation analyses to determine the associations with body composition, markers of appetite dysregulation and health markers.

"It is time to wind back the harms caused by the junk food industry's public relations machinery. Let us bust the myth of physical inactivity and obesity. You cannot outrun a bad diet." (Malhotra et al., 2015, p.968)

5.1 Introduction

Overweight and obesity has more than doubled in the UK in the last 25 years. The relative contribution of reduced EE or increased EI to obesity has been much debated in recent years and has been termed the energy balance wars. The notion that excessive EI is the cause of the current obesity epidemic seems to be more widely accepted over the low activity idea. Despite Cochrane systematic reviews reporting beneficial effects of exercise on weight loss independent of any dietary effect (Shaw et al., 2006) the view persists that being active does not contribute to weight control. This view can be attributed, at least in part, to the way in which the popular press report exercise and weight related research with headlines such as 'Why Exercise Won't Make You Thin' (Time Magazine, 2009) and 'Health Warning: exercise makes you fat' (Telegraph, 2009). These articles suggest that exercise stimulates appetite and promotes overconsumption of food. The view that exercise does not contribute to weight management is not limited to the mainstream media. In a recent editorial in the British Journal of Sports Medicine, a headline title referred to 'the myth of physical inactivity and obesity' and the text categorically stated that 'physical activity does not promote weight loss' (Malhotra et al., 2015). Articles challenging Malhotra's claims

have attempted to prevent further damaging perceptions emanating from the editorial (Blair, 2015, Mahtani et al., 2015).

Observational and longitudinal studies have reported a relationship between free-living sedentary and active behaviours and adiposity. Studies indicate that MVPA is negatively associated with adiposity and SB is positively associated with adiposity, but most studies use questionnaire-based self-report measures of PA and sedentary time (Larsen et al., 2014, McGuire and Ross, 2012). Moreover, after adjusting for MVPA the association between SB and adiposity is nullified (Hamer et al., 2012, Long et al., 2002). Further research is needed to better understand the relationship between objectively measured sedentary and active behaviours with adiposity.

Not only does exercise benefit weight management by increasing EE, it has also been shown to affect eating behaviour and appetite (Caudwell et al., 2013b). Studies examining the relationship between EE and EI were noted as far back as the 1950's. In a study by Mayer et al. (1956) jute mill workers were categorised based on the physical demand of their jobs and the dietary intake of individual workers was calculated. At moderate and high levels of occupation related PA, EI matches EE however, at lower levels of occupation related PA, EI exceeds EE leading to a positive energy balance. This suggests the reciprocal relationship between EI and EE only operates above a certain level of PA (see Figure 2.1). Below that certain level it appears appetite control is lost and EI no longer operates in the interest of energy balance. Mayer called this the 'sedentary zone' and although in this zone much of the jute mill workers day would be spent seated the author is referring to an absence of PA and not SB as it is currently defined. A more appropriate name, given by John Blundell (2011), is the 'non-regulated zone'.

In recent years, much work has focussed on the 'regulated zone' (Figure 2.1) with studies investigating the effect of an acute bout of exercise or whether embarking on a PA regimen can improve appetite control, or alternatively whether those who are habitually physically active exhibit more sensitive appetite control compared to inactive individuals. These studies demonstrate a single bout of exercise has transient effects on appetite and energy expended though exercise is not immediately compensated for by an increase in EI (Broom et al., 2007, King et al., 2010a). Interestingly, longer term exercise interventions have demonstrated large inter-individual differences in weight loss in response to increased exercise that could be explained by differences in appetite sensation and eating behaviour (King et al., 2008, King et al., 2009a). Finally, when habitual PA levels have been examined, individuals who are more physically active appear to have more sensitive control over appetite (Long et al., 2002, Beaulieu et al., 2016). Few studies have examined the effects of moving from the 'regulated zone' to the 'non regulated zone' (Figure 2.1) by reducing PA. One such study by

Stubbs et al. (2004) demonstrated that becoming less active by reducing PA levels from 1.8 to 1.4 times RMR for 7 days did not down regulate EI resulting in a positive energy balance.

Little is known about the effects of SB on appetite control and energy balance. SB has been linked to unhealthy dietary intake such as increased EI and increased intake of snack foods, deserts and added sugars, but the mechanism underlying these associations have received little attention (Pearson and Biddle, 2011, Sisson et al., 2012, Bowman, 2006). Furthermore, a large proportion of studies use TV viewing as a proxy measure of SB. TV viewing is not representative of total sedentary time and has been linked with specific psychological mechanisms, including distraction and disruption of food cues (Tal et al., 2014, Blass et al., 2006), that could lead to the unhealthy dietary patterns and passive overconsumption. Therefore it is not possible to generalise findings from TV viewing studies to SB *per se*. A more recent experimental study examined the effects of breaking up prolonged sitting (5 hours) with two minute bouts of light and moderate intensity activity every 20 minutes on appetite and EI (Bailey et al., 2015). The authors found no difference in absolute EI, gut hormones or subjective appetite sensations between the different conditions. The longer-term effects of this type of intervention are unknown. Further research is needed, both observational and experimental, to assess the relationship between objectively measured SB and appetite control.

Studies indicate that SB is positively and MVPA is negatively associated with adiposity. As well as the reduced EE associated with increased sedentary time and reduced MVPA, the uncoupling of EI to EE in the 'non-regulate zone' of Figure 2.1 may also be driving the associations between inactivity and adiposity; under conditions of low EE, appetite control is weakened causing EI to exceed EE leading to a positive energy balance. Therefore the purpose of this study was to implement advanced motion sensing technology to investigate whether measures of free-living PA and SB were associated with body composition, appetite dysregulation and health markers.

5.1.1 Hypotheses

- Free-living MVPA will be negatively associated with adiposity
- Free-living SB will be positively associated with adiposity
- Free-living PA and SB will be related to markers of appetite dysregulation
- Adiposity will be related to markers of appetite dysregulation

5.2 Methods

5.2.1 Participants

Seventy-one participants (13 men and 58 women) aged 37.4 years (SD = 14.0) with a BMI of 29.9 kg/m^2 (SD = 5.2) were recruited from the University of Leeds, UK, and surrounding area for this cross-sectional study. All participants had valid body composition, TFEQ and BES data, 68 participants had valid SWA data (95.8% compliance), 69 had subjective appetite ratings and 27 participants had valid data for the CoEQ. All participants provided written informed consent before taking part in the study and ethical approval was granted by the School of Psychology Ethical Review Board (14-0099 and 14-0223).

5.2.2 Inclusion criteria

- Healthy men and women
- Aged ≥18 years
- Non smokers
- Not taking any medication known to affect metabolism or appetite

5.2.3 Exclusion criteria

- Recent change in PA or dietary habits (previous four weeks)
- Insufficient language skills to complete study questionnaires
- Contraindications to exercise
- Pregnant, planning to become pregnant or breastfeeding

5.2.4 Design

The study was a cross-sectional study. Participants attended the research unit twice over the course of one week (Figure 5.1). Free-living PA and SB were measured continuously for a minimum of 6 days for >22 hours/d. Participants were fasted for a minimum of 12 hours and had abstained from exercise and alcohol for at least 24 hours before both laboratory visits.

On the morning of day one the following measurements were taken: stature, weight, waist and hip circumference, body composition and RMR. Health markers including, BP, resting HR and fasting BG were also taken. Participants were fitted with a SWA mini (BodyMedia, Inc., Pittsburgh, PA) and advised on proper wear. A PA diary was also provided for participants to record their PA and the time and reason for removal of the SWA. Participants returned to the lab on day seven or eight to complete psychometric questionnaires (fasting hunger and fullness, CoEQ, TFEQ and BES) and to return the activity monitor and completed PA diary. Cardiovascular fitness was also measured.

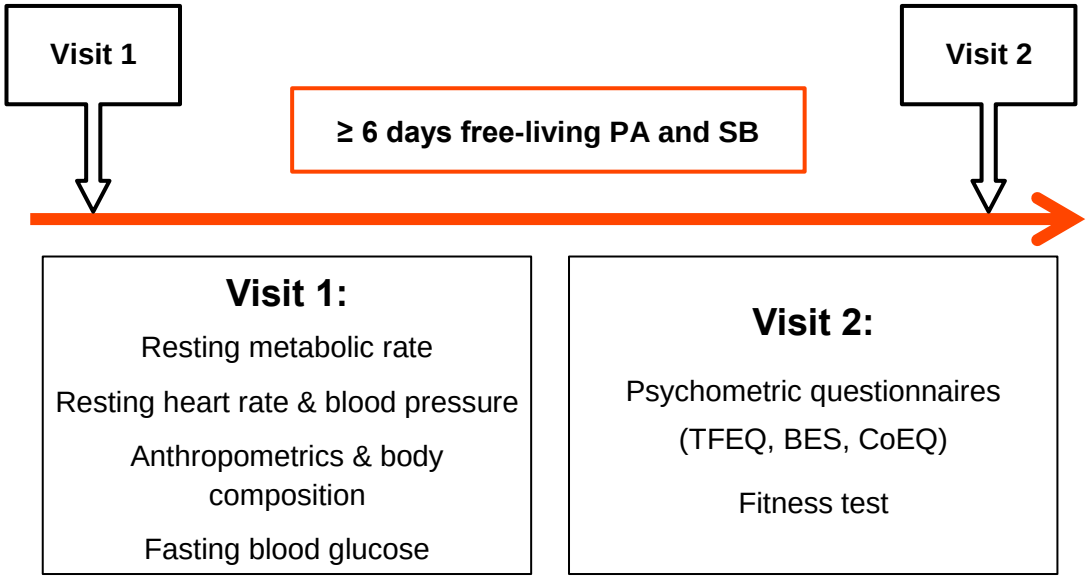


Figure 5.1 Overview of study 1 procedures occurring at two laboratory visits over a one week period

5.2.5 Anthropometrics and body composition

Stature was measured using a stadiometer and body composition was measured using air plethysmography (BOD POD). Body mass was obtained from the BOD POD whilst participants were wearing minimal clothing. Waist and hip circumference was measured using anthropometric measuring tape. A detailed description of all of these measures can be found in Chapter 4.

5.2.6 Resting metabolic rate and health markers

RMR was measured using indirect calorimetry. BP and resting HR were measured using an automatic sphygmomanometer (Omron) immediately after completion of the RMR procedure. Fasting glucose was obtained from a finger prick blood sample and analysed using a BG analyser. For further details on these measurements, see Chapter 4.

5.2.7 Free-living physical activity and sedentary behaviour

Free-living PA and SB was measured objectively using the SWA. Participants wore the armband on the posterior surface of their upper non-dominant arm for a minimum of 22 hours per day for 7 to 8 days in order to obtain data for at least six 24 hour periods from midnight to midnight. Participants were instructed to remove the SWA when showering, bathing or swimming. For the SWA data to be valid there had to be >22 hours of data per day and at least six 24 hour periods (midnight to midnight) including at least one weekend day. Participants completed a PA diary to coincide with the PA monitoring period detailing the intensity, duration and type of activity performed along

with details on removal of the SWA. Further details of the methodological platform for measuring PA and SB can be found in Chapter 6.

5.2.8 Markers of appetite dysregulation

Participants completed several questionnaires including fasting ratings of subjective appetite to assess hunger, fullness, desire to eat and prospective consumption using VAS (Flint et al., 2000); the CoEQ (a 21 items questionnaire designed to assess the severity and type of food cravings experienced over the previous 7 days (Hill et al., 1991)); the TFEQ, a 51 item questionnaire measuring restraint, disinhibition and hunger (Stunkard and Messick, 1985); and the BES, a 16 item questionnaire measuring binge eating behaviour and cognitions indicative of eating disorders (Gormally et al., 1982). Further information about these questionnaires and measures can be found in Chapter 4.

5.2.9 Maximal aerobic capacity

Maximal aerobic capacity ($\dot{V}O_2max$) was measured during an incremental treadmill test with expired air (Sensormedics Vmax29, Yorba Linda, USA) and HR (Polar RS400, Polar, Kempele, Finland) measured continuously. Attainment of true $\dot{V}O_2max$ was determined by a plateau in $\dot{V}O_2$ with an increase in workload, a respiratory quotient of ≥1.1 and a HR within 20 beats of age predicted maximum HR (220-age). Further information about the fitness test is provided in Chapter 4.

5.2.10 Statistical analysis

Data are reported as mean ± SD throughout. Statistical analysis was performed using IBM SPSS for Windows (Chicago, Illinois, Version 21). For reasons of scientific rigour and to reduce the likelihood of false positives, significance was set at $p < .01$. Characteristics of the study population were summarised using descriptive statistics. All variables were checked for outliers and normality was assessed using the Shapiro-Wilk test. Pearson correlation analysis was performed to examine the associations among sedentary and active behaviour, body composition, markers of appetite dysregulation and health markers. In addition partial correlations were also carried out to separate the effects of a third variable acting concurrently on two variables; this involved controlling for body fat, SB, MVPA and sex in different analyses.

5.3 Results

5.3.1 Participant characteristics

Study sample characteristics are displayed in Table 5.1 for men and women separately and combined. Of the 71 participants who took part in the study 68 provided ≥6 days of valid armband data. Average wear time of the SWA was 23.55 hours/d (SD = 0.26) or 98% (SD = 1.2) of the day. Participants were sedentary (excluding sleep) for an average of 11.06 hours/d (SD = 1.72) and recorded 3.26 hours/d (SD = 1.03) in light PA and 2.10 hours/d (SD = 1.40) in MVPA (Figure 5.2). Participants mean age was 37.35 years (SD = 14.01) and their average total EE was 2708.07 kcal/d (SD = 421.81).

Table 5.1 Descriptive statistics for the study sample as a whole and men and women separately

	Combined Mean (SD)	Men Mean (SD)	Women Mean (SD)	*p*
Age (years)	37.35 (14.01)	37.85 (16.33)	37.24 (13.61)	= .889
Stature (m)	1.66 (0.09)	1.78 (0.08)	1.63 (0.07)	**< .001**
PHYSIOLOGICAL				
Body mass (kg)	82.24 (15.26)	80.60 (14.22)	82.61 (15.58)	= .671
BMI (kg/m^2)	29.94 (5.24)	25.53 (3.57)	30.93 (5.07)	**= .001**
FM (kg)	31.79 (13.37)	17.12 (8.09)	35.09 (12.7)	**< .001**
FFM (kg)	50.44 (9.28)	63.45 (7.79)	47.52 (6.75)	**< .001**
WC (cm)	100.23 (12.83)	90.46 (8.74)	102.42 (15.23)	**= .001**
Systolic BP (mm Hg)	118.17 (14.12)	122.31 (10.55)	117.24 (14.70)	= .245
Diastolic BP (mm Hg)	77.80 (10.25)	73.54 (9.30)	78.76 (10.28)	= .097
Resting HR (bpm)	58.56 (9.71)	50.69 (8.20)	60.32 (9.18)	**= .001**
BG (mmol/L)**	4.73 (0.69)	4.87 (0.63)	4.70 (0.71)	= .428
$\dot{V}O_2$max (ml/kg/min)^x	40.99 (7.88)	45.76 (7.03)	36.57 (5.89)	**= .001**
RMR (kcal/d)	1703.72 (299.52)	1876.16 (272.28)	1665.07 (293.71)	= .021
Fasting RQ	0.75 (0.08)	0.76 (0.7)	0.75 (0.08)	= .558
BEHAVIOURAL				
Total EE (kcal/d)^	2708.07 (421.81)	3137.02 (587.01)	2616.20 (314.78)	**< .001**
Activity EE (kcal/d)^	1039.08 (382.22)	1263.05 (491.05)	991.09 (341.25)	= .024
SB (min/d)^	663.50 (103.00)	635.04 (133.18)	669.60 (95.72)	= .295
Light PA (min/d)^	195.45 (61.98)	151.69 (54.15)	204.83 (59.88)	**= .006**
MVPA (min/d)^	125.92 (83.67)	201.10 (99.02)	109.81 (71.21)	**< .001**

PAL		1.62 (0.23)	1.68 (0.25)	1.61 (0.22)	= .279
PSYCHOLOGICAL					
VAS	**Hunger****	64.42 (21.26)	64.46 (17.48)	64.41 (22.19)	= .994
	Fullness**	20.58 (18.67)	21.54 (14.81)	20.36 (19.56)	= .839
	Desire to eat**	66.36 (20.95)	69.62 (15.08)	65.61 (22.13)	= .538
	Prospective consumption **	60.46 (19.39)	60.77 (17.21)	60.39 (20.01)	= .950
TFEQ	**Restraint**	8.21 (3.82)	5.92 (3.53)	8.72 (3.76)	= .016
	Disinhibition	8.85 (3.88)	5.92 (3.93)	9.50 (3.58)	**= .002**
	Hunger	6.00 (3.16)	5.85 (3.34)	6.03 (3.15)	= .848
BES		13.23 (7.30)	9.38 (5.99)	14.09 (7.34)	= .035

** n=69; ^ n=68; ˣ n=27.

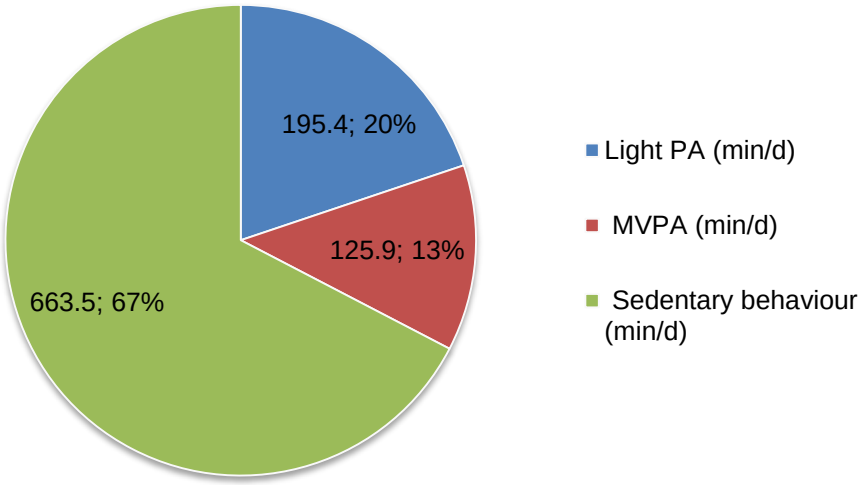


Figure 5.2 The proportion of waking time spent sedentary, in light PA and MVPA. Data presented as percentage of awake time and total minutes on average per day

5.3.2 Association between sedentary behaviour and different categories of physical activity

SB was negatively associated with light [r(66) = -.39, p = .001], moderate [r(66) = -.76, p < .001; see Figure 5.3] and vigorous [r(66) = -.44, p < .001] PA. Light PA was also negatively associated with vigorous PA [r(66) = -.33, p = .006]. Moderate and vigorous PA were positively correlated [r(66) = .65, p < .001].

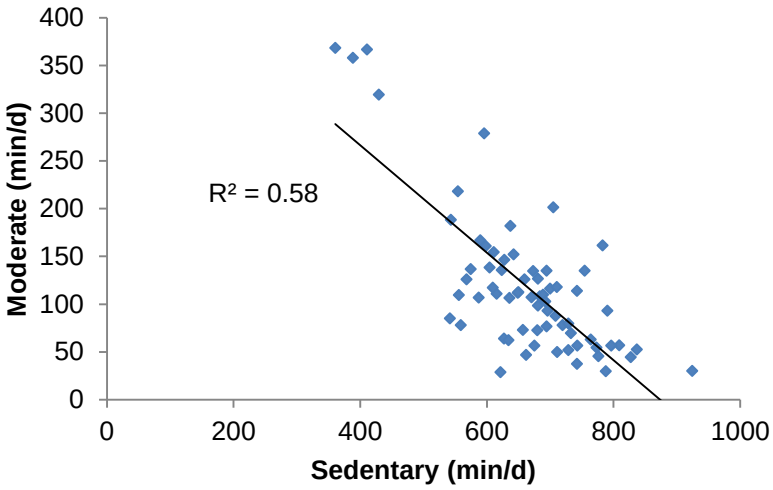


Figure 5.3 The association between SB and moderate PA

5.3.3 Associations between sedentary behaviour, physical activity and body composition

SB was positively correlated with multiple indices of adiposity including body mass, BMI, FM (see Figure 5.4) and WC as shown in Table 5.2. Conversely, MVPA was negatively associated with body mass, BMI, FM (see Figure 5.5) and WC.

Partial correlations were performed to identify the independent effects of SB (controlled for MVPA), light PA (controlled for MVPA and SB, separately) and MVPA (controlled for SB) on body composition (see Table 5.2). After controlling for MVPA the magnitude of the correlations between SB and adiposity were markedly weakened and were no longer statistically significant. However, when the correlations between MVPA and adiposity were adjusted for SB all correlations remained significant. Controlling the correlation between body composition and light PA for SB resulted in significant positive correlation for BMI, FM and WC.

It is noticeable in Figure 5.4 that four participants have low amounts of SB and it was possible that these values were unduly influencing the correlation. When the statistical test was repeated excluding these subjects the correlation remained positive and significant [r(62) = .31, p = .01].

Table 5.2 Correlations between sedentary and active behaviours and body composition

	Body mass (kg)	BMI (kg/m^2)	FM (kg)	WC (cm)	FFM (kg)
SB (min/d)	**.43****	**.51****	**.47****	**.43****	-.02
Light PA (min/d)	.08	.20	.19	.18	-.15
MVPA (min/d)	**-.54****	**-.69****	**-.71****	**-.64****	.15
SB (min/d)	.04	-.01	-.13	-.10	.20
Light PA (min/d)	.03	.19	.18	.16	-.14
Light PA (min/d)	.29	**.50****	**.46****	**.41****	-.16
MVPA (min/d)	**-.37***	**-.54****	**-.61****	**-.53****	.25

Data are Pearson correlation (r). Top panel shows simple correlations; middle panel shows partial correlations controlled for MVPA (min/d); lower panel shows partial correlations controlled for SB (min/d). ** p < .001; * p < .01.

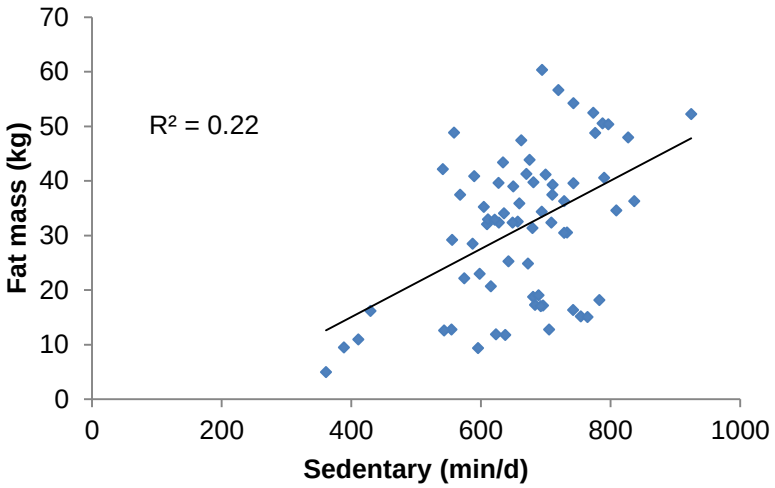


Figure 5.4 The association between SB and FM

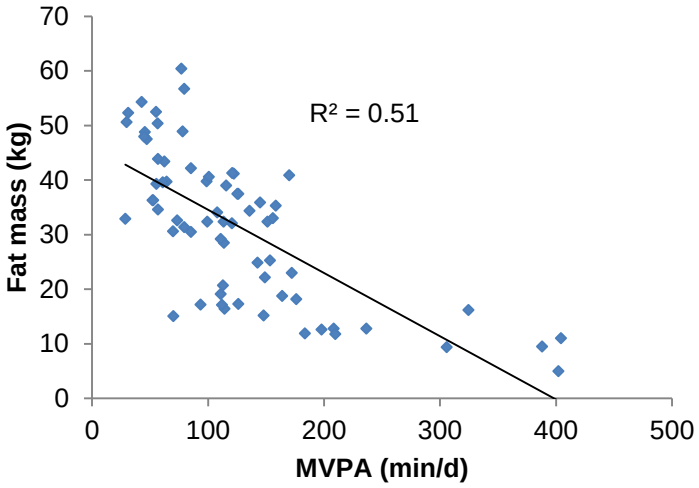


Figure 5.5 The association between MVPA and FM

- **SB was associated with higher adiposity**
- **MVPA was associated with lower adiposity**
- **The relationship between SB and adiposity was no longer significant after controlling for MVPA**
- **The relationship between MVPA and adiposity remained significant after controlling for SB**

5.3.4 Associations between physical activity, sedentary behaviour and energy expenditure

Total EE from the SWA was negatively associated with SB [r(66) = -.35, p = .004; Figure 5.6] and positively associated with MVPA [r(66) = .41, p < .001]. There was no significant association between total EE and light intensity PA [r(66) = .15, p = .23]. In order to investigate whether the relationship between behaviour and adiposity was accounted for by EE, activity EE was calculated as the difference between total EE (SWA) and RMR (directly measured by IC). Activity EE was positively correlated with MVPA [r(66) = .61, p < .001] and negatively associated with time spent sedentary [r(66)=-.61, p<.001]. However, activity EE was not significantly associated with any of the indices of adiposity including body mass [r(66) = -.06, p = .65], BMI [r(66) = -.26, p = .35], FM [r(66) = -.29, p = .16] or WC [r(66) = -.19, p = .12] but it was positively associated with FFM [r(66) = .33, p = .005], see Figure 5.7. Total EE calculated by the SWA was positively associated with body mass [r(66) = .44, p < .001] and FFM [r(66) = .76, p < .001; see Figure 5.8] but not with BMI [r(66) = .05, p = .69], FM [r(66) = -.03, p = .83] or WC [r(66) = .14, p = .26].

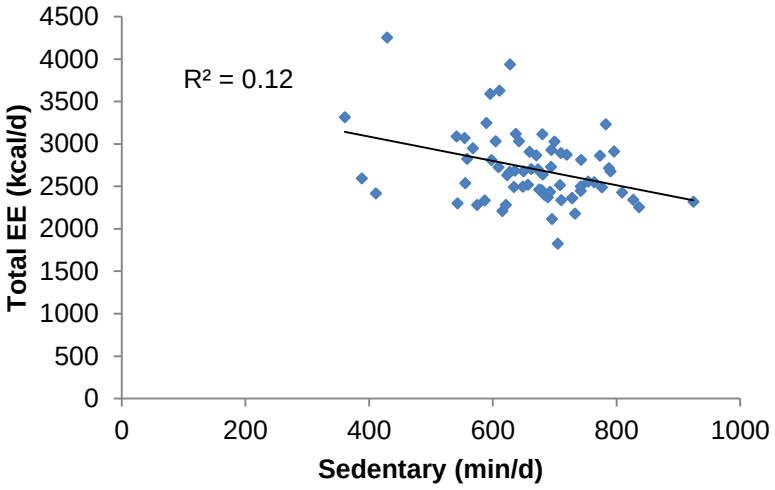


Figure 5.6 The association between SB and total EE

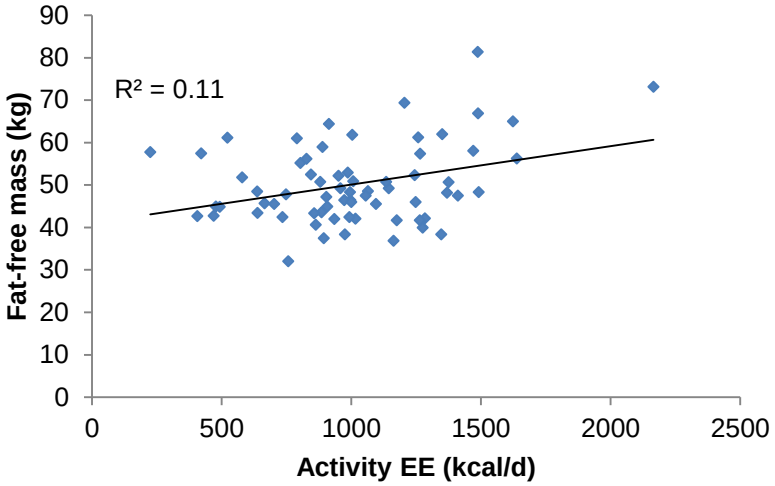


Figure 5.7 The association between activity EE and FFM

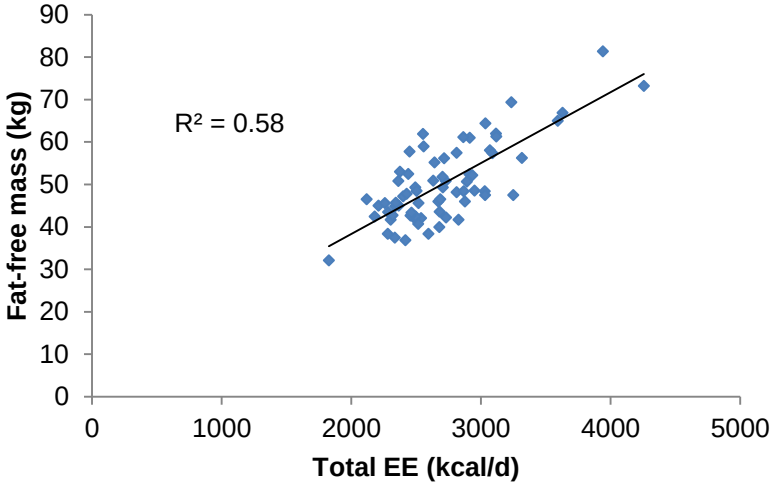


Figure 5.8 The association between total EE and FFM

5.3.5 Associations between sedentary behaviour, physical activity and markers of appetite dysregulation

There were no significant correlations between SB and any of the indices of appetite dysregulation; TFEQ-R, TFEQ-D, TFEQ-H, BES, fasting subjective appetite sensations or CoEQ subscales. However, light PA and MVPA showed some relationship to the TFEQ-D and BES scores, but these were no longer apparent when partial correlations were performed controlling for the amount of body fat (see Table 5.3).

Table 5.3 Correlations between sedentary and active behaviours and markers of appetite dysregulation

	SB (min/d)	Light PA (min/d)	MVPA (min/d)	SB (min/d)	Light PA (min/d)	MVPA (min/d)
TFEQ-R	-.11	.14	.05	-.08	.16	-.01
TFEQ-D	.13	**.34***	**-.44****	-.21	.29	-.05
TFEQ-H	-.05	.22	-.15	-.11	.21	-.12
BES	.08	.22	**-.34***	-.19	.16	-.01
VAS Hunger (mm)	-.05	.31	.05	-.08	.30	.10
VAS Fullness (mm)	-.03	-.20	.01	-.03	-.21	.02
VAS Des (mm)	.02	.20	.02	-.01	.20	.08
VAS Pros (mm)	.03	.22	-.05	-.01	.21	.01
Craving control	.04	-.07	.18	.24	-.01	-.09
Craving sweet	.04	-.04	-.25	-.04	-.07	-.19
Craving savoury	-.16	.05	.11	-.33	.01	.44
Positive mood	-.29	.25	.40	-.18	.33	.27

Data are Pearson correlation (r). Left panel shows simple correlations. Right panel shows partial correlations controlled for body fat (kg). ** p < .001; * p < .01.

- **MVPA was negatively associated with TFEQ-D and BES score**
- **After controlling for adiposity these relationships were no longer significant**

5.3.6 Associations between markers of appetite dysregulation and body composition

TFEQ-D and BES were positively associated with body mass, BMI, FM (see Figure 5.9 and Figure 5.10) and WC. FFM was not significantly associated with any of the measures of appetite dysregulation nor were there any associations between any of the measures of body composition and TFEQ-R, TFEQ-H or any of the subjective appetite ratings (see Table 5.4). FFM and RMR were significantly positively correlated [$r(69) = .67$, $p < .001$]. In order to investigate whether the association between RMR and appetite dysregulation were similar to those between FFM and appetite dysregulation Pearson correlations were performed. RMR was positively correlated with TFEQ-D [$r(69) = .32$, $p = .006$] and BES [$r(69) = .32$, $p = .006$]. After controlling for FM the correlations were no longer significant [$r(68) = .20$, $p = .10$ and $r(68) = .22$, $p = .07$, respectively].

Table 5.4 Correlations between body composition and markers of appetite dysregulation

	Body mass	BMI	FM	WC	FFM
TFEQ-R	-.20	-.05	-.07	-.14	-.23
TFEQ-D	**.51****	**.59****	**.58****	**.56****	.01
TFEQ-H	.18	.12	.10	.12	.15
BES	**.49****	**.45****	**.47****	**.48****	.12

Data are Pearson correlation (r). ** p < .001; * p < .01.

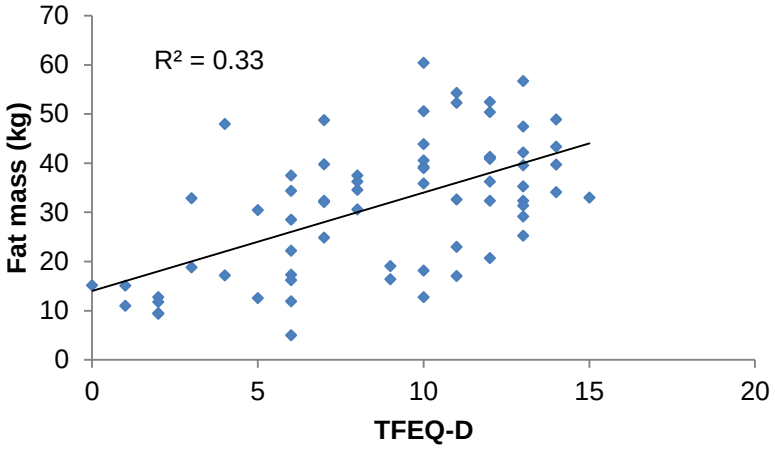


Figure 5.9 The association between FM and the TFEQ-D

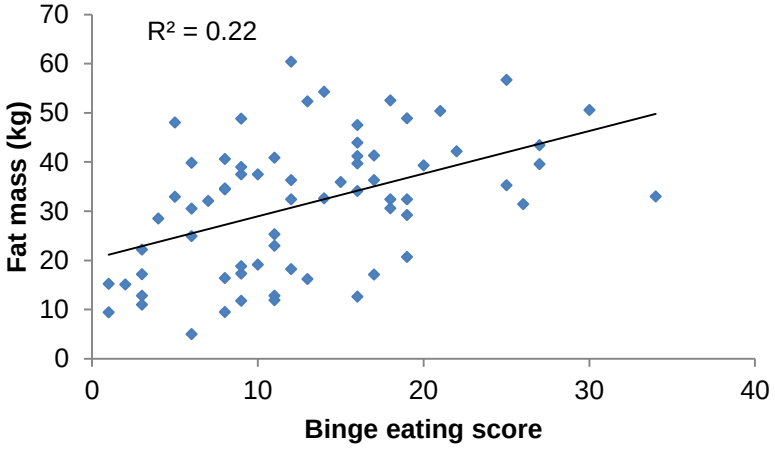


Figure 5.10 The association between FM and the score on the BES

- **Adiposity was positively associated with TFEQ-D and BES score**

5.3.7 Associations between sedentary behaviour, physical activity and health markers

There were no correlations between any of the health markers and SB or light intensity PA. MVPA was negatively correlated with both diastolic BP [r(66) = -.31, p = .01] and resting HR [r(66) = -.43, p < .001] but these were no longer apparent when partial correlations were performed controlling for the amount of body fat (see Table 5.5).

Table 5.5 Correlation between sedentary and active behaviours and health markers

	Systolic BP (mmHg)	Diastolic BP (mmHg)	Resting HR (bpm)	Fasting BG (mmol/L)	$\dot{V}O_2$max (ml/kg/min)	RMR (kcal/d)
SB (min/d)	-.13	.23	.24	.05	-.15	.20
Light PA (min/d)	.11	.14	.25	-.03	-.20	-.08
MVPA (min/d)	.10	**-.31***	**-.43****	-.09	.33	-.12
SB (min/d)	-.17	.04	-.02	.02	.01	.07
MVPA (min/d)	.18	-.02	-.08	-.05	-.03	-.13

Data are Pearson correlation (r). Upper panel shows simple correlations. Lower panel shows correlations controlled for body fat. ** p < .001; * p < .01.

5.3.8 Associations between body composition and health markers

Diastolic BP and resting HR were positively associated with body mass, BMI, FM and WC. (see Table 5.6). RMR showed a positive relationship with body mass, BMI, WC and FFM (see Figure 5.11). Finally, $\dot{V}O_2$max was negatively associated with FM.

Table 5.6 Correlations between body composition and health markers

	Body mass (kg)	BMI (kg/m^2)	FM (kg)	WC (cm)	FFM (kg)
Systolic BP (mmHg)	.12	.02	.04	09	.14
Diastolic BP (mmHg)	**.34***	**.41****	**.42****	**.31***	-.05
Resting HR (bpm)	**.39****	**.54****	**.54****	**.40****	-.13
Fasting BG (mmol/L)	.10	.10	.07	.19	.06
RMR (kcal/d)	**.67****	**.36***	.29	**.41****	**.67****
$\dot{V}O_2$max (kg/ml/min)	.05	-.24	**-.49***	-.13	.43

Data are Pearson correlation (r). ** p < .001; * p < .01.

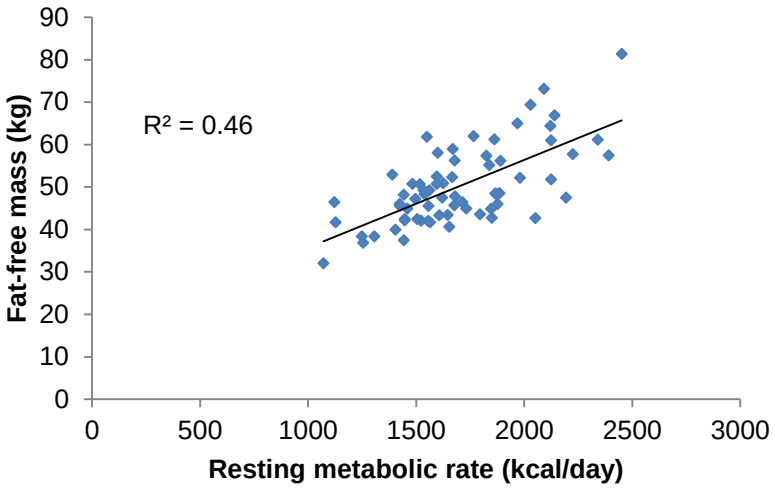


Figure 5.11 The association between FFM and RMR

5.3.9 Sex differences

This study was not designed to assess whether the associations among sedentary and active behaviours, body composition, appetite dysregulation and health differed by gender. However, independent sample t-tests revealed significant differences in some outcome variables (Table 5.1). Therefore, partial correlations controlling for sex were performed where applicable.

The correlations between sedentary and active behaviour and body composition were largely unchanged after controlling for sex. As can be seen in Table 5.7, the magnitude and direction of the partial correlations remain the same.

Table 5.7 Partial correlations between sedentary and active behaviours and body composition controlled for sex

	Body mass (kg)	BMI (kg/m²)	FM (kg)	WC (cm)	FFM (kg)
SB (min/d)	**.42****	**.51****	**.49****	**.41****	.13
Light PA (min/d)	.05	.07	.01	.07	.09
MVPA (min/d)	**-.56****	**-.63****	**-.64****	**-.58****	-.18

Data are Pearson correlation (r). ** p < .001.

When the correlations between sedentary and active behaviours and eating behaviour traits were controlled for sex, light PA was no longer associated with TFEQ-D and the strength of the correlation between MVPA and TFEQ-D was slightly weakened. The correlation between MVPA and BES was reduced to a non-significant level. Adding sex to the partial correlation controlling for body fat in the correlation between

sedentary and active behaviours and appetite dysregulation did not affect the relationships (see Table 5.8).

Table 5.8 Partial correlations between sedentary and active behaviour and markers of appetite dysregulation controlled for sex and FM

	SB (min/d)	Light PA (min/d)	MVPA (min/d)	SB (min/d)	Light PA (min/d)	MVPA (min/d)
TFEQ-D	.09	.25	**-.34***	-.20	.28	-.04
BES	.05	.16	-.27	-.19	.16	-.01

Data are Pearson correlation (r). Left panel shows partial correlations controlled for sex; right panel shows partial correlations controlled for sex and body fat. * p < .01.

The correlation between body composition and eating behaviour traits remained largely unchanged after controlling for sex. The correlations between FFM and TFEQ-D and BES became stronger but remained non-significant (see Table 5.9)

Table 5.9 Partial correlation between body composition and markers of appetite dysregulation controlled for sex

	Body mass	BMI	FM	WC	FFM
TFEQ-D	**.55***	**.67****	**.58***	**.59****	.38
BES	**.57***	**.51***	**.55***	**.55***	.45

Data are Pearson correlation (r). ** p < .001; * p < .01.

Correlations between sedentary and active behaviours and health markers observed before controlling for sex were no longer significant after controlling for sex. MVPA remained negatively associated with resting HR [r(65) = -.32, p = .008]. Only the correlations between body compositions and RMR remained significant when the correlations between body composition and health markers were controlled for sex.

5.4 Discussion

The aim of the present exploratory study was to examine the associations among objectively measured free-living sedentary and active behaviours, body composition, appetite dysregulation and markers of health, and to throw light upon the potential link between physical (in)activity and obesity.

5.4.1 Free-living sedentary and active behaviour and adiposity

These data show SB was associated with higher adiposity. However, after controlling for MVPA the magnitude of the correlation between SB and body fat was weakened and became non-significant. Previous research assessing the relationship between SB and adiposity has yielded similar results. Lynch et al. (2010) reported an association between sedentary time and WC and BMI in breast cancer survivors, furthermore after controlling for MVPA the associations were attenuated. Similarly, when lean and obese individuals were compared the obese group spent around 2 hours/d longer sedentary (Johannsen et al., 2008, Levine et al., 2005). Longitudinal studies have also demonstrated an association between SB and adiposity. Ekelund et al. (2008) found that those who gained weight over a 5 to 6 year period performed significantly more SB than those who lost weight at follow-up. Interestingly, when the correlations between light intensity PA and indices of body fatness were controlled for SB the correlations became significant and positive. Under these circumstances light PA is associated with higher BMI, FM and WC and becomes a marker for SB. There is an inverse association between light and vigorous PA; performing more light intensity PA incurs a reduction is vigorous PA. The lower vigorous PA associated with higher light intensity PA may explain the higher adiposity. The relationship between SB, light PA and adiposity has important implications given that SB and light PA accounts for the majority of the waking day (Dempsey et al., 2014). In the current sample participants spent on average just over 11 hours of their waking day in sedentary activities and over 3 hours in light PA. Similar values have been observed in previous studies (Smith et al., 2014, Hamer et al., 2012), however, some studies report less sedentary time and more light intensity PA perhaps due to variations in measurement techniques (Golubic et al., 2014, McGuire and Ross, 2012).

Our data confirm the association between MVPA and adiposity previously demonstrated (Murabito et al., 2015, Larsen et al., 2014, McGuire and Ross, 2012, Lynch et al., 2010, Healy et al., 2008a). MVPA was inversely associated with body mass, BMI, FM and WC independent of SB. The positive association between MVPA and total EE observed in the current data provides one possible explanation for the relationship with adiposity; PA results in increased EE. After controlling for MVPA all correlations between SB and indices of adiposity were nullified but all correlations remained significant between MVPA and indices of adiposity when controlled for SB. This suggests that the absence of MVPA could be more important than the presence of SB in the accumulation of FM. Recommendation to displace sedentary time with light PA may not be sufficient for weight management and to accrue any benefit PA must be at least moderate intensity in line with current PA guidelines (Department of Health, 2011b).

5.4.2 Free-living sedentary and active behaviour, appetite dysregulation and adiposity

There were no correlations between SB and any of the measures of appetite dysregulation. Light PA was positively associated with TFEQ-D and MVPA was negatively associated with TFEQ-D and BES but these relationships were no longer significant after controlling for body fat. In line with previous research TFEQ-D and BES were associated with multiple indices of adiposity (Lawson et al., 1995, Dykes et al., 2004, Hays et al., 2002, Provencher et al., 2003). TFEQ-D is associated with higher EI (Lindroos et al., 1997) and increased frequency of consumption of high energy dense foods (Lähteenmäki and Tuorila, 1995) which may lead to weight gain and could explain the positive association between TFEQ-D and adiposity. The present study has shown a strong relationship between measures of adiposity and questionnaire measures of eating that imply a loss of control over appetite in the environment. This association is supported by many studies in the literature (Bryant et al., 2008b, Bellisle et al., 2004). This outcome suggests that any observed relationship between free-living PA and trait measures of poor appetite control may be mediated indirectly via mechanisms involved in adipose tissue dynamics. However, this does not rule out the possibility that the amount of free-living sedentary and active behaviour may be linked to objective measures of homeostatic control of meal size and satiety.

5.4.3 Free-living sedentary and active behaviours, adiposity and health

SB had no direct deleterious effect on the health markers measured in this study and MVPA was negatively associated with diastolic BP and resting HR. After controlling for the amount of fat the correlation between MVPA and BP and resting HR were no longer significant. The current study sample was relatively healthy and it is possible that the negative health outcomes associated with SB identified in previous studies (Bakrania et al., 2016) may develop over time. Furthermore, participants in this study performed a high volume of MVPA in comparison to other studies (Shook et al., 2015) and this may have had a protective effect on health. Previous research has demonstrated an inverse relationship between SWA derived MVPA and components of the metabolic syndrome independent of SB (Scheers et al., 2013). On the other hand, the authors reported a negative associations between SB and features of the metabolic syndrome but this was not independent of MVPA. Previous research linking SB with health risks independent of MVPA have used proxy measures of sedentary time such as TV viewing and computer use and could reflect other behaviours which negatively impact on health (Healy et al., 2008b); TV viewing has been associated with increased snacking and being overweight (Bowman, 2006). The beneficial effects of MVPA on health are clear (Warburton et al., 2006), however, the deleterious effects of

SB on health are not well understood. Studies relating objectively measured sedentary and active behaviours with health markers are needed to better understand their independent and combined effects on health.

5.4.4 Conclusion

This study has examined the relationship between objective measures of PA (from sedentary to vigorous) and measures of adiposity under free-living conditions. The outcome has shown that the level of PA is associated with body fatness and is likely to be relevant for obesity. The outcome measures were based on systematic measures taken under natural conditions without any specific intervention. The analysis was derived from correlations (and partial correlations) and the interpretation informed by logic and plausibility. However, correlations are not proof of causation, but they certainly do not rule out the possibility of causal relationships. This study has shown strong and statistically significant links between PA and adiposity; this provides presumptive evidence that SB itself and a low level of PA are relevant for obesity. Bidirectional causality can account for this link. Therefore, low levels of PA involving low EE will lead to a positive energy balance and favour the gain of body fat. In turn a greater degree of adiposity (caused by low activity or by high EI) will serve as a disincentive to perform PA and will favour a positive energy balance and further weight gain. However, these comments are one interpretation of the data and should be clarified with further investigation.

5.5 Outcomes

- **Habitual sedentary time was associated with higher adiposity**
- **Habitual MVPA was associated with lower adiposity**
- **The strongest relationship was with MVPA**
- **FM was positively associated with dysregulated eating (TFEQ-D and BES)**
- **The relationship between physical (in)activity and adiposity is likely to be bidirectional and depends mainly on MVPA**
- **The effect of sedentary and active behaviours on appetite control may not be direct, but may be indirectly influencing appetite through FM accumulation over time**

Chapter 6
Study 2 - Consideration of Free-Living Physical Activity and Sedentary Behaviour Data from the SenseWear Armband and activPAL Separately and Combined; a Novel Integrative Procedure

This chapter will describe, visualise and evaluate data generated by the SenseWear armband mini and activPAL micro. The chapter will also describe the integration procedure for identifying and quantifying sedentary behaviour based on multiple criteria using data from both activity monitors. Data from the SenseWear armband and activPAL will be presented separately and combined to demonstrate the capabilities of the methodological platform to measure free-living sedentary and active behaviours developed as part of this thesis. Differences in weekday compared with weekend day measures of physical activity and sedentary behaviour will be compared and where comparable outputs are available for both activity monitors, comparisons will be made to assess differences in measurement method.

"Several groups have also used multisensor methods with the goal of improving activity classification … These systems typically provide a high level of accuracy that is superior to singlesensor systems and may develop into promising solutions for addressing measurement issues. However, at present, the systems themselves are often poorly suited for typical research studies in which activities will be measured over multiple days under free-living conditions and require higher-level processing skills for working with the data." (Ellingson et al., 2016, p.1636)

6.1 Introduction

There is considerable interest in movement behaviour of contemporary man given the burden of physical inactivity and SB related health problems (Biswas et al., 2015). Free-living PA and SB are complex behaviours that are notoriously difficult to accurately quantify. Until recently, free-living sedentary and active behaviours have been assessed and quantified using questionnaire based self-report measures. As technology has advanced, so too has the ability to measure bodily movement in the free-living environment due to the development of a number of objective measurement devices. Over the last 15-20 years, activity monitors have been used more frequently because of their demonstrated success and the reduced cost associated with such devices (Janz, 2006). These measurement devices overcome some of the limitations

of questionnaires, such as recall and response bias, and offer a more precise measure of free-living activity (Sallis and Saelens, 2000). However, objective measurement devices are not without problems and some activity monitors perform better than others depending on which components of PA and SB are being measured. Two key aspects of free-living PA and SB were important within the context of this thesis; activity intensity and posture during waking hours. The precise measurement of these components was key to investigating the role of free-living PA and SB in appetite control and energy balance.

The most widely used tools to objectively measure free-living movement behaviours are accelerometer based devices, but despite considerable work many challenges remain (Troiano, 2005). For example, accelerometers cannot detect non-ambulatory activities such as cycling and weight-lifting (Butte et al., 2012). In an attempt to overcome these challenges, multi sensor devices such as the SWA, have been developed which combine information from accelerometers with sensors that provide information on physiological parameters associated with PA such as heat production and galvanic skin response (Ainsworth et al., 2015). This approach has been shown to increase the accuracy of the estimated energy cost of PA, but these devices are limited as they do not detect posture (Corder et al., 2007, Calabró et al., 2014, Atkin et al., 2012). There is growing interest in posture allocation under free-living conditions since the emergence of SB as a potentially independent risk factor for negative health outcomes (Owen et al., 2010, Hamilton et al., 2008). The AP is a device that directly measures the postural element of SB. There are a number of different devices available that provide a variety of variables to reflect PA and SB performed under free-living conditions. Two of the most accurate, valid and widely used activity monitors are the SWA and the AP (Edwardson et al., 2016, Bhammar et al., 2016, Johannsen et al., 2010).

Based on the validation studies reviewed in Chapter 2 section 2.2, the SWA mini and AP micro were chosen to measure free-living sedentary and active behaviours throughout this thesis. This chapter will provide detailed technical specifications for both activity monitors and will describe the outcome measures available from proprietary software. Data collection and processing techniques that were developed as part of this thesis will be described as well as the novel integrative procedure to combine information from both activity monitors. Finally, free-living PA and SB will be described and visually represented to illustrate the capabilities of the methodological platform to measure free-living sedentary and active behaviour developed at the outset of this PhD.

6.1.1 Hypotheses

- The SWA and AP will provide realistic and detailed individual profiles of PA and SB under free-living conditions
- The way in which SB is operationally defined and measured will impact on the amount of sedentary time recorded

6.2 Methods

6.2.1 Participants

Seventy-one participants (eight men) with ≥5 days (including ≥1 weekend day) of free-living PA and SB data for the same measurement period from both the SWA and AP were included in this analysis. General recruitment strategies included emails circulated on University mailing lists and poster advertisements around the University of Leeds campus. Data from this study was taken from the three projects outlined in the general methods section of this thesis (Chapter 4). Two of the three projects were intervention studies and baseline data was used in those instances. All participants provided written informed consent before taking part in the study and ethical approval was granted by the School of Psychology Ethical Review Board (14-0099, 14-0223 and 14-0090) and the National Research Ethics Service Committee Yorkshire & the Humber (09/H1307/7).

6.2.2 Inclusion criteria

- Healthy men and women
- Aged ≥18 years
- Non smokers
- Not taking any medication known to affect metabolism or appetite

6.2.3 Exclusion criteria

- Recent change in PA or dietary habits
- Insufficient language skills to complete study questionnaires
- Contraindications to exercise
- Pregnant, planning to become pregnant or breastfeeding

6.2.4 Procedures

The three studies included in this chapter followed the same systematic protocol according to standardised operating procedures. Participants attended the research unit twice over the course of one week. Free-living PA and SB were measured continuously for a minimum of 6 days for >22 hours/d. Participants were provided with a PA diary and fitted with a SWA mini (BodyMedia, Inc., Pittsburgh, PA) and AP micro

(PAL Technologies Ltd, Glasgow, UK) and instructed to continue their normal daily living activities during the measurement period. The SWA only record data when the device is in contact with the skin and for a day to be valid there had to be ≥22 hours of data from the SWA and the AP data had to correspond to the same period without violating the wear time protocol (the procedure to identify AP wear time compliance is described in section 6.2.6.3). For a wear period to be valid there had to be ≥5 days of data including ≥1 weekend day. Participants returned to the laboratory on day 7 or 8 to return the activity monitors and completed PA diary.

6.2.5 Free-living physical activity and sedentary behaviour measurement devices

Only the SWA was used in Study 1 (Chapter 5) as the APs had not yet been purchased at the time of data collection. For the remainder of the studies the SWA and AP were used to measure free-living sedentary and active behaviour and EE. Data generated by these devices can be used to shed light on the associations between free-living sedentary and active behaviours, appetite control and energy balance.

6.2.5.1 SenseWear armband mini technical specification

The SWA measures motion (triaxial accelerometer), galvanic skin response, skin temperature and heat flux (see Figure 6.1). Participants wore the SWA on the posterior surface of their non-dominant arm and were instructed to wear it at all times and only remove it when coming in to contact with water, for example, when bathing or swimming. Classification of different intensities of PA as well as sleep, total EE and step count are calculated from the information gathered from the multiple sensors, along with demographic information (sex, age, stature, weight and handedness), using proprietary algorithms within the accompanying software (SenseWear Professional software version 8.0, algorithm v5.2). However, specific information about the extent each parameter contributes to the prediction equation in not in the public domain. The software enables the user to define cut points for different intensities of activity. Throughout this thesis SB (using the SWA only) was classified as <1.5 METs, light PA 1.5-2.9 METs, moderate PA 3.0-5.9 METs and vigorous PA >6.0 METs (Ainsworth et al., 2011). The SWA has been shown to accurately estimate time in sedentary, light and moderate activities, total EE, EE at rest and during free-living light and moderate intensity PA (Berntsen et al., 2010, Calabró et al., 2014, Malavolti et al., 2007, Johannsen et al., 2010). However, the accuracy of EE estimation is compromised at intensities ≥10 METs (Drenowatz and Eisenmann, 2011). The majority of participants recruited for the studies in this thesis were inactive and therefore were unlikely to engage in activities with an intensity greater than 10 METs.

Figure 6.1. SWA (mini) specifications

6.2.5.2 activPAL micro technical specification

The AP is a small (45 x 25 x 5 mm), light (7.6 g), thigh-mounted triaxial accelerometer worn on the front of the non-dominant leg between the hip and the knee (see Figure 6.2) which directly measures the postural element of SB. The AP was placed in a nitrile sleeve and attached to the leg with a hypafix waterproof dressing. Participants wore the AP at all times apart from when they were in contact with water for prolonged periods, for example, when swimming. The accelerometer senses dynamic accelerations produced by human movements, as well as static acceleration due to gravity and can detect the angle of incline of the thigh, allowing it to distinguish between lying/sitting, standing and stepping. However, since the thigh is horizontal when sitting and lying down, it cannot differentiate these two body postures. Proprietary algorithms (Intelligent Activity Classification) within the accompanying software (activPAL professional software version 7.2.32) classify posture as sitting/lying, standing or stepping and also provides information on the number of transitions between postures and step count per day. The AP has almost perfect correlation and excellent agreement with direct observation for sitting/lying time, upright time, sitting/lying to upright transitions and for detecting reductions in sitting (Kozey-Keadle et al., 2012, Kozey-Keadle et al., 2011, Kim et al., 2015, Grant et al., 2006). Furthermore, it accurately distinguished standing from stepping and stepping cadence, however, accuracy is compromised for detecting stepping at very low walking speeds of < 0.5 m/s (Ryan et al., 2006, Stansfield et al., 2015). It is unlikely that the participants included in this thesis walked at speeds as low as 0.5 m/s as they did not report ambulatory limitations.

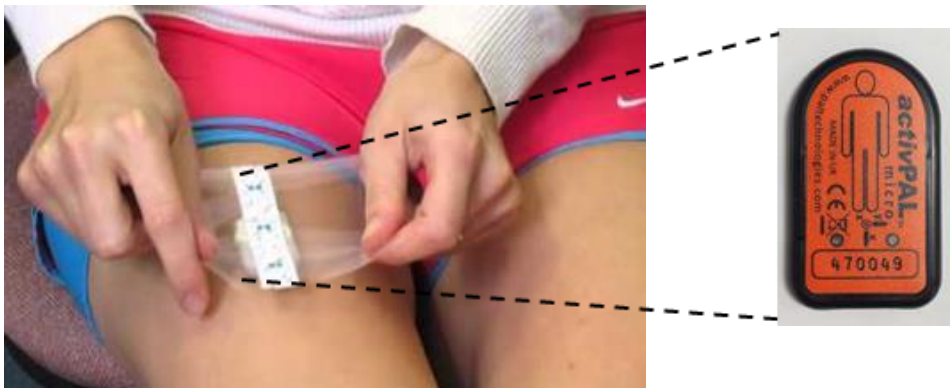


Figure 6.2. AP (micro) positioning and attachment

6.2.6 Description of data outputs using proprietary software

Both the SWA and AP have accompanying software used to initialise, download and process data collected by the devices. The way in which data is presented differs by device and software. This section will provide examples of outputs from both the SWA (SenseWear Professional 8.0) and AP software (activPAL professional 7.2.32) and describe how AP wear time and compliance was determined.

6.2.6.1 SenseWear armband proprietary software outputs

Proprietary algorithms within the SenseWear Professional software estimate steps, total EE, sleep, time spent in SB (including sleep) and light, moderate and vigorous PA. Details of the algorithms used to calculate these outputs are not in the public domain because of the commercial sensitivity of the information. Unlike other activity monitors, the SWA only records information when the sensor is on the body and therefore provides a reliable measure of wear compliance. Figure 6.3 is an example of a 24 hour recording period using the SWA from midnight to midnight of the following day. The figure demonstrates the richness of information available from the SWA. The different variables are displayed down the left hand side of the figure and the grey/blue shaded area indicates activity in each of the categories of activity. SB was interspersed with bouts of light and moderate intensity PA throughout the day, however, no vigorous PA was registered in this 24 hour period. The longest bout of moderate intensity PA occurred between 1:00 pm and 2:00 pm and there was also an increase in step count and EE at the same time which indicates the participant went for a brisk walk or jog. The area with hatched horizontal lines just before 1:00 am represents the time the SWA was off the body.

Subject	Date of Birth	Gender	Weight	Height	Handed	Smoker	BMI	BSA	WHO RMR
	04-Mar-1986 (28)	Female	99.6 kg	175.3 cm	Right	No	32.4	2.2 m²	1,945.0 kcal/day

Start Time	End Time	Duration of View	Duration on-body
Thu 6 Nov 2014 00:00	Fri 7 Nov 2014 00:00	1 day	23 hrs 43 min (98.8%)

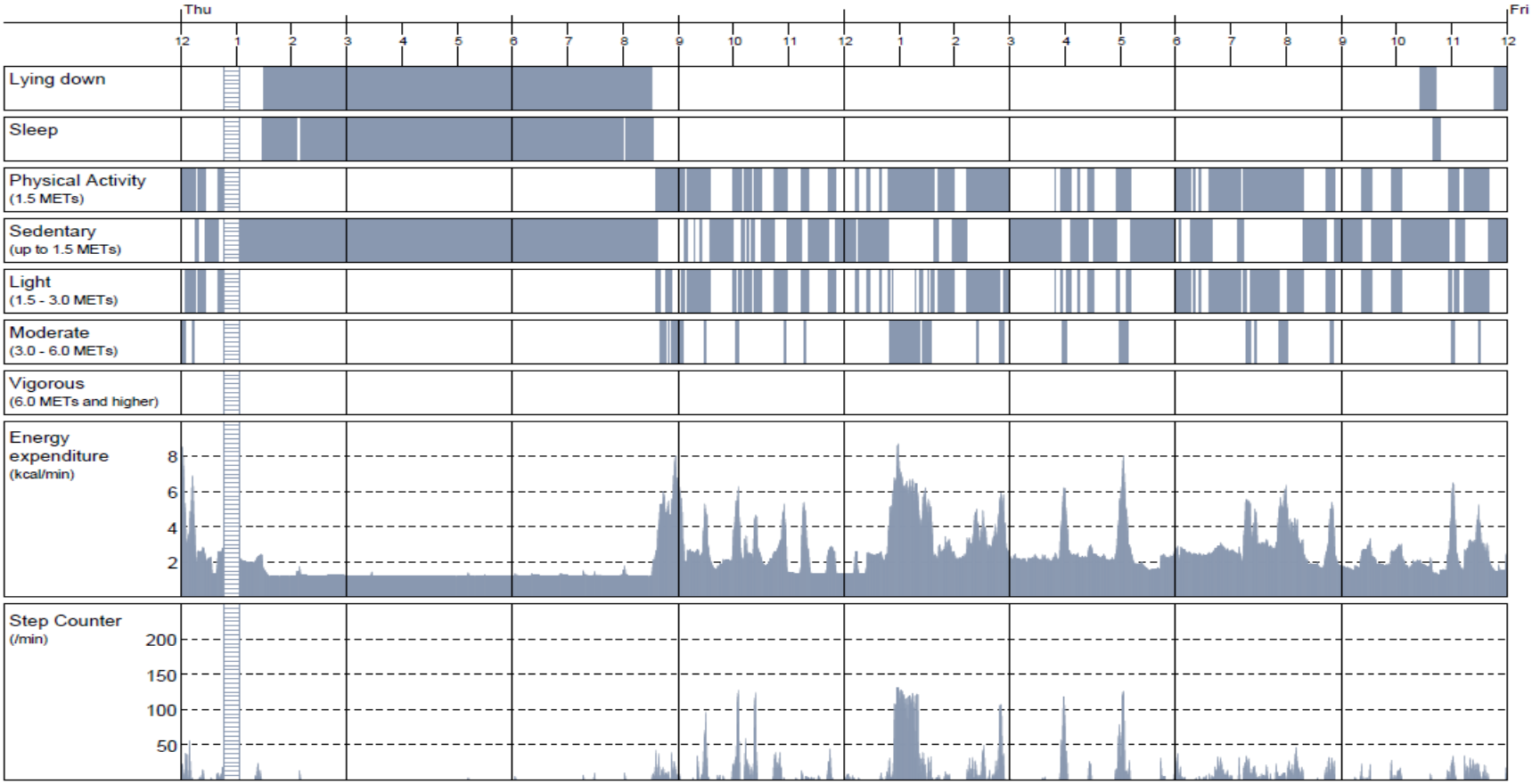


Figure 6.3 Example 24 hour output from the SenseWear Professional software from midnight to midnight

6.2.6.2 activPAL proprietary software outputs

Proprietary algorithms within the AP software use accelerometer-derived information about thigh position and acceleration to determine body posture (sitting/lying, standing and stepping), transitions between postures, number of steps and stepping speed. Figure 6.4 to Figure 6.8 are outputs from the AP software. Figure 6.4 shows the amount of time spent sitting/lying, standing and stepping, the number of upright and seating/lying events and the number of steps accumulated at different stepping speeds. The output also provides an estimate of EE in MET hours (calculated based on stepping rate), however this measure was not reported in this thesis as it does not include factors such as sex, age and body mass and has been shown to differ significantly from IC during treadmill walking and running (Harrington et al., 2011). It is clear from the pie chart that most of the 24 hour period was spent sitting/lying, followed by standing with the smallest proportion of the day occupied by stepping. Stepping rate (cadence) varied during the day from 20-30 steps/min to 140-150 steps/min. For stepping to reach moderate intensity a stepping rate of at least 100 steps/min must be achieved (Tudor-Locke et al., 2005).

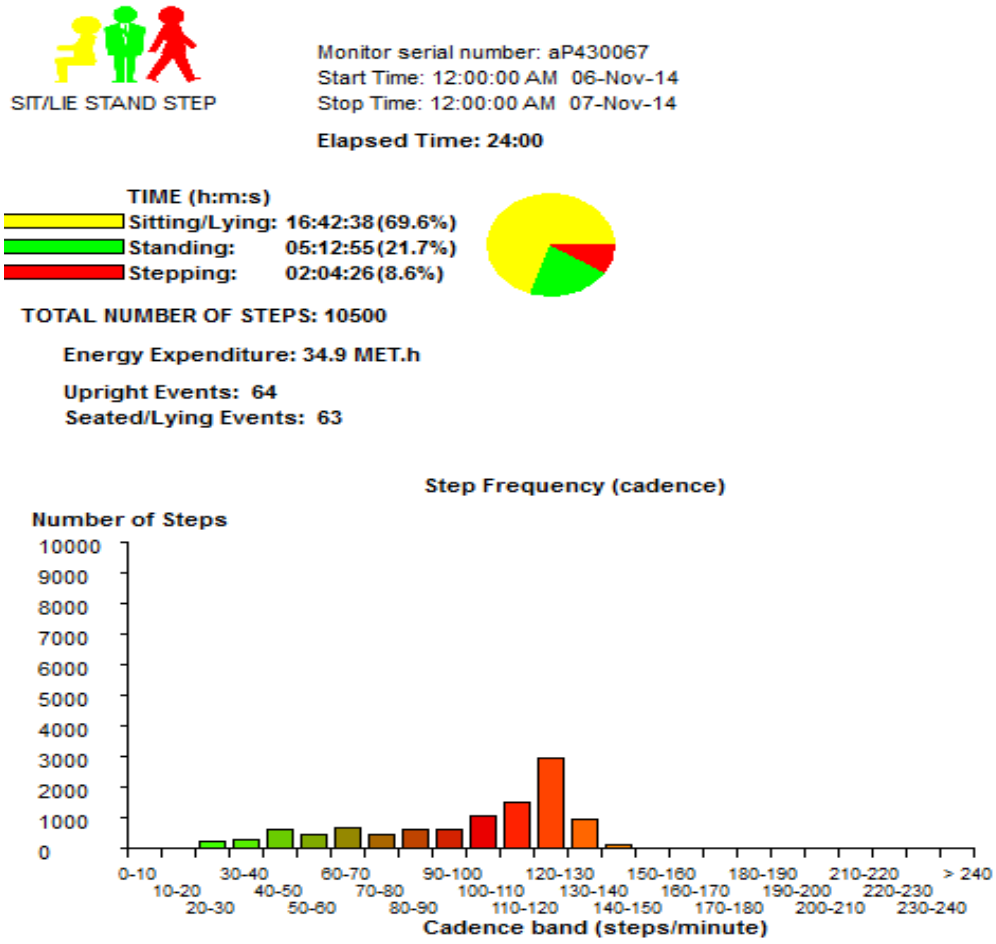


Figure 6.4 Time spent sitting standing and stepping, number of transitions between postures and stepping speed

Figure 6.5 and Figure 6.6 are graphical representations of how time spent standing and sitting/lying was accumulated throughout the day. The number of upright events in bout lengths ranging from 1-10 minutes to >140 minutes are displayed in Figure 6.5. The most frequent bout length is 1-10 minutes and the frequency of events declines as the category duration increases. The least frequent but longest upright event is 40-50 minutes. Similarly, Figure 6.6 shows the majority of seated events are 1-10 minutes long. However, some sitting/lying time is accumulated in prolonged bouts of 60-70 minutes and 90-100 minutes.

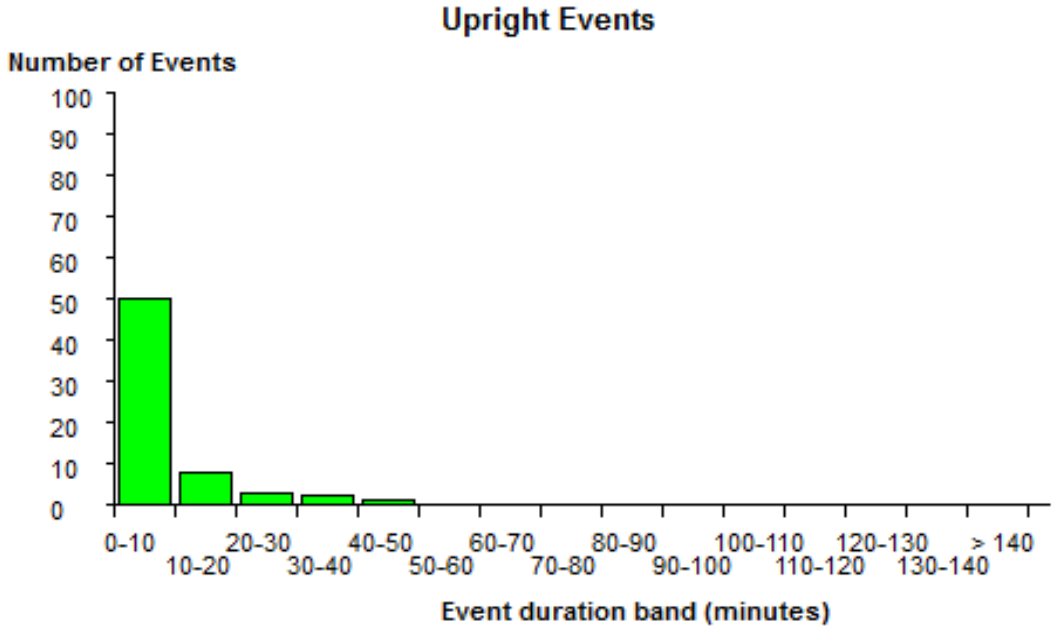


Figure 6.5 Number of upright events categorised by the event duration

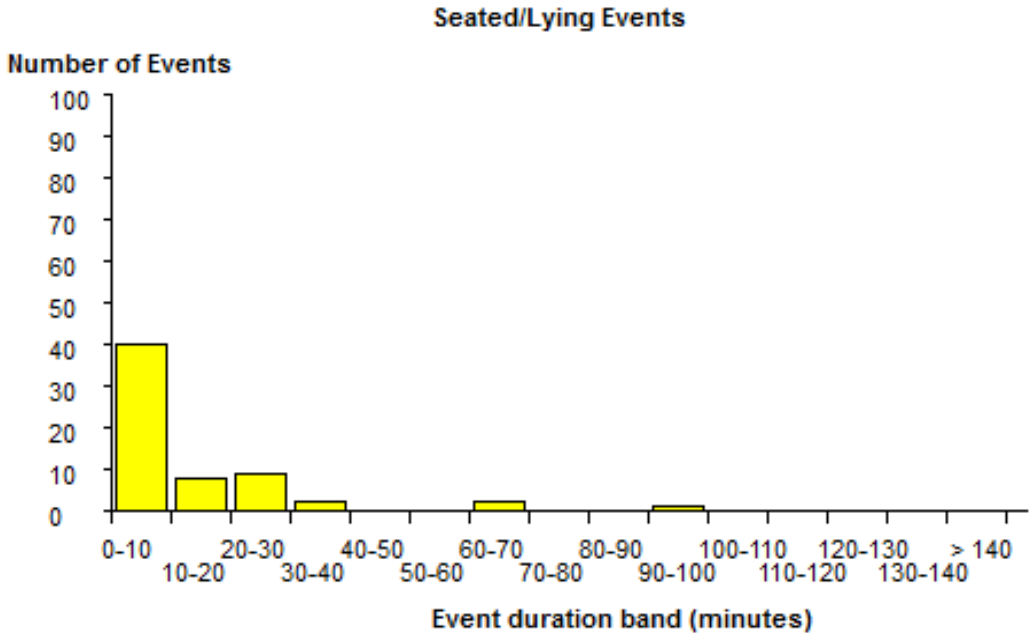


Figure 6.6 Number of seated/lying events categorised by the event duration

Finally, Figure 6.7 and Figure 6.8 show the amount of time spent sitting/lying (yellow), standing (green) or stepping (red) in 15 second epochs for every hour. Figure 6.7 shows the participant was predominantly sitting/lying between 12:00 am and 7:00 am and between 1:30 am and 8:30 am no steps or transitions were registered suggesting the participant was sleeping.

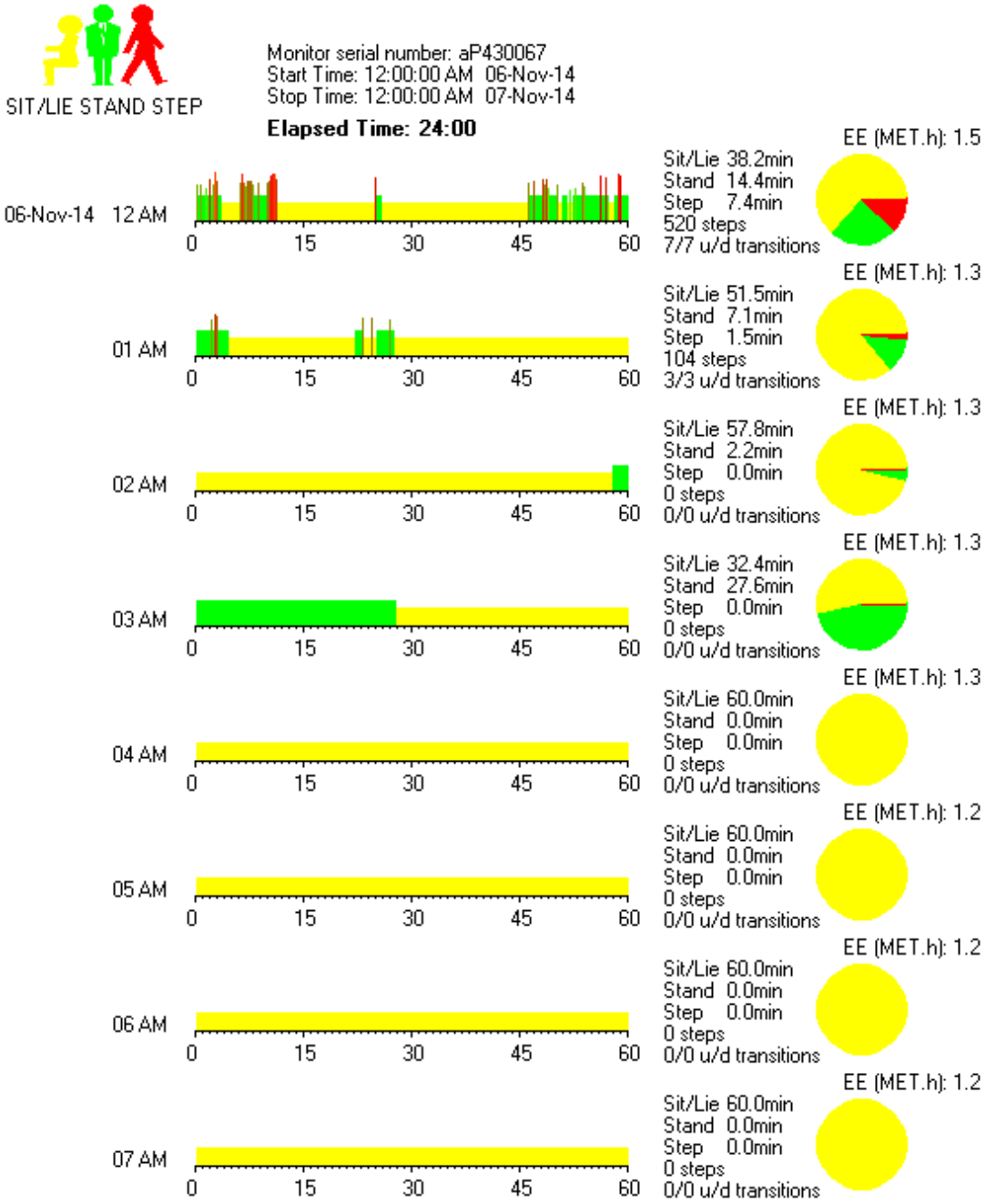


Figure 6.7 Time spent sitting/lying, standing and stepping per hour during the night. Yellow indicates sitting/lying, green indicates standing and red indicated stepping

Figure 6.8 shows waking hours between 8:00 am and 6:00 pm. During waking hours the majority of time was spent sitting/lying and was interspersed with brief periods of

standing or stepping. However, there does appear to be a period of prolonged stepping from 12:55 pm to 1:20 pm and a prolonged period of standing between 6:00 pm and 7:00 pm.

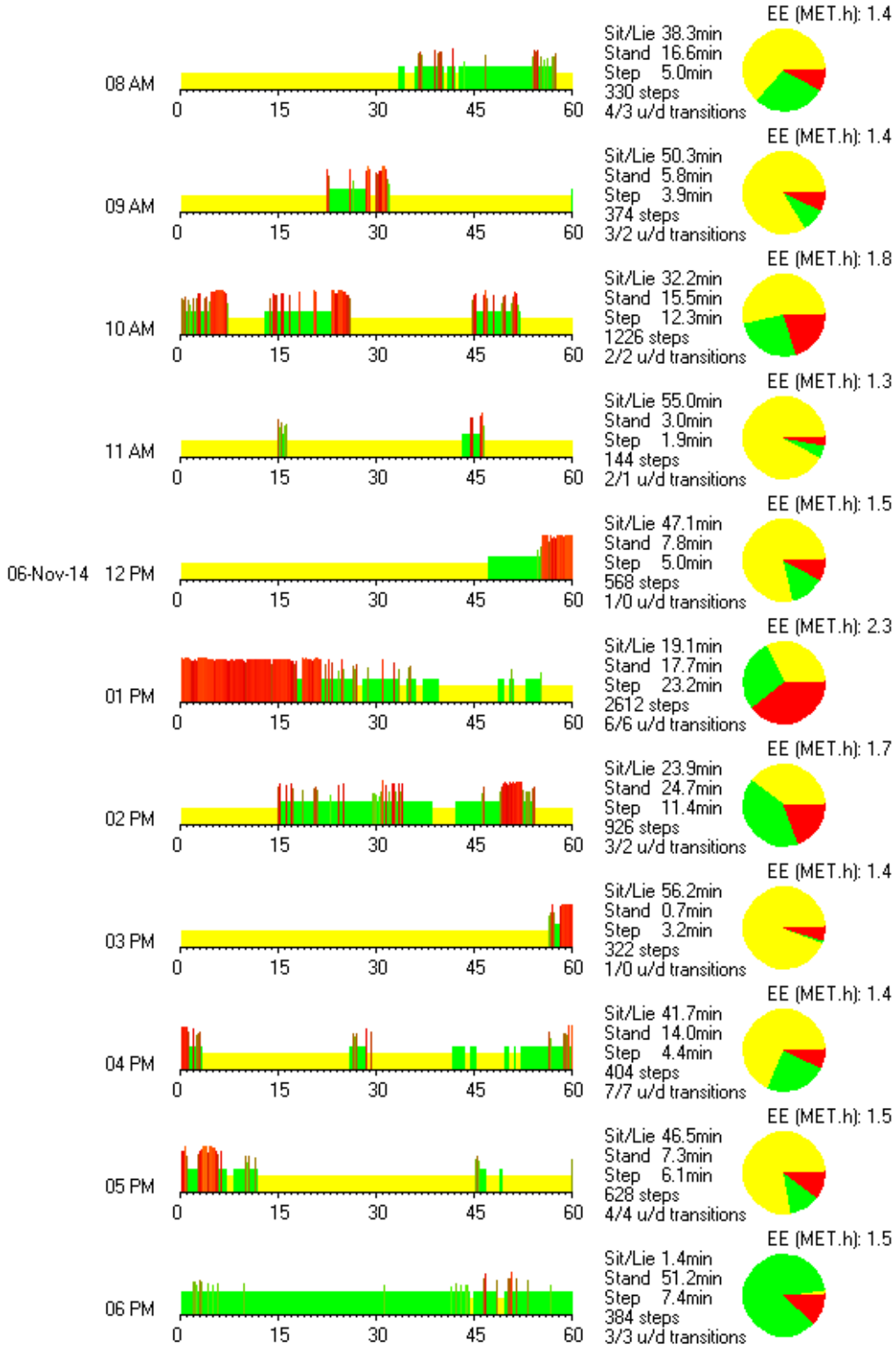


Figure 6.8 Time spent sitting/lying, standing and stepping per hour during the day. Yellow indicates sitting/lying, green indicates standing and red indicated stepping

6.2.6.3 Determining compliance with the activPAL wear protocol

Unlike the SWA, the AP does not indicate when the device has been removed. To determine compliance with the AP wear protocol the downloaded data was visually inspected within the proprietary software for prolonged periods of continuous sitting/lying or standing during waking hours (>2 hours) as this would indicate the device had been removed. To illustrate this process Figure 6.9 shows a 24 hour output from the AP software. Each bar represents one hour, yellow represents sitting/lying, green standing and red stepping. It is evident from the output that between 9:00 am and 12:00 noon there is a prolonged bout of sitting. To check whether this was an indication the participant had removed the AP or if it was a true reflection of behaviour, the corresponding period of time was cross checked within the SWA data. Figure 6.10 shows the SWA also recorded a prolonged period of SB between 9:00 am and 12 noon and indicates the AP data reflect true behaviour rather than non-compliance with the wear protocol. This process was performed for all AP data. If the SWA recorded periods of PA during prolonged periods of sitting/lying measured by the AP it would be deduced that the AP was not on the body and therefore data for that day would be excluded. None of the data collected as part of this PhD were excluded for this reason.

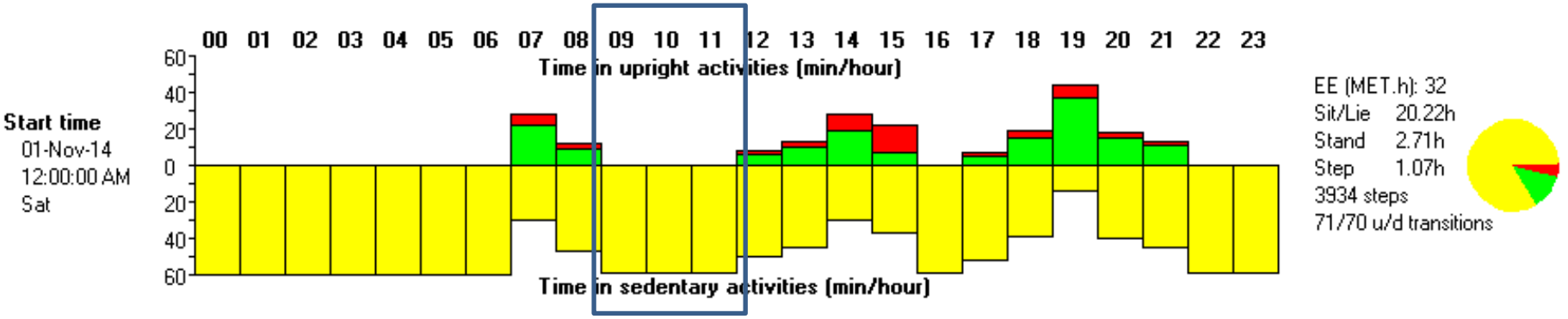


Figure 6.9 Example 24 hour output from the AP. Each bar represents one hour and is comprised of either sitting/lying (yellow), standing (green) or stepping (red) or a combination of all three

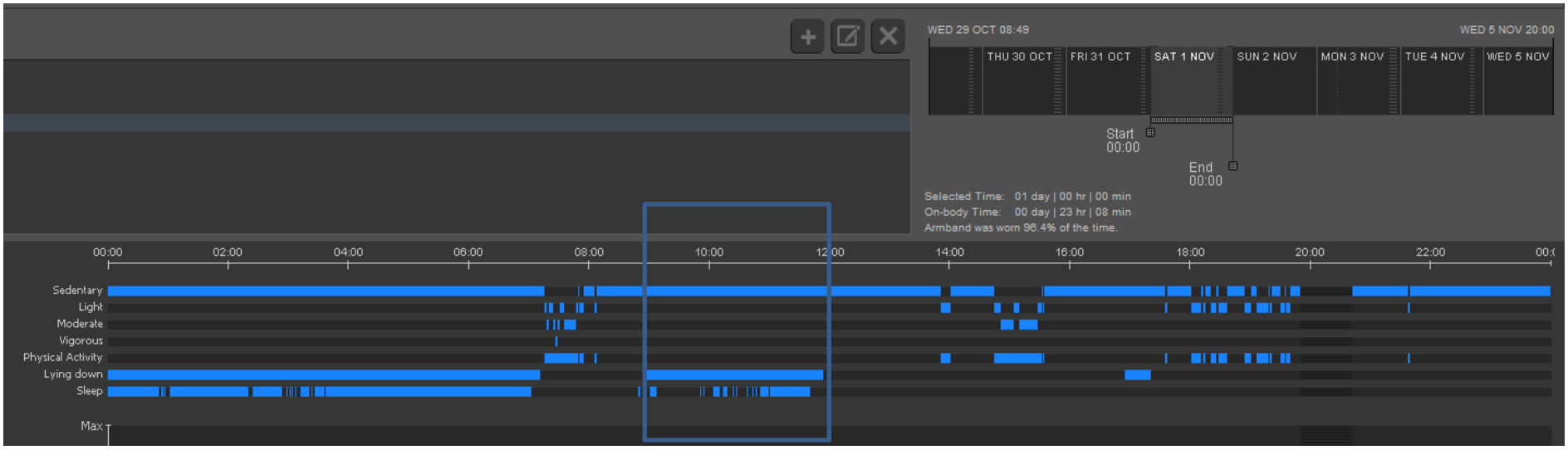


Figure 6.10 Example 24 hour output from the SWA. The blue shaded areas indicate activity in the corresponding category of activity listed down the left hand side of the figure

6.2.7 Data integration: development of a novel set of procedures

6.2.7.1 Integrating data from the SenseWear armband and activPAL

The widely accepted definition of SB includes both activity intensity and postural elements during waking hours. According to the Sedentary Behaviour Research Network (2012) SB is any waking behaviour characterized by an activity intensity of less than 1.5 METs whilst in a sitting or reclining posture. There are no field-based activity monitors available on the market that are capable of accurately and reliably measuring both posture and activity intensity (Edwardson et al., 2016, Gibbs et al., 2015). Indeed, the definition of SB differs depending on which activity monitor is being used to measure it. The AP defines SB based on a sitting or reclining posture, whereas the SWA definition is based on activity intensity (<1.5 METs). Each of the devices alone have limitations when measuring SB defined by both activity intensity and posture in line with the Sedentary Behaviour Research Network (2012) definition. Recent research has demonstrated combining information from multiple devices yields a more accurate measure of sedentary and active behaviour (Kim and Welk, 2015). The authors encouraged further research into the 'multi-method approach'. Furthermore, data from the ActiGraph and AP has been shown to have greater overall accuracy when classifying activity intensity and estimating EE when data from both monitors were integrated (Ellingson et al., 2016). However, the utility of this system has not been tested under free-living conditions. Therefore, an integrated method to combine data from the SWA and AP was developed at the outset of this PhD. Data from the SWA and AP were integrated to enable the classification of SB using the activity intensity and sleep variables from the SWA and the posture allocation variable from the AP during free-living conditions over 5-7 days.

6.2.7.2 Processing integrated data: developing a sedentary behaviour measure based on multiple criteria

Both activity monitors were initialized and downloaded on the same computer such that their internal time stamps would match in order to facilitate data processing. Data from the SWA can only be exported from the proprietary software into Microsoft Excel in 60 second epochs whereas data from the AP can only be exported in 15 second epochs. To integrate data from the two activity monitors a program was developed to condense the 15 second epochs from the AP into 60 second epochs to match the SWA data. Time spent sitting, standing, stepping and number of steps for every four 15 second epochs was summed to create 60 second epochs. See Table 6.1 and Table 6.2 for examples of data before and after converting to 60 second epochs. For a 60 second epoch to be classified as sedentary all 60 seconds had to be spent sitting/lying and awake. This method excludes on average approximately 30-60 minutes of sitting time from the AP sitting/lying variable depending on how sedentary time was

accumulated (frequent short bouts of sitting throughout the day would result in more sedentary time being excluded as there would be more epochs with sitting accounting for <60 seconds). The SWA is likely to exclude a similar amount of sedentary time within the proprietary software. It is not known exactly how the SenseWear software calculates each 60 second epoch but it is probable that an epoch is categorised based on the average intensity of activity performed during those 60 seconds (Chen and Bassett, 2005). Thus, if a 60 second period contained SB and light or moderate PA then the data would be averaged to reflect an intermediate intensity.

Table 6.1 Example of AP data in 15 second epochs before being converted to 60 second epochs

Time	Sitting/lying (seconds)	Standing (seconds)	Stepping (seconds)	Steps
11:42:00	0	15	0	0
11:42:15	0	15	0	0
11:42:30	0	10.1	4.9	6
11:42:45	0	15	0	0
11:43:00	0	6.7	8.3	12
11:43:15	0	13.2	1.8	0
11:43:30	0	15	0	0
11:43:45	0	15	0	0

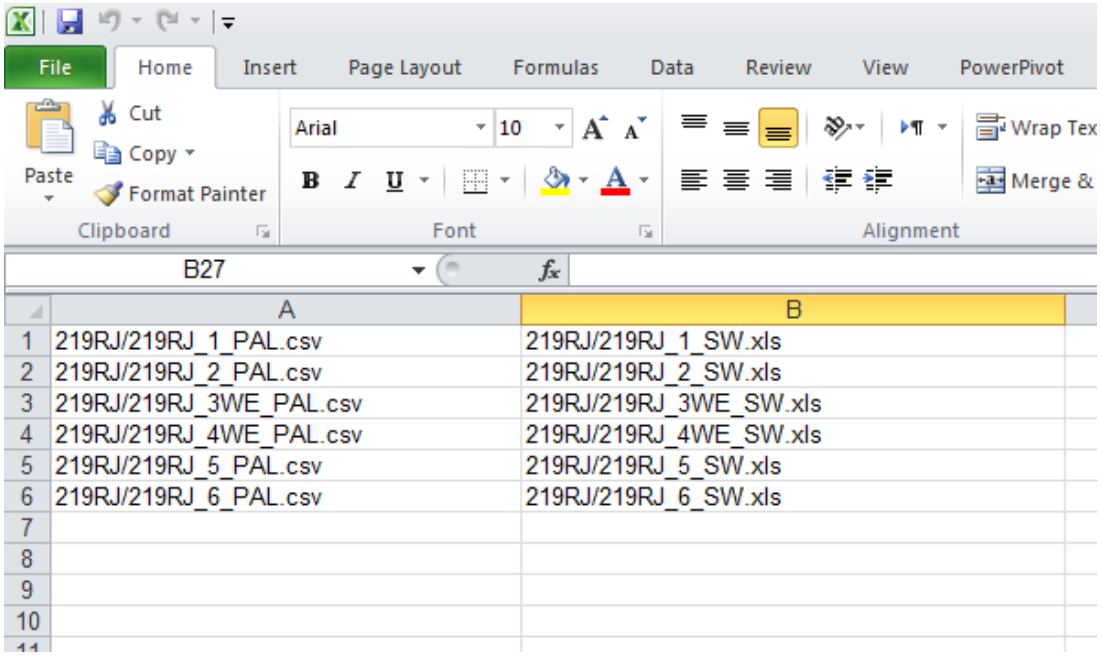
Table 6.2 Example of AP data condensed to 60 second epochs

Time	Sitting/lying (seconds)	Standing (seconds)	Stepping (seconds)	Steps
11:42:00	0	55.1	4.9	6
11:43:00	0	49.9	10.1	12

For a free-living PA and SB monitoring period of 5-7 days there were 5-7 pairs of exported files from the SWA and AP and each pair represented one day of raw data (24 hours from midnight to midnight). To run the program to integrate the data, the pairs of files were placed in a folder and a spreadsheet was created to tell the integration program where the files were stored and the names of the pairs of files to integrate (see Figure 6.11 and Figure 6.12). The data integration program produced one output file for the free-living PA and SB monitoring period and each tab within the file contained data from both activity monitors for one 24 hour period.

Name	Date modified	Type	Size
219RJ_1_PAL.csv	16/06/2015 07:55	Microsoft Excel Com...	157 KB
219RJ_1_SW.xls	19/11/2014 10:22	Microsoft Excel 97-...	517 KB
219RJ_2_PAL.csv	16/06/2015 07:56	Microsoft Excel Com...	157 KB
219RJ_2_SW.xls	19/11/2014 10:22	Microsoft Excel 97-...	514 KB
219RJ_3WE_PAL.csv	16/06/2015 07:56	Microsoft Excel Com...	155 KB
219RJ_3WE_SW.xls	19/11/2014 10:22	Microsoft Excel 97-...	518 KB
219RJ_4WE_PAL.csv	16/06/2015 07:57	Microsoft Excel Com...	155 KB
219RJ_4WE_SW.xls	19/11/2014 10:23	Microsoft Excel 97-...	503 KB
219RJ_5_PAL.csv	16/06/2015 07:57	Microsoft Excel Com...	156 KB
219RJ_5_SW.xls	19/11/2014 10:23	Microsoft Excel 97-...	522 KB
219RJ_6_PAL.csv	16/06/2015 07:58	Microsoft Excel Com...	155 KB
219RJ_6_SW.xls	19/11/2014 10:26	Microsoft Excel 97-...	516 KB

Figure 6.11 Pairs of PA monitor data files to be integrated. Each .xls file contained 24 hours of data exported from the SWA software and each .csv file contained 24 hours of data exported from the AP software



	A	B
1	219RJ/219RJ_1_PAL.csv	219RJ/219RJ_1_SW.xls
2	219RJ/219RJ_2_PAL.csv	219RJ/219RJ_2_SW.xls
3	219RJ/219RJ_3WE_PAL.csv	219RJ/219RJ_3WE_SW.xls
4	219RJ/219RJ_4WE_PAL.csv	219RJ/219RJ_4WE_SW.xls
5	219RJ/219RJ_5_PAL.csv	219RJ/219RJ_5_SW.xls
6	219RJ/219RJ_6_PAL.csv	219RJ/219RJ_6_SW.xls
7		
8		
9		
10		

Figure 6.12 Example of data integration program input file. Input file tells the integration program where the files that are to be integrated are located. Each cell contains the folder name followed by file name. The name and location of the AP files was written in column A and the corresponding SWA file name and location was written in column B

The resultant output file from the integration program contained both SWA and AP data with each row of data corresponding to 60 seconds. Integrated data was then processed using a Microsoft Excel template containing formulae to calculate the following:

- **SWA data**: total (per week) and average (per day) minutes of sleep, SB excluding sleep (SED^{SWA}), light, moderate and vigorous PA; number of sedentary periods in bout lengths ranging from 1-5 minutes to >40 minutes and the number of minutes accumulated in each category; total (per week) and average (per day) total EE[1] and energy expended during sleep, sedentary, light, moderate and vigorous PA.
- **AP data**: total (per week) and average (per day) number of sit-to-stand movements; total (per week) and average (per day) minutes spent sedentary (SED^{AP}; sleep variable from the SWA was used to exclude sit/lie time registered during sleep) sitting or lying (including sleep), standing, stepping, number of steps; number of sedentary periods in bout lengths ranging from 1-5 minutes to >40 minutes and the number of minutes accumulated in each category. **For a minute to be classified as sedentary using the condensed 60 second epoch data, the behaviour classification had to be sitting for the full 60 seconds and awake (from the SWA sleep detection variable). If any other behaviour was detected during that minute the SB that did occur was not counted.
- **Integrated data**: total (per week) and average (per day) minutes spent sedentary excluding sleep (SED^{INT}); number of sedentary periods in bout lengths ranging from 1-5 minutes to >40 minutes and the number of minutes accumulated in each category.

The variables described above were used in analyses presented throughout this thesis. The SB and PA variables were calculated as averages per day (total minutes for each category of activity over the whole monitoring period divided by the number of days the activity monitor was worn). Sedentary time measured using the SWA, AP and integrated data will be referred to as SED^{SWA}, SED^{AP} and SED^{INT}, respectively.

6.2.8 Statistical Analysis

Data are reported as mean ± SD throughout. Statistical analysis was performed using IBM SPSS for Windows (Chicago, Illinois, Version 21). Characteristics of the study population and time spent sedentary and in different intensities of PA were summarised using descriptive statistics. Paired sample t-tests were performed to identify differences in steps measured by the SWA and AP, and PA and SB performed on weekdays compared with weekend days. Differences in sedentary time using SED^{SWA}, SED^{AP} and SED^{INT} methods were examined using repeated measures

[1] Diet-induced thermogenesis (DIT) was not measured in any of the studies in this thesis. In healthy subjects consuming a mixed diet, DIT represents about 10% of the total amount of energy ingested over 24 hours. Therefore, when an individual is in energy balance, DIT is 10% of daily EE.

ANOVA with Bonferroni post-hoc tests. Relationships were regarded as significant with a p value < .05.

6.3 Results

6.3.1 Description and visualisation of a typical weeks free-living physical activity and sedentary behaviour; averages from an adult sample

Study sample characteristics are presented in Table 6.3. Of the 71 participants, eight were men.

Table 6.3. Descriptive statistics of study sample

	Mean (SD)	Range
Age (years)	36.97 (13.74)	19.00 – 69.00
Stature (m)	1.65 (0.08)	1.49 – 1.89
Body mass (kg)	79.35 (13.90)	44.90 – 115.80
BMI (kg/m^2)	29.02 (4.78)	19.00 – 42.50
Monitoring period (days)	6.48 (0.67)	5.00 – 7.00

6.3.1.1 SenseWear armband data description

Data presented in this section are calculated from raw data from the SWA using the Microsoft Excel template described in section 6.2.7.2.

6.3.1.1.1 Average minutes per day spent in sleep, SED^{SWA}, light, moderate and vigorous physical activity

Behaviour can be split in to five distinct categories: sleep, SB, light, moderate and vigorous PA. These categories are mutually exclusive; change in time spent in one category will inevitably lead to a change in time spent in at least one of the other four categories. The sum of the amount of time per day spent in each of these activities when measured using the SWA equates to 24 hours (1,440 minutes) minus the time the armband was off the body (see Table 6.4).

Table 6.4. Average time per day spent in different categories of activity measured using the SWA

	Min/d		Hours/d	
	Mean (SD)	**Range**	**Mean (SD)**	**Range**
SWA wear time	1416.76 (15.86)	1362.00 – 1440.00	23.61 (0.26)	22.70 – 24.00
Sleep	444.35 (56.78)	330.00 – 594.00	7.41 (0.95)	5.50 – 9.90
SED^{SWA}	697.07 (101.34)	361.00 – 883.00	11.62 (1.69)	6.02 – 14.72
Light PA	171.97 (63.71)	59.00 – 342.00	2.87 (1.06)	0.98 – 5.70
Moderate PA	97.07 (61.43)	15.00 – 369.00	1.62 (1.02)	0.25 – 6.15
Vigorous PA	6.23 (9.51)	0.00 – 48.00	0.10 (0.16)	0.00 – 0.80
MVPA	103.30 (67.72)	15.00 – 402.00	1.72 (1.13)	0.25 – 6.70

Figure 6.13 shows the amount of time the current sample spent on average per day in sleep, SED^{SWA}, light, moderate and vigorous PA. SED^{SWA} accounted for the majority of the waking day. As the intensity of activity increased, the time spent in that category decreased. Some participants did not register any vigorous PA during the full 5-7 day monitoring period.

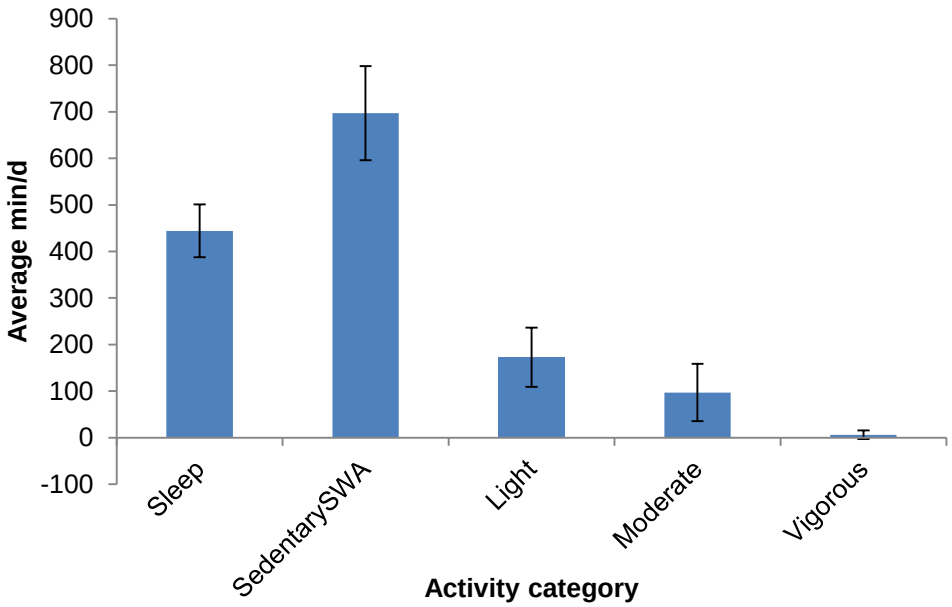


Figure 6.13 Average minutes per day spent in different categories of activity measured using the SWA

Studies have reported differences in activity patterns on weekdays and weekend days. Figure 6.14 shows the difference in sleep, SED^{SWA} and light, moderate and vigorous PA on weekdays compared with weekend days. Paired sample t-tests revealed there

was no significant difference in the amount of light [p = .71], moderate [p = .68] or vigorous PA [p = .28] performed on weekdays compared with weekend days. Participants slept significantly longer on weekend days (M = 469.67 min/d, SD = 83.51) compared with weekdays (M = 433.57 min/d, SD = 61.37) [t(70) = -3.54, p < .001] and the longer sleep duration on weekend days displaced sedentary time as the difference in SED^{SWA} on weekdays (M = 708.48 min/d, SD = 111.08) compared with weekend days (M = 671.98 min/d, SD = 130.24) was also significant [t(70) = 2.45, p = .02].

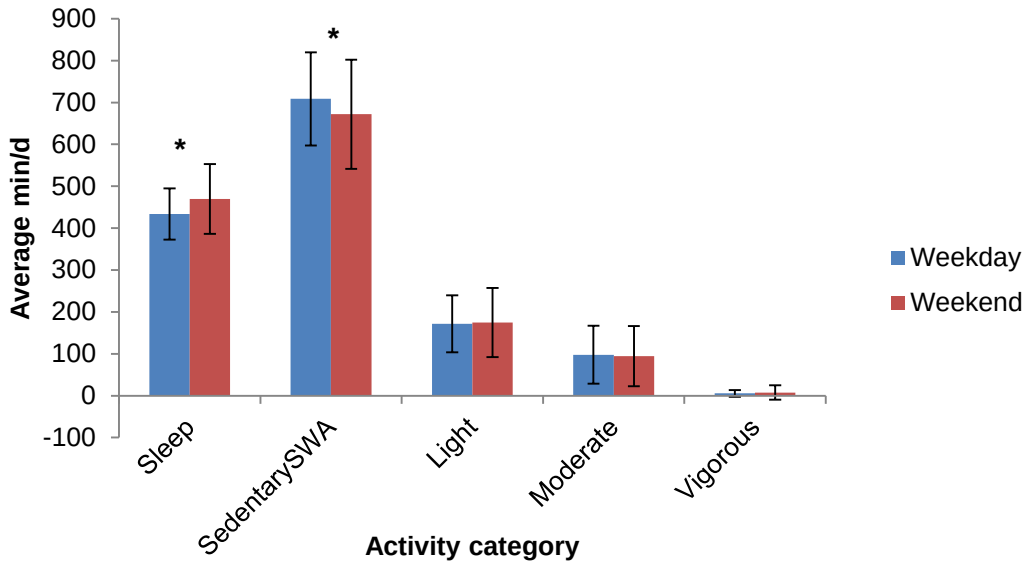


Figure 6.14 Difference in average time per day spent in different categories of activity on weekdays and weekend days measured using the SWA

6.3.1.1.2 Energy expenditure during sleep, sedentary behaviour and light moderate and vigorous physical activity

The SWA also provides information about EE. The proprietary software provides data on total EE, measured EE (EE whilst the SWA is on the body) and estimated RMR (WHO equation). In addition to the information on EE available from the proprietary software, it was possible to calculate EE in different intensities of activity from the raw data from the SWA. Below are graphical representations of energy expended whilst in different intensities of activity. Data are presented as averages per day. SED^{SWA} accounts for the largest proportion of the day and Figure 6.15 shows that the majority of daily EE occurs during sedentary activities. Vigorous intensity PA contributed the least to total EE. As the intensity of activity increased, the amount of time required to expend a given number of calories decreased (see Figure 6.15). The number of calories per minute on average expended in sleep, SED^{SWA} and in light, moderate and

vigorous PA was 1.04 kcal/min (SD = 0.17), 1.46 kcal/min (SD = 0.21), 2.71 kcal/min (SD = 0.55), 5.08 kcal/min (SD = 0.72) and 8.28 kcal/min (SD = 1.78), respectively.

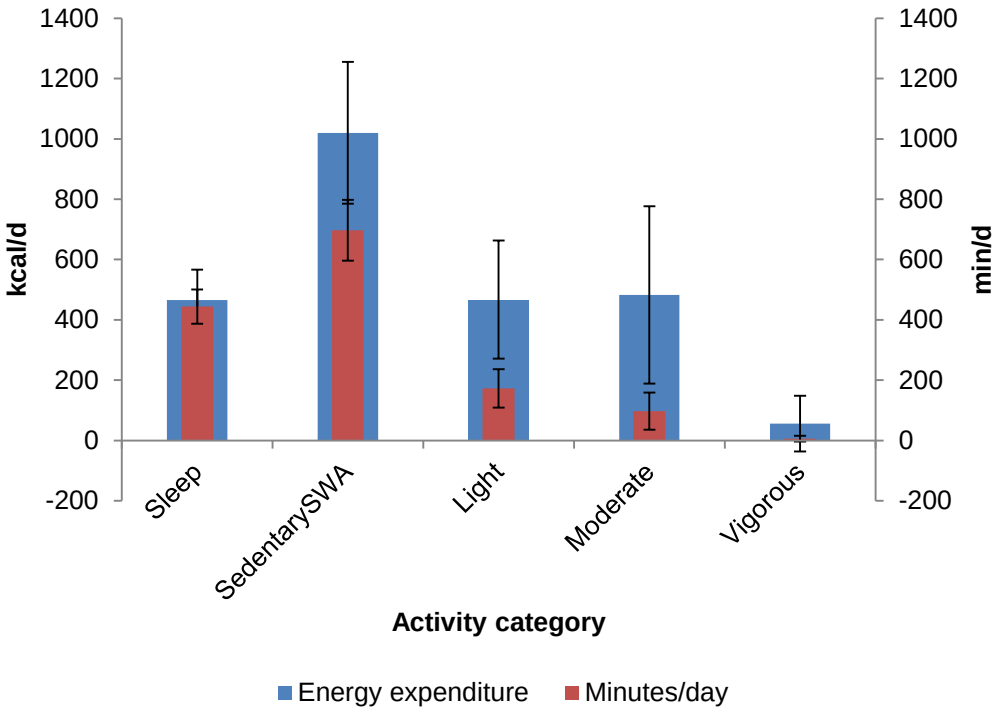


Figure 6.15 Time and EE in different categories of activity measured using the SWA

6.3.1.2 activPAL data description

Data presented in this section were collected using the AP. The AP does not differentiate between sleep and awake time therefore, the sleep variable from the SWA was used to remove sitting/lying whilst asleep from the AP data and is represented by SED^{AP}.

6.3.1.2.1 Average minutes per day spent sitting/lying, standing or stepping, number of steps and transitions from stand to sit

During the course of a 24 hour monitoring period an individual can be either sleeping, SED^{AP}, standing or stepping when activity is measured using the AP. As with categories of behaviour based on activity intensity, categories based on posture allocation are also related. Time spent in these behaviours are collinear, that is, every increase in the total time spent in one behaviour necessarily causes a decrease in the total time spent in one or more of the other behaviours. The sum of the amount of time per day spent in each of these activities equates to approximately 24 hours (1,440 minutes). Some of the SED^{AP} time is excluded when it is integrated with the SWA

sleep variable as only epochs with a full 60 seconds of sitting/lying are classified as sedentary (see section 6.2.7.2 for further explanation). Although the AP does not indicate when the device has been removed from the thigh, participants were instructed to wear the device at all times and visual inspection of the AP data indicated compliance with the continuous wear time protocol. The amount of waking time spent SED^{AP}, standing and stepping is displayed in Table 6.5, and Table 6.6 shows the average number of steps and transitions between postures per day.

Table 6.5 Average time per day spent SED^{AP}, standing and stepping measured using the AP

	Min/d		Hours/d	
	Mean (SD)	**Range**	**Mean (SD)**	**Range**
SED^{AP}	612.90 (102.28)	348.00 - 864.00	10.22 (1.71)	5.80 - 14.40
Standing	235.66 (84.25)	105.00 - 482.00	3.93 (1.40)	1.75 - 8.00
Stepping	107.68 (32.03)	51.00 - 195.00	1.80 (0.53)	0.85 - 3.25

Table 6.6 Average number of steps and transitions between postures per day measured using the AP

	Mean (SD)	**Range**
Steps/d	9302.76 (3176.19)	3770.00 - 18768.00
Sit to stand transitions/d	52.17 (12.91)	20.00 - 86.00

The amount of time the current sample spent on average per day SED^{AP}, standing and stepping are displayed in Figure 6.16. The amount of time spent SED^{AP} accounted for the largest portion of the day followed by standing and the least amount of time was occupied by stepping. As with the SWA derived measures of PA, as the intensity of the activity increased the time spent in that activity category decreased.

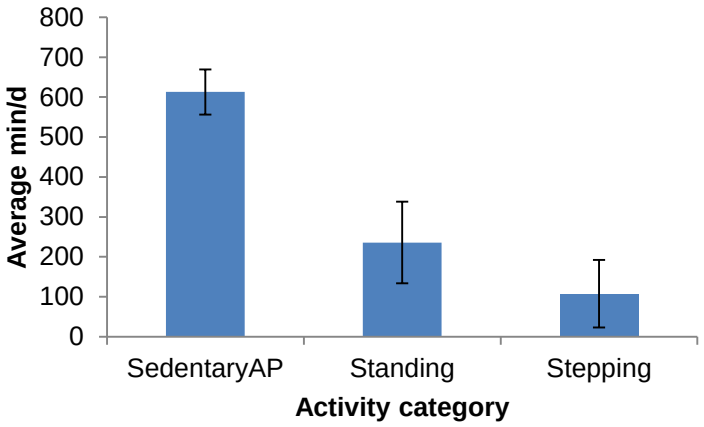


Figure 6.16 Average time per day spent sedentary, standing and stepping measured using the AP

Differences in PA and SB on weekdays compared with weekend days when measured using the AP are displayed in Figure 6.17. Paired sample t-tests revealed that weekday and weekend day time spent standing [p = .55] and stepping [p = .30] were not significantly different. Participants spent 28 minutes per day longer SED^{AP} on weekdays compared with weekend days and this difference was statistically significant [t(70) = 2.12, p = .03].

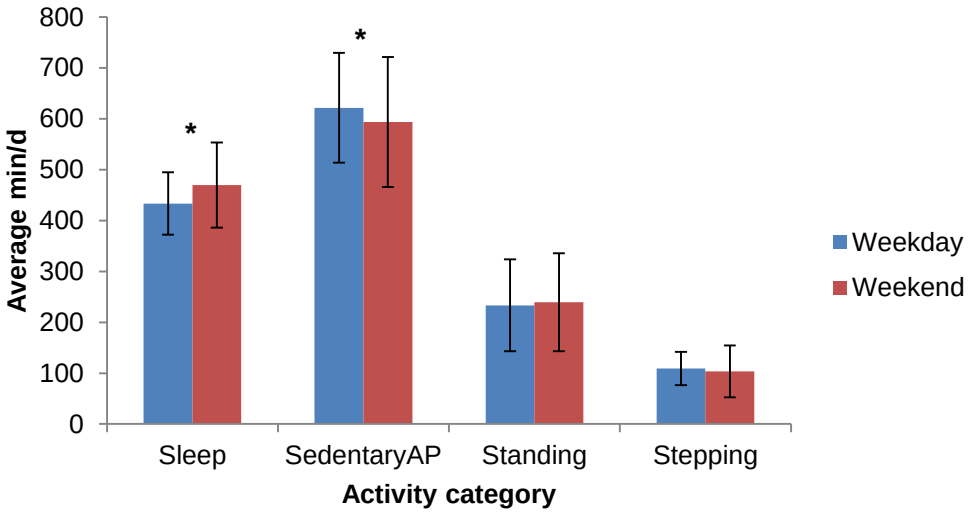


Figure 6.17 Difference in average time per day spent in different categories of activity during weekdays and weekend days measured using the AP

- **The SWA and AP provide realistic and detailed profiles of average daily PA and SB**
- **The amount of PA did not differ between weekdays and weekend days**
- **Sleep displaced sedentary time on weekend days**

6.3.1.3 A novel integrative method to classify sedentary behaviour based on activity intensity and posture during waking hours

Integrated data combines information from both the SWA and the AP to generate a SB variable based on activity intensity and posture during waking hours (see section 6.2.7 for more details). SED^{INT} excludes sitting >1.5 METs (which would be included in SED^{AP}) and standing <1.5 METs (which would be included in SED^{SWA}). As shown in Figure 6.18, even when SB is classified based on multiple criteria it still accounts for around 9 hours/d (M = 546.00 min/d, SD = 99.19). Figure 6.18 shows the difference in SED^{INT} on weekdays (M = 552.95 min/d, SD = 108.52) compared with weekend days (M = 531.32 min/d, SD = 126.49). As with SED^{SWA} and SED^{AP} participants accumulated more sedentary time on weekdays compared with weekend days when it was measured using the integrated method but the difference was not significant [p = .14].

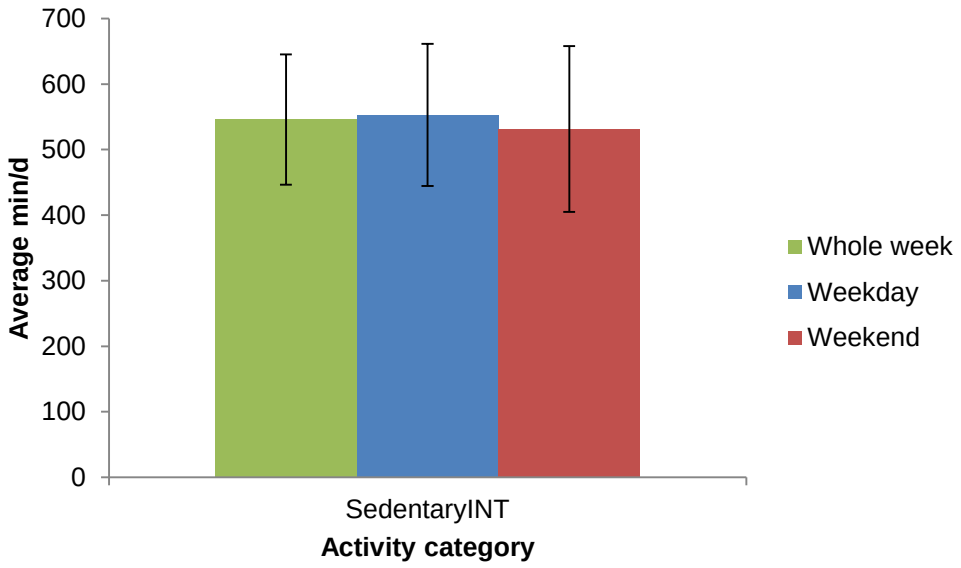


Figure 6.18 Average time per day spent sedentary measured using integrated data from the SWA and AP on average for weekdays and weekend days separately and combined

6.3.1.4 Difference in sedentary time and steps when measured using the SenseWear armband and activPAL

Both the SWA (M = 8354.42 steps/d, SD = 3037.16) and the AP (M = 9302.76 steps/d, SD = 176.19) provide a measure of steps per day. The paired sample t-test revealed the AP produced a significantly higher estimation of steps per day compared with the SWA [t(70) = -.6.81, p < .001]. The difference between the two devices was 948 steps/d.

There was a significant difference between average daily sedentary time determined by the different measurement methods for week days and weekend days combined [F(1.19, 83.51) = 106.14, p < .001]. Sedentary time measured using the three different methods on weekdays [F(1.21, 84.41) = 92.75, p < .001] and weekend days [F(1.24, 87.03) = 103.31, p < .001] separately also produced significantly different values. Post-hoc tests using the Bonferroni correction revealed all three methods were significantly different from each other (p < .001). SED^{SWA} recorded the most sedentary time, followed by SED^{AP}, and the least amount of sedentary time was recorded using the SED^{INT} method (see Figure 6.19).

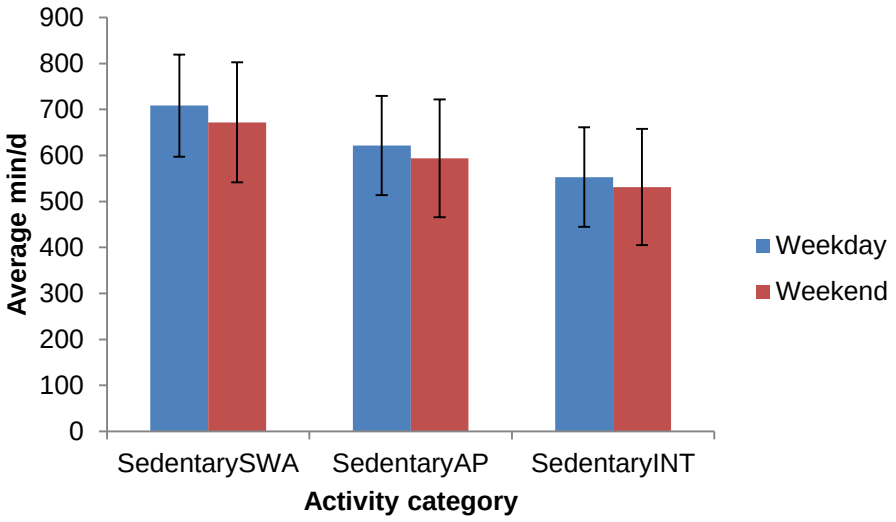


Figure 6.19 Difference in sedentary time when measured using the SWA, AP and INT

- **A procedure has been developed to integrate information on two dimensions of free-living SB (sitting posture and low activity intensity) during waking hours using two validated activity monitors**
- **Amount of sedentary time differs according to the measurement method**

6.3.1.5 Accumulation of sedentary behaviour

The Microsoft Excel template described in 6.2.7.2 was used to categorize sedentary periods by specific duration for the three different measures of SB. The duration of bout categories were 1-5, 6-10, 11-20, 21-40 and >40 minutes. The average number of sedentary periods and the number of minutes accumulated in each bout category were averaged across the 5-7 day monitoring period for each participant to obtain an

average per day for each participant and then averaged across the whole sample to obtain the sample average.

6.3.1.5.1 SenseWear armband sedentary time accumulation

Figure 6.20 and Figure 6.23A are visual representations of how SED^{SWA} data was accumulated. All 71 participants registered at least one sedentary period in all 5 of the sedentary bout categories. The number of sedentary periods is highest in the shortest sedentary bout category (1-5 minutes) and the number of sedentary bouts declines as the bout category duration increases. The largest amount of sedentary time was accumulated in bouts lasting >40 minutes.

Data presented in Figure 6.23A resembles a power law distribution; larger number of short periods accounts for a small amount of time, while a small amount of long periods accounts for a large amount of time. Sedentary periods of 1-5 minutes accounted for 9%, 6-10 minute periods accounted for 7%, 11-20 minute periods accounted for 12%, 21-40 minute periods accounted for 19% and >40 minute periods accounted for 53% of total SED^{SWA} per day.

6.3.1.5.2 activPAL sedentary time accumulation

Figure 6.21 and Figure 6.23B are visual representations of how SED^{AP} data was accumulated. All 71 participants registered at least one period of SB in bout lengths ranging from 1-5 minutes to >40 minutes. As with SED^{SWA}, SED^{AP} periods of SB became less common as the bout length increased. Total SED^{AP} was less than total SED^{SWA} but the number of periods of SB and the number of minutes accumulated in bout lengths of 6-10, 11-20 and 21-40 minutes was greater for the AP. Sedentary time accumulation measured by the AP also resembles a power law distribution (see Figure 6.23B). Sedentary periods of 1-5 minutes accounted for 9%, 6-10 minute periods accounted for 9%, 11-20 minute periods accounted for 17%, 21-40 minute periods accounted for 25% and >40 minute periods accounted for 41% of total SED^{AP} per day.

6.3.1.5.3 Integrated data sedentary time accumulation

Figure 6.22 and Figure 6.23C are visual representations of how SED^{INT} data was accumulated. All 71 participants registered at least one period of SB in bout lengths ranging from 1-5 minutes to >40 minutes. As with SWA and AP determined sedentary periods, SED^{INT} periods became less common as the bout length increased. The largest amount of SED^{INT} was accumulated in bouts lasting >40 minutes. Sedentary periods of 1-5 minutes accounted for 11%, 6-10 minute periods accounted for 10%, 11-20 minute periods accounted for 18%, 21-40 minute periods accounted for 25% and >40 minute periods accounted for 35% of total SED^{INT} per day.

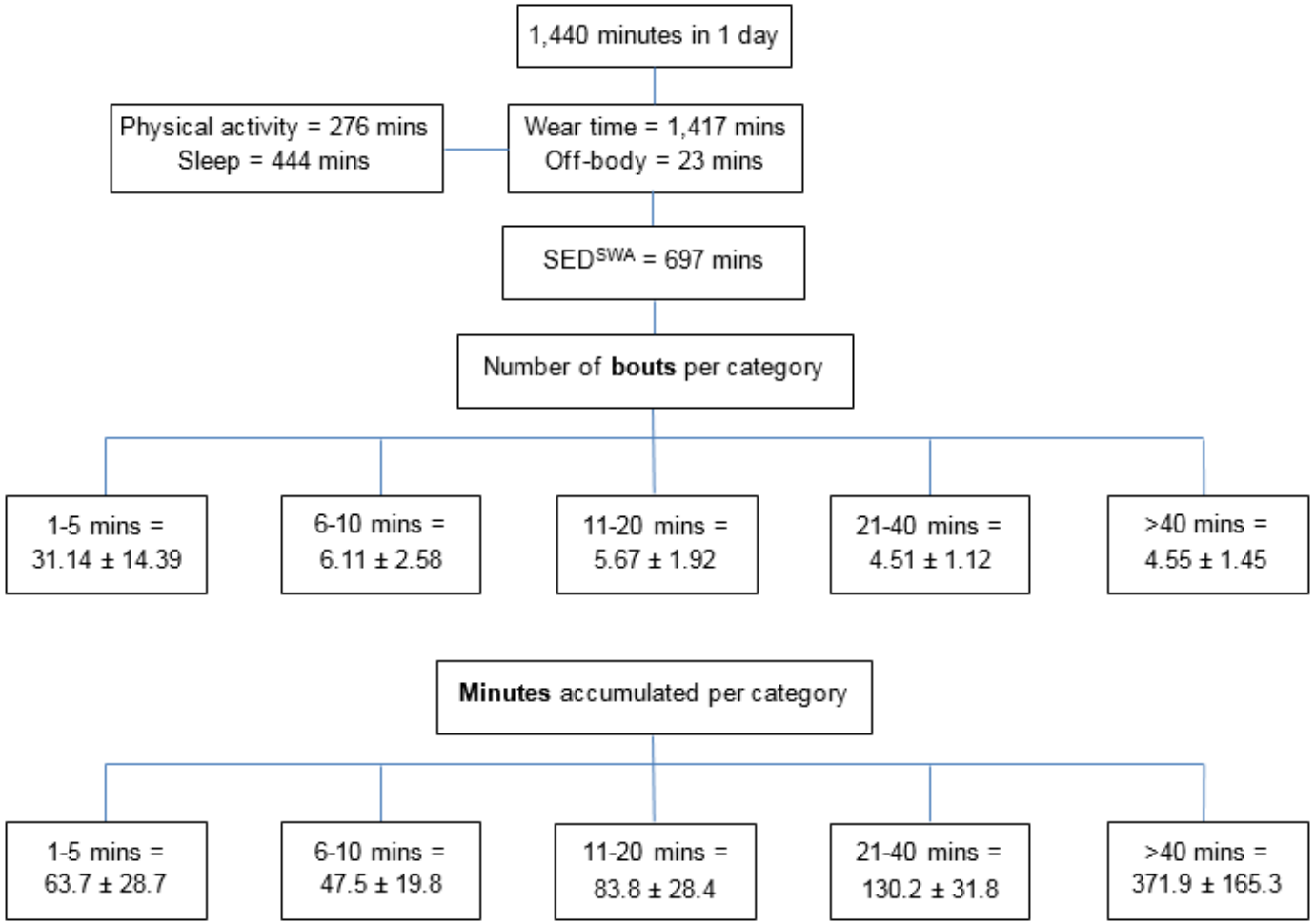


Figure 6.20 SED^{SWA} - sedentary time categorised into different bout lengths and presented as average number of bouts per day and amount of time per day accumulated in different sedentary bout categories

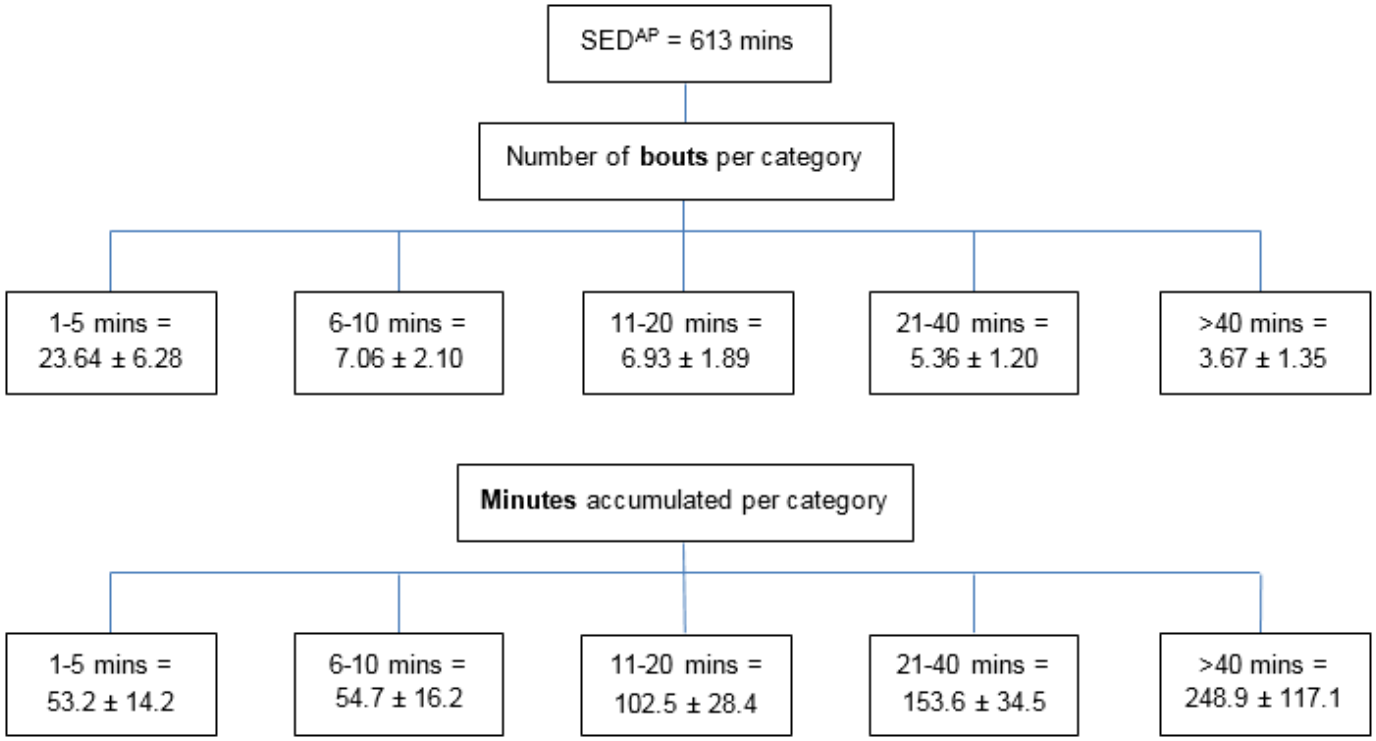


Figure 6.21 SED^{AP} - sedentary time categorised into different bout lengths and presented as average number of bouts per day and amount of time per day accumulated in different sedentary bout categories

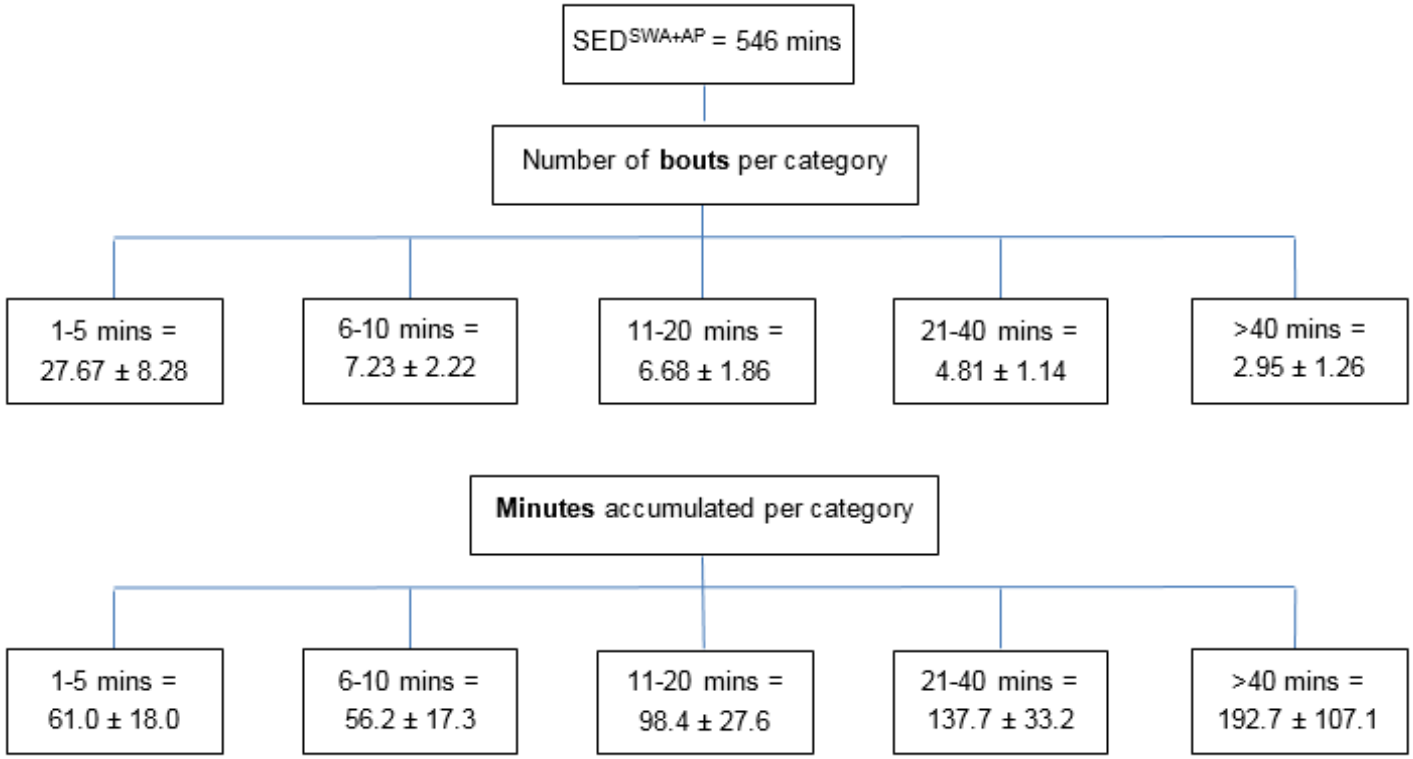


Figure 6.22 SED^{INT} - sedentary time categorised into different bout lengths and presented as average number of bouts per day and amount of time per day accumulated in different sedentary bout categories

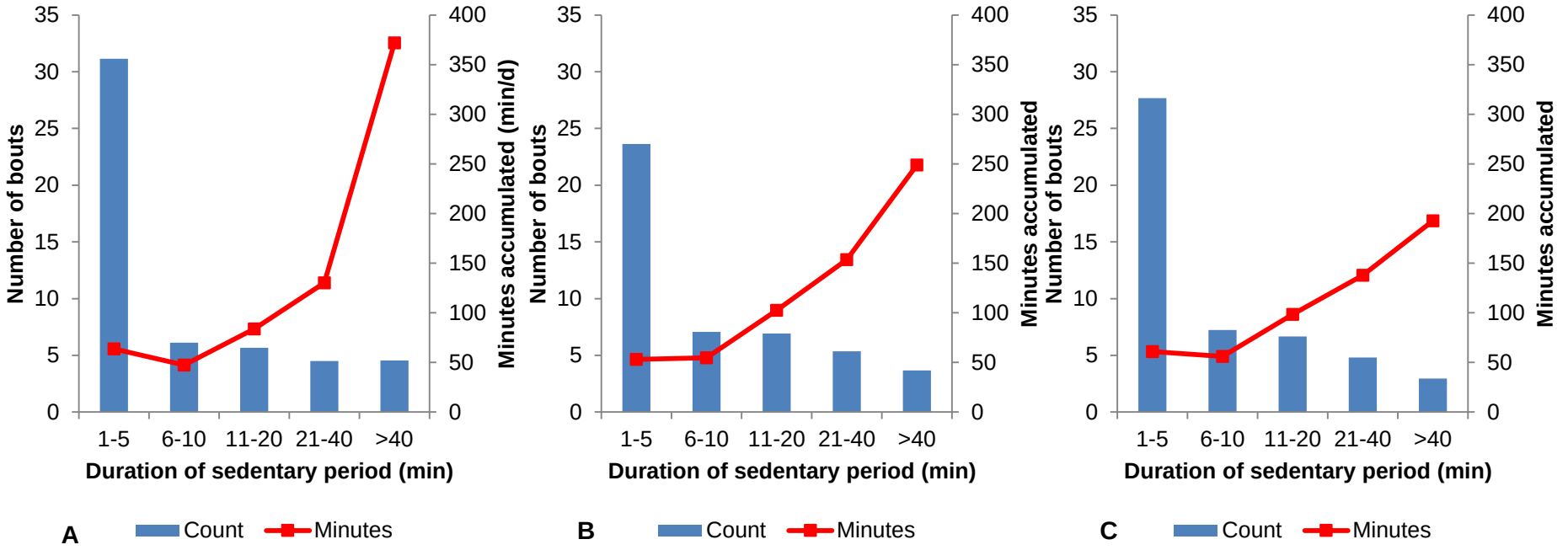


Figure 6.23 (A) SED^{SWA}, (B) SED^{AP} and (C) SED^{INT} - Average number of sedentary periods per day and average total amount of time spent in each sedentary period per day

6.3.1.5.4 Comparison of the three sedentary behaviour measures when determining sedentary time accumulation

SED^{INT} provided the lowest estimate of sedentary time followed by SED^{AP} and then SED^{SWA}. A greater proportion of sedentary time was accumulated in shorter sedentary periods when SB was determined based on activity intensity and posture combined (SED^{INT}) compared with SB based on each component alone (see Figure 6.24). A smaller proportion of time was accumulated in the more prolonged sedentary categories when the integrated method was used to classify SB. A greater proportion of sedentary time was accumulated in prolonger bouts (>40 minutes) when using the SED^{SWA} method compared with SED^{AP} and SED^{INT}.

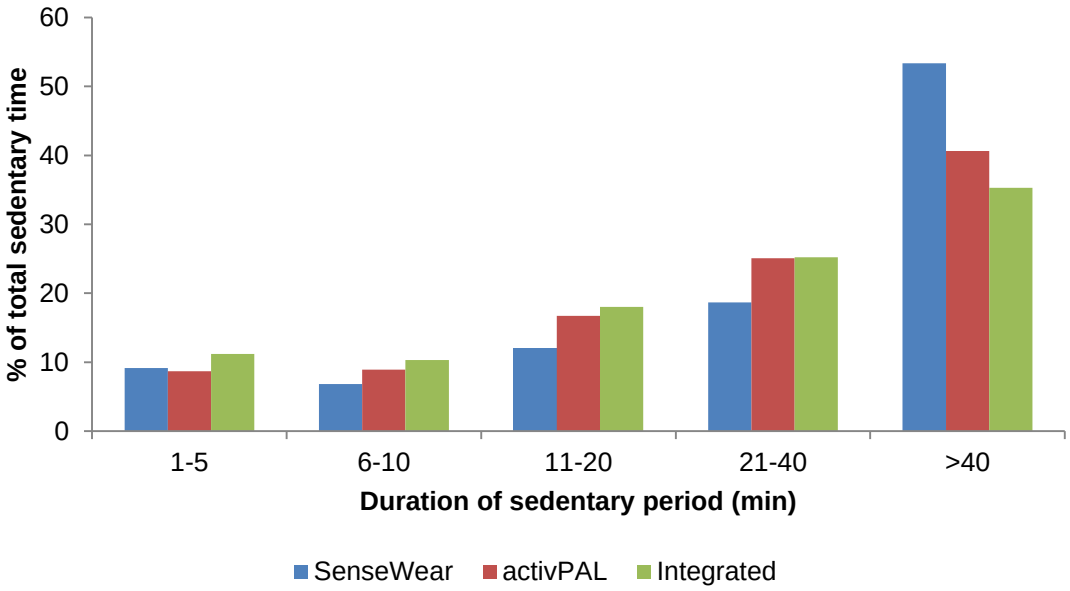


Figure 6.24 Percentage of total sedentary time accumulated in different bout categories by SB measurment method

- **Sedentary time accumulation resembles a power law distribution; larger number of short periods accounts for a small amount of time, while a small amount of long periods accounts for a large amount of time**

6.4 Discussion

The methodological platform for measuring free-living PA presented in this chapter provides detailed and realistic individual and group profiles of free-living SB and PA measured over a 5-7 day period and are similar to previously reported data (Scheers

et al., 2012, Healy et al., 2008c, Alkhajah et al., 2012). Group averages for the PA and SB variables can be used to investigate the relationship between free-living movement behaviour and outcome measures of interest, such as body composition or eating behaviour. The data presented in this chapter demonstrate it is possible to identify differences in patterns of free-living PA and SB on weekdays compared with weekend days. Participants displaced some sedentary time with sleep on weekend days compared with weekdays, an observation that has previously been reported in a sample of healthy young adults (Drenowatz et al., 2016). In agreement with previous studies, there were no differences in measures of PA measured using the SWA (light, moderate and vigorous PA) or the AP (standing and stepping) on weekdays compared with weekend days (Drenowatz et al., 2016, Smith et al., 2015).

Data from two widely validated activity monitors can be integrated to i) exclude sleep time coded as sitting/lying from SED^{AP} (the AP alone does not distinguish between sitting/lying performed when asleep and awake), and ii) measure SB based on both posture and activity intensity during waking hours. The integration procedure provides three measures of SB during waking hours based on i) activity intensity, ii) posture, and iii) activity intensity and posture. Indeed, consideration should be given to the different definitions of SB upon which the three measures are based. It has been acknowledged that the specific properties of SB that contribute to diminished health outcomes needs further investigation (Byrom et al., 2016). Indeed, investigating how the three SB measures relate to outcome measures such as adiposity and appetite control will shed light on which components of SB are most relevant to health. This issue will be addressed in the next chapter (Chapter 7).

At present there is no single activity monitor available that accurately estimates posture and activity intensity of movement behaviours in a free-living environment. Until such a device is developed the ‘multi-method approach’ can overcome the limitations inherent when measuring SB with devices capable of measuring only one component of SB; posture or activity intensity. The Microsoft Excel template developed at the outset of this PhD provides information on sedentary time accumulation based on predetermined bout lengths for all three SB variables: SED^{SWA}, SED^{AP} and SED^{INT}. All three measures of SB resembled a power law distribution; larger number of short periods accounting for a small amount of time, while a small amount of long periods accounted for a large amount of time. Similar observations for the distribution of SB accumulation have previously been highlighted (Dowd et al., 2012). It was apparent that a larger proportion of sedentary time was accumulated in shorter bout lengths when SB was classified based on multiple criteria using integrated data from the SWA and AP compared with SB determined by each activity monitor alone. The integrated method was able to detect breaks in SB occurring as a result of postural transitioning

from sitting to standing or a rise in EE above 1.5 METs whilst remaining seated/lying that the SWA or AP alone may not detect.

6.4.1 Conclusion

The aim of this study was to develop a methodological platform to quantify free-living PA and to integrate data from both activity monitors using an integration program and custom made Microsoft Excel spreadsheet to classify SB based on activity intensity and posture during waking hours. Measures of free-living PA will be used from each activity monitor alone; the SWA will provide a measure of total EE, number of steps and time spent in light, moderate and vigorous PA, and the AP will provide number of steps and time spent standing and stepping. Whether SB based on low activity intensity and posture is more strongly related to the outcome measures of interest in this thesis compared with SB based on activity intensity (regardless of posture) or posture (regardless of activity intensity) is not clear. Therefore, where data are available from both activity monitors, all three measures of SB will be included in analyses in order to determine which procedure provides the most biologically and psychologically meaningful understanding of SB. This will involve assessing the associations with relevant endpoints, such as adiposity, which is addressed in the next chapter.

6.5 Outcomes

- **This study has objectively quantified free-living sedentary and active behaviours using the SWA and AP. Both activity monitors provide realistic and detailed profiles of average daily PA and SB**
- **A procedure has been developed to integrate information on two dimensions of free-living SB (sitting posture and low activity intensity) during waking hours using two validated activity monitors**
- **Amount of sedentary time differs according to the measurement method**
- **Subsequent studies in this thesis will clarify which dimensions of SB are associated with appetite control and energy balance outcomes; low activity intensity (and therefore EE) or sitting**

Chapter 7
Study 3 - A Novel Integrative Procedure for Identifying and Characterising Objectively Measured Free-Living Sedentary Time: Disentangling the Relationship Between Sedentariness and Obesity

This chapter will examine whether the association between free-living sedentary behaviour and body composition differs depending on the way in which sedentary behaviour is operationally defined and measured. Three measures of sedentary behaviour, defined by i) activity intensity (<1.5 METs), ii) posture (sitting/reclining) and iii) activity intensity and posture, will be used in correlation analyses to determine the relationship with body composition. Ultimately the question of whether low activity intensity, posture or both are associated with levels of adiposity will be determined.

"while there is agreement that sedentary behaviour certainly includes sitting at <1.5 METS while awake, establishing whether low-intensity behaviours at <1.5 METS (e.g. standing) should also be included in the definition is a priority … In support of this effort, when possible, further research using both definitions in epidemiological studies or evaluating physiological differences between the two definitions in field and laboratory-based studies would be useful." (Gibbs et al., 2015, p.1297)

7.1 Introduction

SB is common in the 21st century accounting for between 46% - 72% of the waking day (Owen et al., 2014, Henson et al., 2013, Jefferis et al., 2015). Many studies use TV viewing as a proxy measure to reflect total sedentary time, however, TV viewing does not appear to be representative of overall sedentary time and is also associated with other health related behaviours such as higher EI, particularly from fat (Ford and Caspersen, 2012, Dunstan et al., 2005, Atkin et al., 2012, Gore et al., 2003). To address the limitations of self-report proxy measures of SB, objective measurement methods are increasingly being used (Healy et al., 2008c, Healy et al., 2008a, Henson et al., 2013). However, objective measurement devices are not without limitations. The devices used to measure movement behaviours throughout this thesis both feature a triaxial accelerometer, however, they measure different facets of SB as described in Chapter 6. The inconsistencies between studies in the way SB is defined and measured make it difficult to deduce which components of SB are driving the negative relationship with health outcomes reported in the literature. A standardized definition of

SB has obvious benefits for clarifying the impact of SB on health outcomes. A recent project, carried out by the Sedentary Behaviour Research Network, provided a consensus definition of SB (the same as the definition proposed by the network in 2012) and other terms relating to SB research based on expert opinion (Tremblay et al., 2017). However, the study did not address whether posture contributes to negative health outcomes or whether it is the low activity intensity driving the relationships. Indeed, different facets of SB may be associated with some health outcomes and not others. Several definitions of SB exist in the literature based on activity intensity, posture and a combination of both:

Activity intensity:

> *"Sedentary behaviour includes activities that involve energy expenditure at the level of 1.0-1.5 METs." (Pate et al., 2008, p.174)*

Posture:

> *'Operationally defined as 'sitting' time. Sedentary behaviours are multi-faceted. Typically, key sedentary behaviours include screen-time (TV viewing, computer use), motorised transport, and sitting to read, talk, and do homework, or listen to music.' (Biddle et al., 2010, p.67)*

Activity intensity and posture:

> *"Sedentary behaviour refers to any waking behaviour characterized by an energy expenditure ≤1.5 METs while in a sitting or reclining posture." (Sedentary Behaviour Research Network, 2012, p.540)*

Despite the Sedentary Behaviour Research Network's (2012) attempt to consolidate the two ways in which SB has previously been reported in scientific literature, there remains no consensus definition of SB (Gibbs et al., 2015). The word 'sedentary' originates from the Latin word 'sedere', which means to sit, and implies posture is a fundamental construct of SB. However, it is unclear whether the postural element of SB is important or whether standing with an activity intensity of <1.5 METs also has a negative impact on health. Thus, it is of paramount importance to evaluate whether posture should be included in the SB definition (Gibbs et al., 2015). Indeed, it has been acknowledged that the specific properties of SB that contribute to diminished health outcomes needs further investigation and experts have encouraged the inclusion of different SB definitions in studies to identify whether they have different relationships with health outcomes (Byrom et al., 2016, Gibbs et al., 2015). Furthermore, if SB is defined by both activity intensity and posture, it is yet to be determined what activities performed in a standing posture with an intensity of <1.5 METs should be categorised as. Figure 7.1 illustrates how the different definitions of SB fit with an overall human movement spectrum. When posture is included in the definition the boundary between SB and light intensity becomes less apparent, for example, activities in a sitting posture can result in an energy expenditure (EE) >1.5 METs and activities in a standing posture might result in an activity intensity of <1.5

METs. In other words it is possible to be inactive whilst not in a seated posture; and alternatively to show some activity (>1.5 METs) whilst actually being seated. This issue reflected by the concepts of passive standing and active sitting is incorporated into the very recently published Terminology Consensus from the Sedentary Behaviour Research Network (Tremblay et al., 2017).

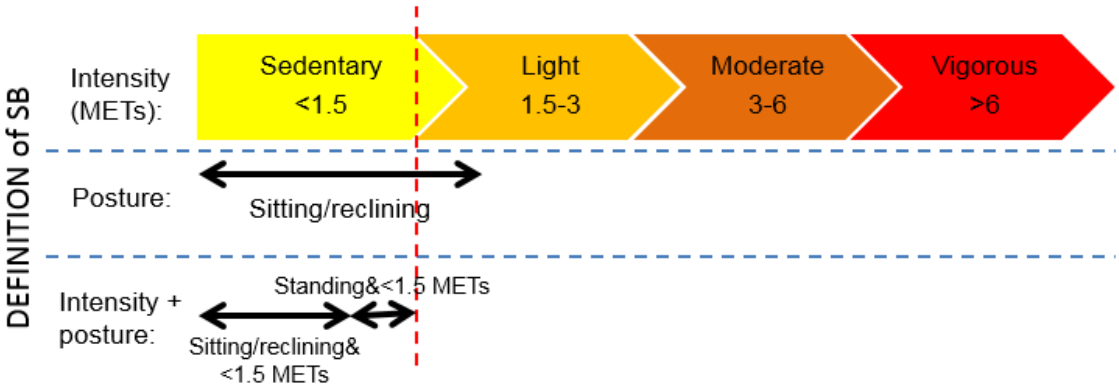


Figure 7.1 The activity intensity only definition fits well within the spectrum where non-sedentary behaviour is classified as light (1.5-2.9 METs), moderate (3-5.9 METs) and vigorous PA (>6 METs) (Ainsworth et al., 2011), however the postural definition of SB could in theory encompass activities >1.5 METs, for example, active video gaming. The posture and intensity definition of SB is also incongruent with the human movement spectrum as activities in a standing posture which do not cross the 1.5 METs threshold, such as standing still, will not be classified as sedentary or light PA

Previous research has investigated whether standing is more healthful than sitting. Thorp et al. (2014) performed a randomised crossover trial to investigate the effects of reductions in sitting on postprandial triglycerides, glucose and insulin in overweight/obese office workers. The authors found modest improvements in glucose response when sitting was interrupted with standing compared with uninterrupted sitting, but there were no differences in triglycerides or insulin. This study indicates that interrupting sitting with standing is beneficial for metabolic health when compared with prolonged sitting, however it is unclear whether these benefits are due to the change in posture or an increase in EE as a result of the transition from sitting to standing. As participants were permitted to move around during the standing breaks it is probable that the EE was greater in the standing condition compared with the seated condition (EE was not measured) making it difficult to determine whether the improvements in post prandial glucose metabolism was due to a change in posture or increased EE. Indeed, it is currently unknown whether the positive benefits of reduced SB on metabolic health are primarily driven by increased EE that accompany the transition into light activity, or to differences in postural allocation, or a combination of both. To

investigate whether the postural element of SB is impacting on health outcomes the activity intensity would need to be held constant whilst manipulating posture only. Bailey and Locke (2015) concluded interruptions in sitting every 20 minutes with 2 minutes of standing (still) during a 5 hour period did not improve postprandial glucose response to a standardised test drink whereas interruptions in sitting with light intensity walking did. However, the authors did not measure the EE of the different experimental condition. In a similar study Pulsford et al. (2016) investigated whether interruptions in sitting need to involve PA (walking) or just a change in posture (standing still) to impact on glucose and insulin response. The authors concluded interruptions to a 7 hour day of prolonged sitting in a simulated office environment with 2 minutes bouts of light intensity walking every 20 minutes (average measured intensity 2.7 ± 0.4 METs) improved postprandial insulin and glucose response whereas interrupting sitting with standing (average measured intensity 1.1 ± 0.2 METs) did not. Taken together, these studies indicate that standing *per se* may not be more beneficial than sitting at least for metabolic health markers. Rather, standing provides greater opportunity for spontaneous light intensity PA, which in turn increases EE. The energy cost of standing for obese individuals is greater than for their normal weight counterparts and the transition from sitting to standing (without walking) may be sufficient in this population to increase EE to >1.5 METs (Mansoubi et al., 2015).

Although experimental studies have begun to investigate whether the postural element of activities that engender <1.5 METs is important, it remains unclear how these nuances in SB impact on health outcomes under free-living conditions. The available tools to objectively quantify free-living SB limit researchers ability to address these questions. It has been noted that there is no single measurement device that provides an accurate measure of both posture and activity intensity (Edwardson et al., 2016, Gibbs et al., 2015). Study 2 (Chapter 6) demonstrated it is possible to integrate data from the SWA and AP to measure SB defined by an activity intensity of <1.5 METs whilst in a seated or reclining posture. Furthermore, Study 1 (Chapter 5) identified a negative association between SB and adiposity when defined by activity intensity alone (Myers et al., 2016). The aim of this study was to explore whether the relationship between SB and body composition differed depending on the way in which SB was measured and defined. The three measures of SB were defined by i) activity intensity, ii) posture and iii) activity intensity and posture.

7.1.1 Hypothesis

- SB defined by posture and activity intensity will be more strongly related to indices of adiposity than measures of SB defined by posture or activity intensity alone

7.2 Methods

7.2.1 Participants

Participants in the current study are the same as in Study 2 (Chapter 6), however, men were excluded from analyses in this chapter as the sample was unbalanced with only eight men. All participants provided written informed consent before taking part in the study and ethical approval was granted by the School of Psychology Ethical Review Board (14-0099, 14-0223 and 14-0090) and the National Research Ethics Service Committee Yorkshire & the Humber (09/H1307/7).

7.2.2 Study design

The study design has previously been described in Study 1 (Chapter 5) and therefore will not be described in detail in this chapter. Briefly, prior to testing sessions, participants were instructed to be fasted overnight (no food or drink except water from 9:00 pm the evening before), avoid exercise and alcohol for the previous 24 hours, and avoid caffeine for the previous 12 hours. Participants attended the research unit twice over the course of one week. On the morning of visit 1 the following measurements were taken: stature, weight, waist circumference, body composition and resting metabolism. Free-living SB was measured continuously for a minimum of 6 days for >22 hours/d. In addition to the SWA, participants were also provided with an AP to measure the postural element of SB and Study 2 (Chapter 6) describes the procedures for measuring free-living PA and SB with the SWA and AP. For the purposes of this chapter all three of the SB outputs were included from the activity monitors and were represented by SED^{SWA}, SED^{AP} and SED^{INT}, when referring to data from the SWA, AP and integrated data from both activity monitors, respectively. By subtracting SED^{INT} from SED^{SWA} it was also possible to identify time spent standing at an intensity of <1.5 METs (SED^{STAND}). PA variables were also included to examine whether participants exhibiting different types of low intensity activity had different PA levels. More specifically, whether the amount PA differed between participants who performed more SED^{SWA} than SED^{AP} (sedentary standers) and those who performed more SED^{AP} than SED^{SWA} (active sitters).

7.2.3 Statistical analysis

Data are reported as mean ± SD throughout. Statistical analysis was performed using IBM SPSS for Windows (Chicago, Illinois, Version 21). Relationships were regarded as significant with a p value < .05. All variables were checked for outliers and normality was assessed using the Shapiro-Wilk test. Characteristics of the study population were summarised using descriptive statistics. Differences in SED^{SWA}, SED^{AP} and SED^{INT} methods were examined using repeated measures analysis of variance (ANOVA) with Bonferroni post-hoc tests. Additionally, a Bland-Altman plots was

reported to identify any systematic bias and limits of agreement between SED^{SWA} and SED^{AP} measures of sedentary time. Pearson correlations were performed to examine the associations between SB and body composition. Independent sample t-tests were performed to examine differences in time spent in different intensities of PA between those who registered more SED^{SWA} than SED^{AP} and those who performed more SED^{AP} than SED^{SWA}.

7.3 Results

7.3.1 Participant characteristics

Study sample characteristics are displayed in Table 7.1. Sixty-three participants (women) had ≥5 days (including at least one weekend day) of valid SWA and AP data. Average wear time for the SWA was 23.61 hours/d (SD = 0.27) and the average wear period was 6.48 days (SD = 0.67).

Table 7.1 Descriptive statistics of study sample

Variable	Mean (SD)	Range		
Age (years)	37.08 (13.58)	19.00 – 69.00		
Stature (m)	1.64 (0.06)	1.49 – 1.79		
Body mass (kg)	79.51 (13.81)	44.90 – 115.80		
BMI (kg/m^2)	29.57 (4.67)	19.00 – 42.50		
FM (kg)	33.29 (11.23)	11.90 – 62.90		
FFM (kg)	46.22 (5.19)	32.10 – 57.40		
WC (cm)	98.28 (13.58)	69.00 – 139.00		
	Min/d		**Hours/d**	
	Mean (SD)	**Range**	**Mean (SD)**	**Range**
Wear time^{SWA}	1416.76 (15.86)	1362.00 – 1440.00	23.61 (0.27)	22.70 – 24.00
Sleep^{SWA}	444.35 (56.78)	330.00 – 594.00	7.38 (0.99)	5.50 – 9.90
SED^{SWA}	697.07 (101.34)	361.00 – 883.00	11.74 (1.60)	8.27 – 14.72
SED^{AP}	612.90 (102.28)	348.00 – 864.00	10.16 (1.75)	6.40 – 14.40
SED^{INT}	546.00 (99.19)	301.00 – 778.00	9.10 (1.67)	5.02 – 12.97
SED^{STAND}	158.65 (90.36)	48 – 447.00	2.64 (1.51)	0.80 – 7.45

7.3.2 Difference in measures of sedentary behaviour defined by low activity intensity (SWA), posture (AP) and a combination of both (INT)

There was a significant difference between average daily sedentary time determined by the different measurement methods; participants were sedentary (excluding sleep) for an average of 11.74 hours/d (SD = 1.60), 10.16 hours/d (SD = 1.75) and 9.10 hours/d (SD = 1.67) when determined by the SED^{SWA}, SED^{AP} and SED^{INT} methods, respectively [$F(1.18, 73.15) = 104.70$, $p < .001$]. Post-hoc tests using the Bonferroni correction revealed all three methods were significantly different from each other [$p < .001$]. SED^{SWA} recorded the most sedentary time, followed by SED^{AP}, and the least amount of sedentary time was recorded using the SED^{INT} method (see Figure 7.2).

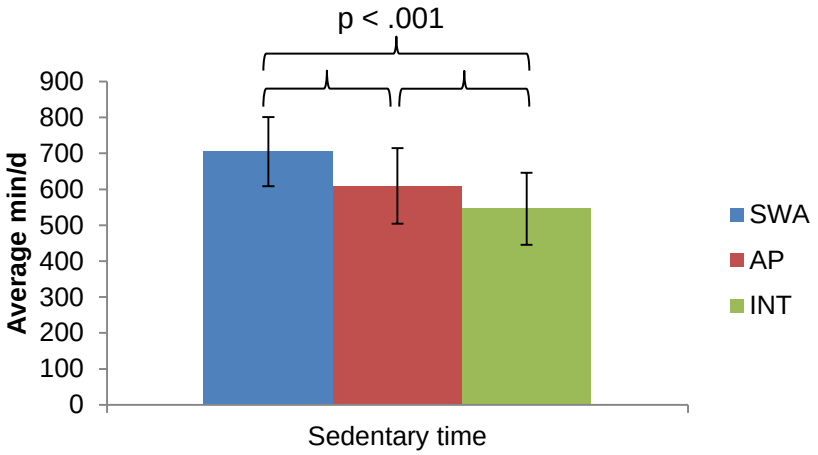


Figure 7.2 Difference in sedentary time when measured using the SWA, AP and INT

Figure 7.3 displays a Bland-Altman plot to compare the difference in sedentary time when measured using the SWA and the AP plotted against the average of the two measures. The plot does not indicate the presence of any systematic bias.

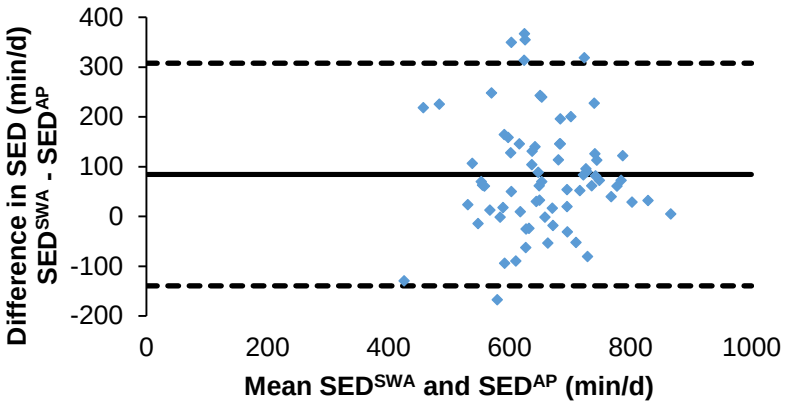


Figure 7.3 Bland-Altman plot of the difference in sedentary time when measured using the SWA and AP against the mean of the two measures. The solid black line represent the mean difference (bias) and the upper and lower dashed black line represent the upper and lower 95% limits of agreement (LOA).

7.3.3 Associations between the different measures of free-living sedentary time

The associations between the different measures of free-living SB are displayed in Table 7.2. All three measures of SB were significantly positively correlated. The weakest association was between SED^{SWA} and SED^{AP} [p = .003], followed by SED^{SWA} and SED^{INT} [p < .001] and the strongest association was between SED^{AP} and SED^{INT} [p < .001].

Table 7.2 Correlation between different measures of free-living SB

	SED^{SWA} (min/d)	SED^{AP} (min/d)	SED^{INT} (min/d)
SED^{SWA} (min/d)	-	**.37***	**.58****
SED^{AP} (min/d)	**.37***	-	**.91****
SED^{INT} (min/d)	**.58****	**.91****	-

Data are Pearson correlation (r). ** p < .001; * p < .01.

7.3.4 Associations between free-living sedentary behaviour and body composition

There was a positive correlations between SED^{SWA} and body mass [p = .02], BMI [p = .009] and FM [p = .01]. However, there were no correlations between SED^{AP} and SED^{INT} and any of the measures of body composition (see Table 7.3). Figure 7.4, Figure 7.5 and Figure 7.6 are visual representations of the relationship between sedentary time and body fat when SB is defined by either an activity intensity of <1.5

METs, a sitting or reclining posture or a combination of both. It was also possible to examine the relationship between SED^{STAND} and body composition. There was a positive correlation between SED^{STAND} and BMI [r(61) = .32, p = .012] and FM [r(61) = .26, p = .039].

Table 7.3 Correlation between different measures of free-living SB and body composition

	Body mass (kg)	BMI (kg/m^2)	FM (kg)	WC (cm)	FFM (kg)
SED^{SWA} (min/d)	**.29†**	**.33***	**.32***	.23	.08
SED^{AP} (min/d)	.05	-.02	.02	-.05	.10
SED^{INT} (min/d)	.09	.03	.08	.01	.08

Data are Pearson correlation (r). ** p < .001; * p < .01; † p < .05.

Participants were categorised based on whether they performed more SED^{SWA} than SED^{AP} (sedentary standers; n = 52) or those who performed more SED^{AP} than SED^{SWA} (active sitters; n = 11). Independent sample t-tests revealed that sedentary standers performed less total PA [t(61) = 4.18, p < .001], light PA [t(61) = 3.78, p < .001] and MVPA [t(61) = 2.51, p = .015] than active sitters.

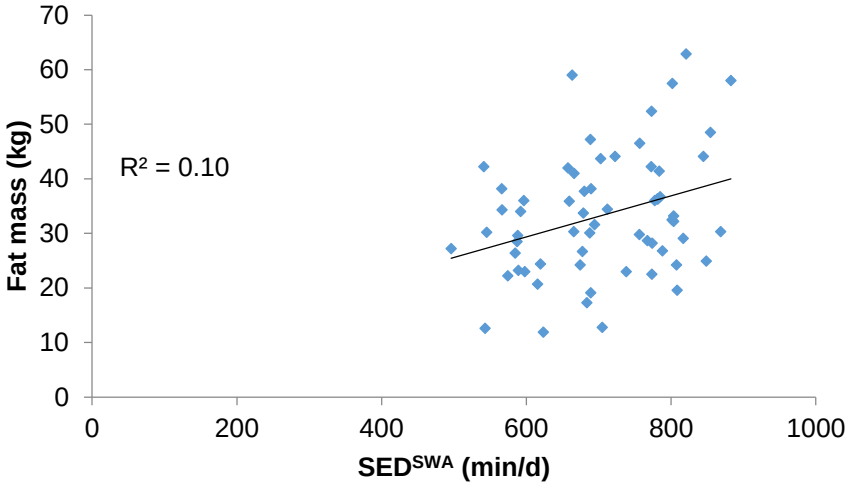


Figure 7.4 The association between SED^{SWA} and FM

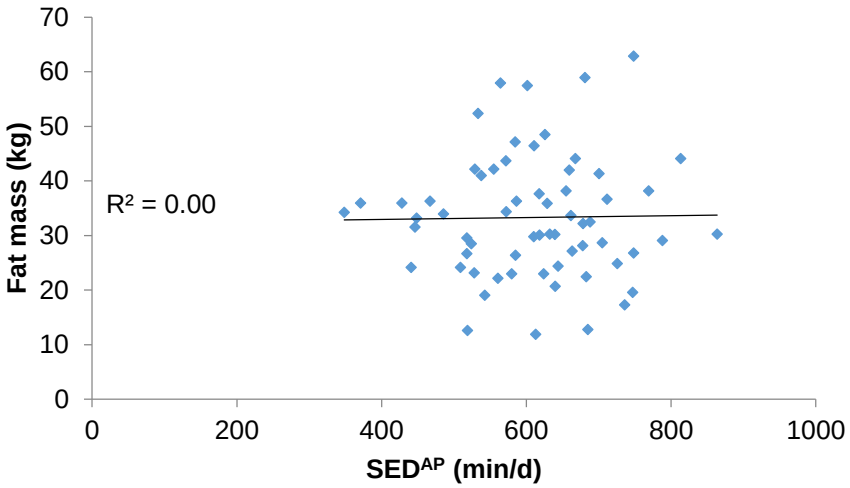


Figure 7.5 The association between SED^{AP} and FM

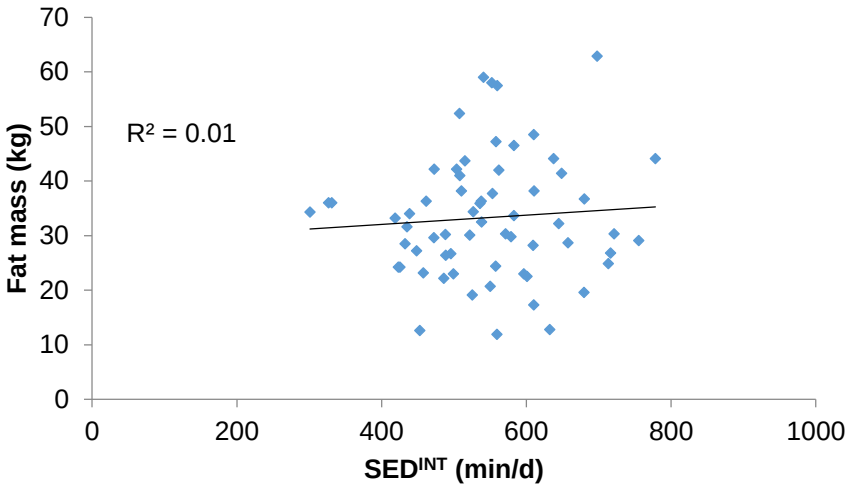


Figure 7.6 The association between SED^{INT} and FM

- **Only SB defined by an activity intensity of <1.5 METs was associated with measures of adiposity**

7.4 Discussion

The aim of the current study was to examine whether the addition of posture to low activity intensity explained more variance in indices of adiposity than measures of low activity intensity and posture alone. Using the methodological platform described in chapter 6 to combine information from two validated activity monitors using a novel integrative procedure, three measures of SB were defined by i) activity intensity (<1.5 METs), ii) posture (sitting/reclining) and iii) activity intensity and posture.

There was a significant difference between average daily sedentary time determined by the different measurement methods. SED^{SWA} recorded the most sedentary time, followed by SED^{AP} and the least amount of sedentary time was recorded by SED^{INT}. The difference in sedentary time when determined by the different measurement methods has important implications for the association between SB and health outcomes. Studies reporting the relationship between SB and health outcomes may differ depending on the component of SB being measured. Indeed, previous research has identified differences in associations between SB and cardiometabolic risk when measuring SB subjectively and objectively (Stamatakis et al., 2012). However, differences in associations between health outcomes and different measures of objectively measured SB have not been examined within the same study. Research to determine the specific properties of SB that relate to diminished health is a key priority (Gibbs et al., 2015, Byrom et al., 2016). This will inform researcher's decisions on the most appropriate device to use for their specific research question and aid policy makers to develop effective SB recommendations.

SB accounted for the majority of the waking day and was similar to previously reported data (Smith et al., 2014, Varela-Mato et al., 2016, Myers et al., 2016). Participants were sedentary for between 11.7 hours/d and 9.1 hours/d depending on the measurement criteria. Even the most stringent measure, defined by both activity intensity and posture, accounted for 55% of the waking day. Previous studies have examined the relationship between objectively measured free-living SB and body fatness and have produced mixed findings (Hamer et al., 2012, Healy et al., 2008c, Lynch et al., 2010, Van Dyck et al., 2015, McGuire and Ross, 2012, Murabito et al., 2015). The inconsistencies between studies could be explained by the different measurement methods used to quantify both SB and adiposity. In the current study, there was a positive correlation between SED^{SWA} and indices of adiposity. Similarly, Study 1 (Chapter 5) demonstrated a positive association between multiple indices of adiposity and SB defined by an activity intensity <1.5 METs using the SWA. Interestingly, SED^{AP} and SED^{INT} were not significantly associated with any measures of adiposity.

The absence of an association between measures of sitting/reclining and sitting/reclining plus low activity intensity and adiposity in our data suggests that the postural element of SB is not sufficient for FM accumulation. SED^{SWA} captures some standing with an activity intensity of <1.5 METs as well as sitting/reclining. A recent study found that compared to sitting, standing did not cause a sustained increase in EE in 81% of the study sample (n = 36) and EE did not exceed 1.5 METs in any of the participants (Miles-Chan et al., 2017). In light of this, recommendations to reduce sitting by increasing standing (Buckley et al., 2015) may not cause a significant enough increase in EE to produce health benefits. The relationship between activities

of low EE in a standing posture with health related outcomes needs exploring. It was possible to calculate SED^{STAND} by subtracting SED^{INT} from SED^{SWA} and correlation analysis revealed there was a positive correlation between SED^{STAND} and BMI and FM. The absence of an association between activity of <1.5 METs in a sitting posture but the presence of a relationship between activity of <1.5 METs in a standing posture seemed counter intuitive. Further analysis revealed that those who performed more SED^{SWA} than SED^{AP} (spent more time standing at an EE of <1.5 METs) performed less total PA, light PA and MVPA than those who performed more SED^{AP} than SED^{SWA} (spent more time sitting at an EE of >1.5 METs). Therefore, the positive association between SED^{STAND} and BMI and FM could be driven by lower levels of PA rather than standing at an EE of <1.5 METs. Furthermore, when the correlation was controlled for MVPA it was no longer significant. When relating SB to adiposity, the definition of SB based on activity intensity only by Pate et al. (2008) seems most appropriate.

However, this does not rule out the role of posture in the development of other cardiometabolic health outcomes (Young et al., 2016). Laboratory studies examining the mechanisms underlying negative health outcomes associated with SB indicate that prolonged sitting may trigger a chain of unhealthy molecular responses, including down regulation of lipoprotein lipase activity, impacting physiological outcomes (Hamilton et al., 2007). It remains unclear whether a change in posture is sufficient to induce improvements in biological markers of metabolic health or whether a change in posture must result in increased EE before any benefit is accrued. Pulsford et al. (2016) recently found that interrupting sitting with repeated short bouts of light intensity walking improved insulin sensitivity, whereas repeated short bouts of standing did not. Furthermore, breaking up SB with 5 minutes of upper body exercise every 30 minutes whilst remaining seated attenuated post-prandial glycaemia (McCarthy et al., 2017). As with the results of the present study, these findings indicate that the postural element of SB is not driving the relationship between SB and negative health outcomes reported in the literature and in fact it is low EE as a result of a lack of movement. The current study demonstrates the associations between SB and body composition differ depending on the measurement technique used to quantify and define SB. This is a pertinent issue given that the research in this area employs a plethora of measurement techniques to measure SB; from self-report questionnaires focusing on screen-based activities such as TV viewing (Healy et al., 2008b, Healy et al., 2011b), to objective measures of activity intensity and posture (Lynch et al., 2010, Myers et al., 2016, Smith et al., 2014). The present study suggests low EE, as a result of high volumes of behaviours expending <1.5 METs (either sitting or standing), is detrimental to body composition.

7.4.1 Conclusion

It is possible to obtain a measure of free-living SB based on both activity intensity and posture by integrating data from two validated activity monitors. Measures of SB using different objective measurement techniques are not measuring the same phenomenon. Indeed, the three measures of SB in the current study differed significantly. Of the three measures of SB included in this study, only low activity intensity (<1.5 METs) was associated with adiposity. This suggests that the postural element of SB is not sufficient for the accumulation of adiposity, rather low EE, as a result of high volumes of low intensity activity, may be driving the association. The present research indicates that the relationship between SB and adiposity depends on the measurement device used to measure behaviour and therefore which aspect of SB the device captures. Researchers should be clear about which component of SB is being captured by the measurement device used when reporting research. Although the integrated measure of SB was not associated with adiposity, this does not rule out the possibility of an association with other biological or health markers.

7.4.2 Outcomes

- **Only the activity intensity (<1.5 METs) measure of SB was associated with measures of adiposity**
- **Posture alone is not a good indicator of the tendency to accumulate FM**
- **The SWA is a more appropriate instrument than the AP for measuring sedentary time in energy balance studies**

Chapter 8
Study 4 - Relating Energy Intake to Energy Expenditure: The Associations Among Metabolic and Behavioural Components of Energy Expenditure and Homeostatic Appetite Control in Overweight and Obese Inactive Women

This chapter will examine the relationship between components of energy expenditure and homeostatic appetite control. More specifically, it will examine whether energy expenditure resulting from metabolic (fat-free mass and resting metabolic rate) and behavioural (time spent in different intensities of free-living physical activity and sedentary behaviour) components of total energy expenditure are associated with measures of subjective appetite and energy intake in overweight and obese inactive women. To achieve this, correlation analyses will be performed to examine the relationship between free-living energy expenditure, resting metabolic rate, fat-free mass, time spent in different intensities of activity and homeostatic appetite control (subjective appetite sensations and energy intake) measured under controlled laboratory conditions.

"the differences between the intakes of food [of individuals] must originate in differences in the expenditure of energy" (Edholm et al., 1955, p.297)

8.1 Introduction

To date research has centred on the physiological processes involved in meal initiation and termination (satiation), and the suppression of hunger between eating episodes (satiety). Together, these processes have been identified and described using the Satiety Cascade (Blundell et al., 1987). However, the source and nature of the ongoing and recurring excitatory drive to eat is less well understood (Halford and Blundell, 2000). From a homeostatic perspective, a drive to seek out food emanating from the energy required to sustain key metabolic and behavioural processes would seem plausible and logical. Indeed, recent formulations of the processes involved in appetite control have emphasised the role of RMR, which contributes heavily (50-70%) to total EE, and FFM in driving EI to meet the body's energy needs (Hopkins and Blundell, 2016, Goran, 2000, Shetty, 2005).

8.1.1 Metabolic energy expenditure

Daily EE can be roughly divided into behavioural (PA EE) and metabolic (RMR, FFM) components. There is evidence to support the role of FFM in driving EI. Indeed, FFM, but not FM and BMI, is positively associated with meal size and EI (Blundell et al., 2012b, Weise et al., 2014). Furthermore, RMR is also associated with fasting levels of hunger and EI, mirroring the relationship between FFM and EI, which is unsurprising given FFM contributes around 60-70% to RMR (Caudwell et al., 2013a, Johnstone et al., 2005). It has been suggested that RMR acts as a mediating variable in the relationship between FFM and EI and this was recently demonstrated using path analysis whereby the effects of FFM on EI were fully mediated through its effect on RMR (Hopkins et al., 2016).

The issue of whether EE drives EI is not a new concept. This topic was investigated over 60 years ago in a series of studies by Edholm et al. (1955) who measured the EI and EE of army cadets and found no relationship between total EE and EI within a single day. The lack of a relationship during a single day is not surprising given the variability in both total daily EE (mainly PA EE) and EI (Donahoo et al., 2004, Bray et al., 2008). For example, a marked increase in EE towards the end of a day would not leave time for the increase to be matched by the consumption of food. However, over the course of one week there was a strong relationship between total EE and EI (Edholm et al., 1955, Edholm et al., 1970, Edholm, 1977). In addition, the authors observed a positive relationship between daily body mass change and daily EI and a negative relationship with EE demonstrating the importance of energy balance for body mass regulation (Edholm et al., 1970).

In keeping with the work of Edholm et al. (1955), Mayer et al. (1956) demonstrated that in workers in the Kolkata jute mills with more physically demanding jobs (higher EE) had a greater EI compared with those in less physically demanding occupations (lower EE). However, this association was only apparent above a certain level of physical exertion, below which, EI increased resulting in a positive energy balance. This suggests that appetite control is desensitised to physiological signals below a certain level of physical exertion and no longer operates in the interest of energy balance (Blundell, 2011). Those with less physically active jobs also had a higher body mass than their more active counterparts, however, body composition was not measured. Further support for a curvilinear or ‘j shaped’ relationship between EI and EE has been reported recently in experimental studies and a systematic review of the literature (Harrington et al., 2013, Shook et al., 2015, Beaulieu et al., 2016).

8.1.2 Body fat and appetite dysregulation

The mechanisms underlying the apparent uncoupling of EI to EE at low levels of PA (and a high volume of SB) are not well understood. A plausible explanation for the apparent appetite dysregulation in less active individuals could arise from differences in body composition and sensitivity to tonic peptides (Blundell et al., 2012a, Hopkins and Blundell, 2016). Leptin, an appetite hormone produced by adipose tissue, relays information about the state of the body's energy stores (fat) to the central nervous system (CNS) to suppress food intake and promote EE (Myers et al., 2008). However, despite elevated circulating leptin levels in proportion to the increase in FM observed in overweight and obese individuals, appetite and feeding are not supressed (Ostlund Jr et al., 1996). This paradoxical observation implies a resistance to leptin with elevated FM (Considine et al., 1996) whereby the tonic inhibition of appetite, brought about by the action of leptin on the CNS, is reduced in overweight and obese individuals (Blundell et al., 2012a, Blundell et al., 2015a, Steinberg et al., 2002). In addition to leptin resistance, it has been proposed that overweight and obese individuals exhibit an increased drive to eat because of their elevated FFM and RMR in comparison to their normal weight counterparts (Hopkins and Blundell, 2016). Therefore, the development of obesity may further promote over consumption and appetite dysregulation due to the mismatch between the tonic inhibition of food intake and the excitatory drive to eat arising from FM and FFM, respectively (Hopkins and Blundell, 2016). The uncoupling of EI to EE at low levels of PA may, in part, be explained by the accumulation of adipose tissue associated with physical inactivity and SB (Shook et al., 2015, Myers et al., 2016).

8.1.3 Behavioural energy expenditure

The behavioural component of EE (discretionary PA) is highly variable and contributes 20-40% to total EE depending on PA level. When compared with the more constant energetic demand generated by RMR, PA EE is more sporadic and involves many physiological processes in addition to an increase in EE and therefore it could exert a different type of control over appetite compared to RMR and FFM. As well as the direct effects of PA on EE and energy balance, there is accumulating evidence to suggest PA also contributes to energy balance via its effects on appetite control as was discussed in section 5.1. Whilst being more physically active seems to improve the sensitivity of the appetite control system, becoming less active (and performing more SB) does not down regulate EI resulting in a positive energy balance (Stubbs et al., 2004). It follows that the regulation of body mass is asymmetrical; a negative energy balance (weight loss) is strongly defended against, on the other hand a positive energy balance is permitted (weight gain) (Blundell et al., 2008).

8.1.4 Sedentary behaviour and appetite control

Recently, the effects of SB on EI have been investigated. Pearson and Biddle (2011) conducted a systematic review on SB and dietary intake and found that SB, usually assessed by screen-time, was associated with unhealthy dietary intake such as higher consumption of energy dense snacks and lower consumption of fruit and vegetables. Furthermore, Chaput et al. (2011) concluded modern sedentary behaviours (i.e. video gaming, TV viewing, cognitive working) promote overconsumption of food in the absence of hunger. TV viewing has also been associated with increased snacking on fast-foods and increased EI (Scully et al., 2009, Bowman, 2006, Gore et al., 2003). However, using proxy measures of SB such as TV viewing and screen time may be problematic because those specific behaviours could be driving the observed associations and not SB *per se*. For example, exposure to TV advertisements promoting food related items or distraction associated with TV viewing have been shown to promote EI (Boulos et al., 2012). A more recent experimental study found no effect of breaking up prolonged sitting (5 hours) with light or moderate PA (2 min every 20 min) on subjective appetite sensations, gut hormones or absolute energy intake (Bailey et al., 2015). However, the longer-term effects of such an intervention to break up sedentary time are unknown. There is a paucity of studies that have objectively measured SB in the free-living environment and its association with measures of homeostatic appetite control and objectively measured EI. It is therefore unclear whether SB exerts effects on energy balance beyond low EE.

There is accumulating evidence to suggest EI is related to EE but the behavioural and metabolic components of daily EE seem to contribute differently to energy balance. The relationship between objectively quantified time spent in different intensities of activity (from sedentary to vigorous) and laboratory measures of 24 hour EI and subjective appetite sensations over the course of a day have not been examined. Further research is needed to establish the relationship of behavioural and metabolic components of EE to EI using accurate measurement techniques to quantify both. Therefore, the aim of this study was to investigate the associations among behavioural (free-living sedentary and active behaviours) and metabolic (RMR and FFM) components of EE and laboratory measures of homeostatic appetite control and EI in overweight and obese inactive women.

8.1.5 Hypotheses

- Total EE, RMR and FFM will be positively associated with EI and hunger reflected in fasting VAS rating and AUC
- Time spent in MVPA will be positively associated with EI and hunger reflected in fasting VAS rating and AUC
- Time spent sedentary will be positively associated with EI

8.2 Methods

8.2.1 Participants

Baseline data from the 12-week exercise intervention study formed the basis of this cross-sectional study. Participants were included in the current study if they completed baseline measures and probe day visits and had ≥5 days (including at least one weekend day) of valid SWA and AP data from the first free-living PA and SB measurement period. Thirty-two (women) participants aged 32.00 years (SD = 11.36) with a BMI of 28.21 kg/m^2 (SD = 2.76) were included in this study.

8.2.2 Inclusion criteria

Participants were eligible for this study if they met the following inclusion criteria:

- Provided written informed consent
- Healthy women
- Aged 18-55 years
- BMI between 25-34.9 kg/m^2
- Not currently dieting to lose or gain weight
- Not increased PA levels in the past 2-4 weeks
- Regular breakfast eaters
- Non-smokers
- ≥3 liking of study foods (on 7-point Likert scale)

8.2.3 Exclusion criteria

- Significant health problems that will affect study outcomes or constitute a risk to the participant
- Taking any medication or supplements known to affect appetite or weight within the past month and/or during the study
- History of anaphylaxis to food
- Any known food allergies or food intolerance
- Vegetarians
- Smokers and those who have recently ceased smoking (within the last 3 months)
- BMI <25 kg/m^2 or >34.9 kg/m^2
- Volunteers self-reporting currently dieting or having lost significant amount of weight in the previous 6 months (5%)
- Volunteers who have significantly changed their PA patterns in the past 4 weeks (defined as change in MVPA of >150 minutes per week)
- Participants receiving systemic or local treatment likely to interfere with evaluation of the study parameters
- Participants (e.g. staff/students) who work in appetite or feeding related areas

- Non-breakfast eaters

8.2.4 Study design

Study 5 (Chapter 9) provides a detailed description of the 12-week exercise intervention study protocol and therefore a detailed description will not be provided in this chapter. This section will describe the procedures for measuring 24 hour EI and subjective appetite sensations during the probe days. Where measurement procedures have been described previously the relevant chapter will be cross-referenced.

On completion of eligibility checks participants were provided with the SWA and AP to wear for the following seven days and were instructed to continue their normal daily activity (the procedures for measuring free-living PA and SB with the SWA and AP simultaneously are outlined in detail in Study 2, Chapter 6). Participants returned to the research unit seven to eight days later to return the activity monitors and the following measurements were taken: stature, weight, BMI, RMR and body composition (a detailed descriptions of each of these procedures is provided in Chapter 4). Participants then completed two probe days in the week after the measures day, separated by ≥3 days, to measure 24 hour eating behaviour and subjective appetite sensations throughout the day. An average of the EI and subjective appetite sensation data from the two probe days was calculated and used in the analysis in this study. Figure 8.1 provides an overview of the study protocol.

In addition to the free-living PA variables outlined in Study 2 (Chapter 6), activity EE was also calculated in the current study to identify the relationship between the behavioural component of total EE and homeostatic appetite control. This was calculated by summing energy expended whilst performing activities >1.5 METs.

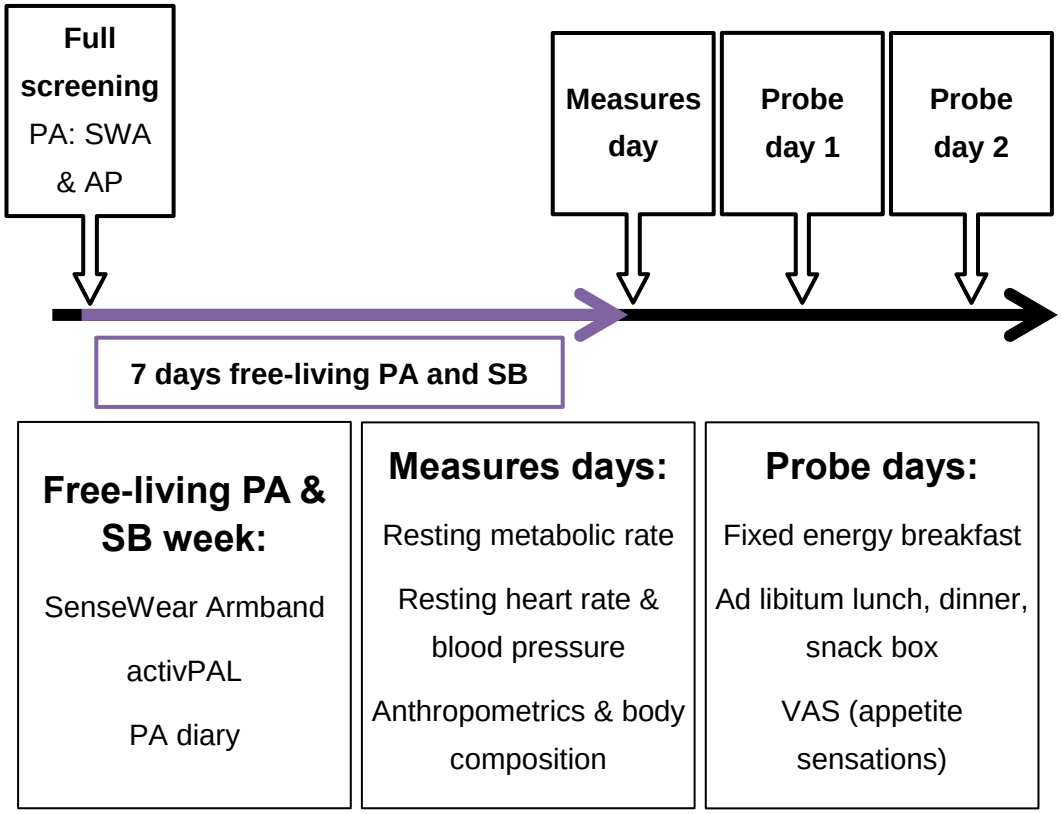


Figure 8.1 Overview of Study 4 protocol

8.2.5 Probe day procedures

Participants arrived at the laboratory between 7:00 am and 9:00 am. Once they were settled in their cubicle and the probe day had been explained, they completed the first VAS ratings on the EARS-II. Participants completed a further VAS ratings immediately before breakfast was provided and 10 minutes after breakfast was served (0 min). Seven further VAS rating were taken throughout the morning between breakfast and lunch at the following intervals: +15 min, +30 min, +60 min, +90 min, +120 min, +180 min and +230 min. Between breakfast and lunch, participants remained in the research unit in their private cubicle and were able to use a desktop computer/laptop, listen to music or read. The *ad libitum* lunch was then served immediately after the +230 min VAS ratings were completed. VAS ratings were completed again post-lunch. Participants were free to leave the research unit between lunch and dinner. Participants were instructed to drink only water and not to consume any food in the interim period. They were provided with a water bottle and instructed to record the number of times the water bottle was refilled and VAS ratings were completed hourly. Participants returned four hours later for their *ad libitum* dinner. Immediately after dinner, participants were instructed to complete the VAS ratings and informed the hand-held EARS-II device should be completed at hourly intervals for the 2 hours after dinner. Before leaving the research unit, participants were provided with a snack box

and the End of Day Questionnaire (EoDQ) to take home and arrangements were made to return these items the following day. Participants were instructed they could eat as much or as little of the snack box foods as they wanted but not to share foods and to return any uneaten food and empty packaging. Figure 8.2 provides an overview of the probe day procedures.

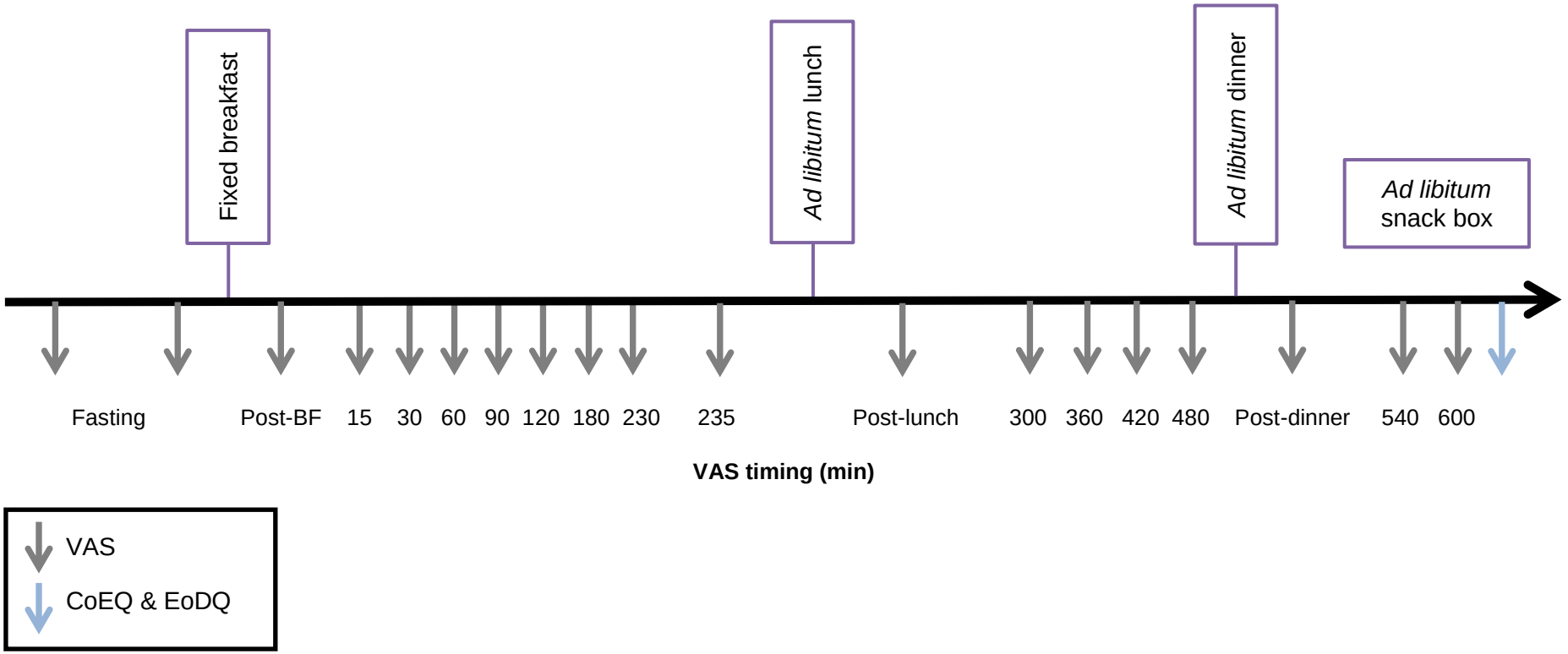


Figure 8.2 Exercise intervention study probe day procedures

8.2.5.1 Subjective appetite sensations

The EARS-II (Gibbons et al., 2011) was used throughout the probe days to assess subjective appetite sensations in response to food consumption. Participants answered the following questions:

- How HUNGRY do you feel now?
- How FULL do you feel now?
- How strong is your DESIRE TO EAT?
- How MUCH food could you eat now?

VAS ratings were completed at 19 time points throughout the probe days. The first rating was completed when the participant arrived to the research unit on the morning of the probe day prior to the cannula being fitted. Ratings were completed before and after test meals and immediately prior to each blood sample. The EARS-II system was programmed to prompt the participants to complete VAS rating every hour whilst the participant was away from the laboratory between lunch and dinner (4 occasions) and after dinner (2 occasions). For further details on VAS subjective appetite sensations see Chapter 4.

8.2.5.1.1 Satiety Quotient

SQ is a measure of the satiating capacity of foods relative to their energy content. The SQ has previously been validated and SQ for fullness has been shown to be associated with *ad libitum* and self-reported EI (Drapeau et al., 2007, Drapeau et al., 2005). The SQ was calculated to assess the satiating efficiency of the fixed (relative to energy requirements) breakfast test meal at baseline and again following the 12 week exercise intervention. EI at breakfast and the following hunger, fullness, desire to eat and prospective consumption VAS ratings were used to calculate SQ: Fasting, post-breakfast, +15 minutes, +30 minutes, +60 minutes, +90 minutes, +120 minutes, +180 minutes, +230 minutes and +235 minutes. SQ is calculated as follows:

$$SQ(mm/kcal) = \frac{(\text{rating before eating episode} - \text{rating after eating episode})}{\text{Energy intake of eating episode}} * 100$$

This resulted in a single SQ value for each of the appetite sensations: hunger, fullness, desire to eat and prospective consumption. For further details on SQ see Chapter 4.

8.2.5.1.2 Area under the curve

AUC was calculated for the whole day for hunger, fullness, desire to eat and prospective consumption using the trapezoid method (see Chapter 4). Fasting VAS rating were excluded to remove differences in fasting levels of appetite sensations that might artificially alter the mean AUC. The following VAS rating were used in the calculation: 0 minutes (post-breakfast), +15 minutes, +30 minutes, +60 minutes, +90

minutes, +120 minutes, +180 minutes, +230 minutes, +235 minutes, +260 minutes (post-lunch), +300 minutes, +360 minutes, +420 minutes, +480 minutes, +500 minutes (post-dinner), +540 minutes, +600 minutes.

8.2.5.2 Probe day energy intake

Participants were asked to rate how much they liked all study foods on a 7-point Likert scale with 1 representing 'not at all' and 7 representing 'extremely'. To be included in the study, participants had to rate all study foods ≥3.

8.2.5.2.1 Individualised fixed energy breakfast

Breakfast was consumed in two parts; a standardised fixed energy breakfast and a fixed portion of active or control yoghurt/pudding.

The fixed energy breakfast test meal provided 25% of participants' measured resting energy requirements (measured using IC). A choice of tea, coffee or water was given with breakfast. Participants consumed the same drink at all probe days. Participants were instructed to consume all the food and drink provided within 10 minutes (see Figure 8.3). If the participant had not consumed everything at the end of the 10 minutes the researcher instructed them to consume what was left and provided more time. The macronutrient composition of the breakfast was fixed at 55%, 30% and 15% for carbohydrate, fat and protein, respectively. Table 8.1 provides the macronutrient composition and energy content of study foods provided at breakfast.

Table 8.1 Macronutrient composition and energy content of fixed energy breakfast foods

Food	kcal/100g	Fat/100g	CHO/100g	PRO/100g
Muesli base	348.6	4.6	70.3	10.9
Raisins	271.9	0.4	69.3	2.1
Sultanas	274.7	0.4	69.4	2.7
Almonds	607.5	49.0	22.0	21.0
Semi-skimmed milk	45.7	1.6	6.8	3.4

Participants were provided with 150 g of active or control yoghurt/pudding[1] (see Table 8.2 and Figure 8.3) after completion of the fixed energy breakfast. This included 100 g of pudding as the bottom layer and 50 g of yoghurt as the top layer. Fifteen drops of

[1] The yoghurt/pudding product was developed as part of the larger SATIN project and was included in the study to investigate whether the active product had an effect on satiety and satiation. There was no difference in outcome measures between active and control yoghurt/pudding probe days and therefore both probe days at baseline and post-intervention were averaged.

fruit flavoured syrup was added to the yoghurt to sweeten and then poured over the pudding that was cut in to approximately 1 cm cubes. Participants were given 10 minutes to eat the yoghurt pudding. If the participant had not consumed everything at the end of the 10 minutes the researcher instructed them to consume what was left and provided more time.

Table 8.2 Macronutrient composition and energy content of active and control yoghurt pudding

Food	Serving (g)	kcal/serving	Fat (g/serving)	CHO (g/serving)	PRO (g/serving)
Active					
Yoghurt	50	24	0.8	2.2	1.9
Pudding	100	63	1.6	8.0	3.3
Total	**150**	**87**	**2.4**	**10.2**	**5.2**
Control					
Yoghurt	50	23	0.8	1.8	1.8
Pudding	100	61	1.4	6.7	5.5
Total	**150**	**84**	**2.2**	**8.5**	**7.3**

Figure 8.3 Standardised fixed energy breakfast and a fixed portion of yoghurt/pudding

8.2.5.2.2 *Ad libitum* lunch

The *ad libitum* lunch test meal was provided four hours after breakfast and consisted of chilli con carne with rice, strawberry yoghurt and water (see Table 8.3 and Figure 8.4). Participants were instructed to consume as much or as little as they wanted but to eat until they reached a comfortable level of fullness. Food was weighed before and after consumption to the nearest 0.1 g to determine food intake.

Table 8.3 Serving size, macronutrient composition and energy content of lunch foods

Food	Serving (g)	kcal/serving	Fat (g/serving)	CHO (g/serving)	PRO (g/serving)
Stagg chilli	650	791.4	32.5	84.5	45.5
Uncle Ben's basmati rice	250	358.7	4.0	77.3	8.3
Yeo valley Strawberry yoghurt	425	435.6	16.2	56.1	20.0
Sainsbury's double cream	45	197.6	21.4	0.7	0.7
Water	500	-	-	-	-

Figure 8.4 *Ad libitum* lunch

8.2.5.2.3 *Ad libitum* dinner

The *ad libitum* dinner test meal was served four hours after lunch and consisted of tomato and herb risotto, salad items, garlic bread, chocolate brownies and water (see Table 8.4 and Figure 8.5). Participants were again instructed to consume as much or as little as they like but to eat until they reached a comfortable level of fullness. Food was weighed before and after consumption to the nearest 0.1 g to determine food intake.

Table 8.4 Serving size, macronutrient composition and energy content of dinner foods

Food	Serving (g)	kcal/serving	Fat (g/serving)	CHO (g/serving)	PRO (g/serving)
Tomato and herb risotto	900	1508.9	35.1	282.6	33.3
Olive oil	45	372.3	41.2	0.2	0.2
Garlic bread	260	902.4	53.8	95.9	14.6
Lettuce	50	7.2	0.3	1.0	0.4
Cucumber	115	14.4	0.6	1.6	0.8
Tomatoes	115	21.8	0.6	3.6	0.8
Chocolate brownies	140	584.7	28.0	80.4	7.8
Water	500	-	-	-	-

Figure 8.5 *Ad libitum* dinner

8.2.5.2.4 *Ad libitum* snack box

Participants were provided with a snack box to take home with them after dinner to measure free-living *ad libitum* snack food intake. The snack box contained the following: 1 apple, 2 mandarins, ham, grated cheese, bread, crisps, margarine, chocolate buttons and a yoghurt (see Table 8.5 and Figure 8.6). Participants were instructed they could eat as much or as little as they liked from the selection of foods, but they should not share or dispose of any foods. Participants were instructed to return the snack box the next day containing any packaging from foods they had eaten and any uneaten food. Food was weighed before and after consumption to the nearest 0.1 g to determine food intake.

Table 8.5 Serving size, macronutrient composition and energy content of snack box foods

Food	Provided	kcal/100g	Fat (g)/serving	CHO (g)/serving	PRO (g)/serving
Apple	1	46.8	0.1	11.8	0.4
Mandarin	2	40.7	0.5	8.7	0.9
Ham	4 slices	119.7	2.8	1.4	22.3
Grated cheese	75 g	389.0	31.4	1.7	25.0
Bread	4 slices	212.1	2.2	40.4	10.2
Crisps	24 g	526.0	31.9	51.5	6.1
Margarine	20 g	408.9	45.0	0.5	0.5
Chocolate buttons	50 g	516.8	30.5	56.5	7.6
Yoghurt	1 pot	46.6	0.1	7.5	4.4

Figure 8.6 Snack box foods

8.2.6 Statistical analysis

Data are reported as mean ± SD throughout, unless otherwise stated. Statistical analysis was performed using IBM SPSS for Windows (Chicago, Illinois, Version 21). As the sample size is smaller in the current study compared with Study 1, significance was set at $p < .05$ to reduce the risk of committing a type two error. All variables were checked for outliers and normality was checked using the Shapiro-Wilk test. Several variables were not normally distributed and were therefore log transformed (natural log). Characteristics of the study population were summarised using descriptive statistics. Pearson correlations were performed to examine the associations of

behavioural (activity EE and time in different intensities of activity from sedentary to vigorous) and metabolic (RMR and FFM) components of total EE with EI and subjective appetite sensations (VAS). Pearson correlations performed on the transformed data only affected one correlation (total EE with snack box EI), therefore correlations are reported using the untransformed data as the untransformed data is easier to interpret and more meaningful. Furthermore, after performing the log transformation, snack box EI was still non-normally distributed so the non-parametric Spearman rank order correlation was performed on this variable.

8.3 Results

Table 8.6 provides sample characteristics for the 32 women participants who completed baseline measurements in the 12-week exercise intervention study Chapter 9). To be included in the analysis participants had to have baseline measures of free-living PA and SB, RMR[2], body composition, probe day EI and subjective appetite sensations. AP data was not included for one participant because the AP and SWA data did not represent the same monitoring period. Participants consumed more calories during the probe days (M = +269.94, SD = 528.37 kcal/d) than they expended on average per day during the free-living PA and SB monitoring period.

Table 8.6 Descriptive statistics of study sample

	Mean (SD)	Range
Age (years)	32.00 (11.36)	19.00 – 55.00
Stature (m)	1.65 (0.05)	1.56 – 1.79
Body mass (kg)	77.04 (9.63)	63.10 – 112.40
BMI (kg/m^2)[3]	28.21 (2.76)	24.70 – 35.10
FM (kg)	30.79 (7.49)	19.60 – 58.00
% FM	39.60 (5.09)	29.90 – 51.60
FFM (kg)	46.24 (3.96)	39.80 – 55.00
WC (cm)	94.81 (9.40)	76.60 – 111.30

[2] After the study was completed, RMR measured by the GEM was found to be unreliable and therefore the WHO RMR calculation was used in this study.

[3] One participant was recruited on the basis of being overweight at screening (wearing light clothing) but when body mass was measured using the BOD POD wearing minimal clothing during the baseline measures visit their BMI was <25 kg/m^2.

RMR (kcal/d)	1568.13 (137.36)	1400.30 - 2127.80
PA level	1.50 (0.17)	1.24 - 1.84
FREE-LIVING PA AND SB		
SWA wear time (hours/d)	23.61 (0.27)	22.80 – 24.00
Total EE (kcal/d)	2346.32 (310.52)	1811.30 – 3135.00
Activity EE (kcal/d)	819.00 (348.28)	318.00 – 1499.00
Light PA (min/d)	164.27 (65.55)	58.60 – 341.60
Moderate PA (min/d)	78.07 (43.60)	20.00 – 176.40
Vigorous PA (min/d)	3.82 (4.24)	0.00 – 14.60
MVPA (min/d)	81.88 (45.50)	23.00 – 188.80
Steps^{SWA}	7887.90 (2997.58)	3561.30 – 17474.60
Steps^{AP} ^	8700.09 (3553.15)	3770.00 – 18768.00
Standing (min/d) ^	235.80 (91.91)	105.20 – 481.90
Stepping (min/d) ^	100.71 (36.93)	50.90 – 195.10
SED^{SWA} (min/d)	729.25 (96.15)	496.10 – 882.60
SED^{AP} (min/d) ^	625.88 (110.48)	427.80 – 863.70
SED^{INT} (min/d) ^	561.80 (106.87)	331.20 – 755.40
ENERGY INTAKE[4]		
Total EI (kcal/d)	2616.26 (569.84)	1575.15 – 3825.20
***Ad libitum* EI (kcal/d)**	2112.18 (554.83)	1135.15 – 3353.20
Lunch EI (kcal/d)	800.89 (224.50)	383.95 – 1289.60
Dinner EI (kcal/d)	928.20 (282.98)	487.45 – 1693.40
Snack box EI (kcal/d)	383.10 (238.72)	0.00 – 902.90

^ n=31

[4] Expressed as the average calculated from two probe days

8.3.1 Relationship between behavioural and metabolic components of energy expenditure and objectively measured probe day energy intake

Table 8.7 displays the correlations between EE, free-living PA, SB and EI. There was a significant positive correlation between SWA measured total EE and probe day total EI (Figure 8.7), *ad libitum* EI and snack box EI (Figure 8.8). Spearman rank order correlation analysis also resulted in a significant positive correlation between total EE and snack box EI [$r_s(30) = .38$, $p = .03$]. The magnitude and direction of the Spearman correlation was the same as the Pearson correlation (see Table 8.7). Although total EE was significantly correlated with EI, activity EE, time spent in different intensities of PA, time spent in different postures and in SB were not significantly associated with probe day EI.

Table 8.7 Correlations between behavioural EE, time spent in different intensities of activity and EI

	Total EI (kcal/d)	*Ad libitum* EI (kcal/d)	Lunch EI (kcal/d)	Dinner EI (kcal/d)	Snack box EI (kcal/d)
Activity EE (kcal/d)	.17	.16	.03	.05	.28
Total EE (kcal/d)	**.40***	**.37***	.21	.25	**.38***
Light PA (min/d)	.14	.16	.03	.19	.11
Moderate PA (min/d)	.07	.05	.01	-.09	.22
Vigorous (min/d)	-.08	-.09	-.21	-.05	.05
MVPA (min/d)	.06	.04	-.01	-.10	.21
Steps^{SWA}	.12	.11	.01	.05	.19
Steps^{AP}	.13	.12	.07	-.02	.24
Standing (min/d)	-.01	-.04	-.13	-.01	.05
Stepping (min/d)	.18	.16	.10	.03	.25
SED^{SWA} (min/d)	-.05	-.07	-.03	-.11	-.02
SED^{AP} (min/d)	-.02	-.01	.03	-.03	-.02
SED^{INT} (min/d)	-.05	-.04	.00	.01	-.10
PA level	.14	.12	.06	-.05	.28

Data are Pearson correlation (r). * p<.05.

Table 8.8 displays correlations between RMR, FFM, FM and EI. RMR and FFM were significantly positively correlated with total EI, *ad libitum* EI and EI at dinner. However, FM was not significantly correlated with any of the measures of probe day EI. FFM was positively correlated with RMR [r(30) = .69, p < .001] and total EE [r(30) = .50, p = .003].

In passing it can be noted that in this study, with a small homogeneous sample of obese women, the amount of vigorous PA was negatively associated with adiposity (FM) [r(30) = -.44, p = .012]. Although the amount of vigorous PA was low in this sample this outcome confirms the relationship found in Study 1.

Table 8.8 Correlation between contributors to metabolic EE, body composition and EI

	Total EI (kcal/d)	*Ad libitum* EI (kcal/d)	Lunch EI (kcal/d)	Dinner EI (kcal/d)	Snack box EI (kcal/d)
RMR (kcal/d)	**.43***	**.41***	.26	**.43***	.20
FFM (kg)	**.51****	**.50****	.33	**.52****	.25
FM (kg)	.31	.29	.26	.14	.27

Data are Pearson correlation (r). ** p<.01; * p<.05.

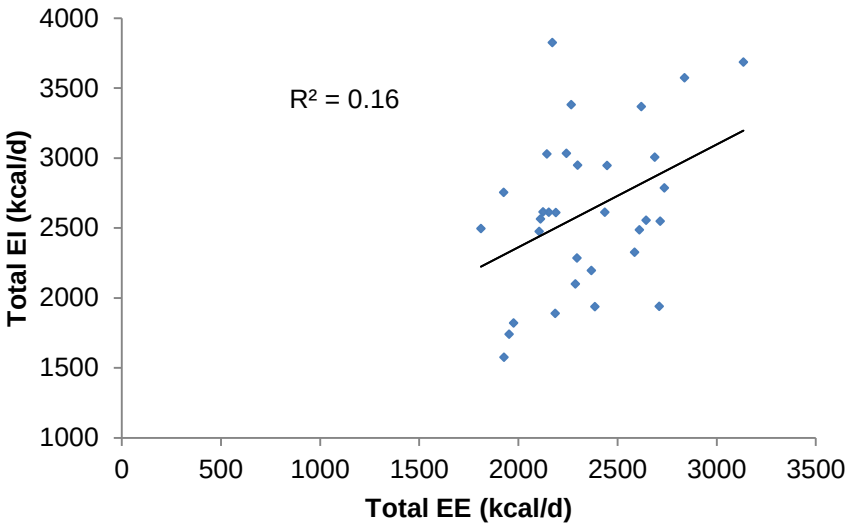


Figure 8.7 The association between total EE and total EI

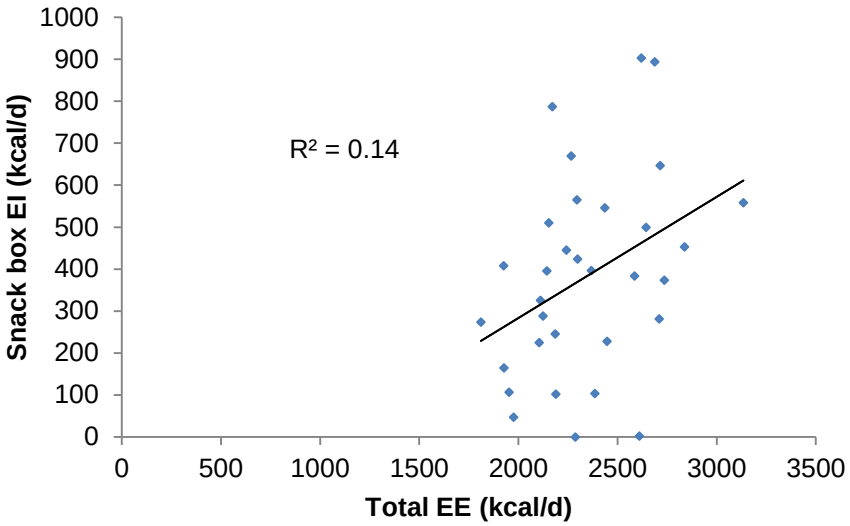


Figure 8.8 The association between total EE and snack box EI

- **Total EE was positively associated with total EI, *ad libitum* EI and snacking**
- **Activity EE and time spent in different intensities of activity (from sedentary to vigorous) were not systematically related to EI**
- **RMR and FFM were positively associated with total EI**
- **Vigorous PA was negatively associated with FM (adiposity)**
- **There was no significant association between FM (adiposity) and EI**

8.3.2 Relationship between behavioural and metabolic contributors to energy expenditure and subjective appetite sensations

Total EE was negatively associated with fasting levels of fullness [r(30) = -.36, p = .05], see Figure 8.9. There was a significant positive association between time spent in light intensity PA and fasting levels of hunger [r(30) = .37, p = .04]. There were no other significant correlations between measures of free-living sedentary and active behaviours, EE and fasting appetite sensations.

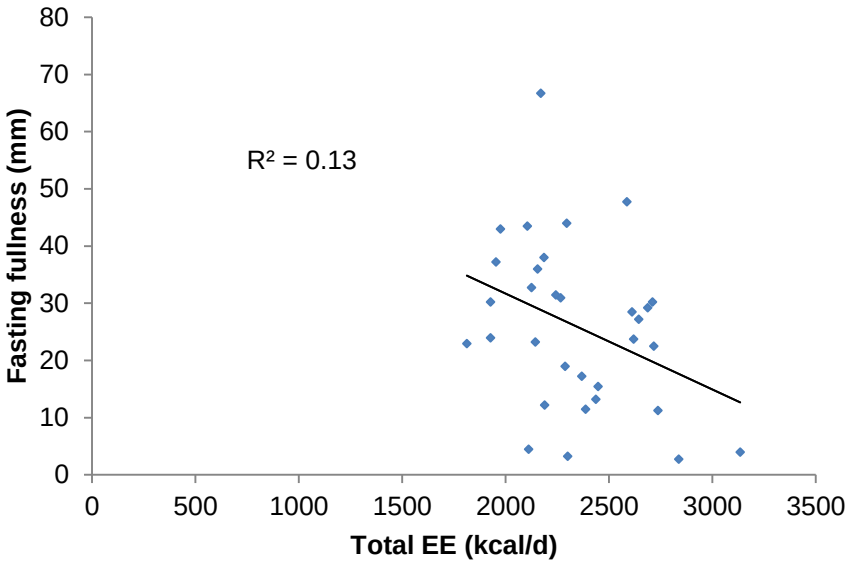


Figure 8.9 The association between total EE and fasting fullness

VAS scores throughout the probe day were used to calculate AUC to provide an aggregate value for each appetite rating for the whole day. Correlation analysis revealed total EE was significantly positively associated with AUC hunger (see Figure 8.10) and desire to eat. Furthermore, RMR and active EE were positively associated with AUC desire to eat. There were no other significant relationships between measures of free-living sedentary and active behaviours and appetite ratings as can be seen in Table 8.9.

Table 8.9 Correlations between components of EE and AUC for hunger, fullness, desire to eat and prospective food consumption

	AUC hunger (mm x min)	AUC fullness (mm x min)	AUC desire to eat (mm x min)	AUC PFC (mm x min)
FFM (kg)	.26	-.29	.33	.27
RMR (kcal/d)	.28	-.18	**.37***	.20
Activity EE (kcal/d)	.31	-.14	**.36***	.19
Total EE (kcal/d)	**.36***	-.27	**.48****	.31
Light PA (min/d)	.28	-.14	.24	.06
Moderate PA (min/d)	.23	-.09	.32	.15

Vigorous (min/d)	.29	-.07	.29	.27
MVPA (min/d)	.25	-.10	.33	.17
Steps^{SWA}	.24	-.14	.30	.14
Steps^{AP}	.25	-.10	.28	.11
Standing (min/d)	-.09	.16	-.05	-.17
Stepping (min/d)	.24	-.15	.31	.10
SED^{SWA} (min/d)	-.13	-.03	-.11	.06
SED^{AP} (min/d)	.15	-.22	.13	.31
SED^{INT} (min/d)	.00	-.21	.01	.21

Data are Pearson correlation (r). *** p<.001; ** p<.01; * p<.05.

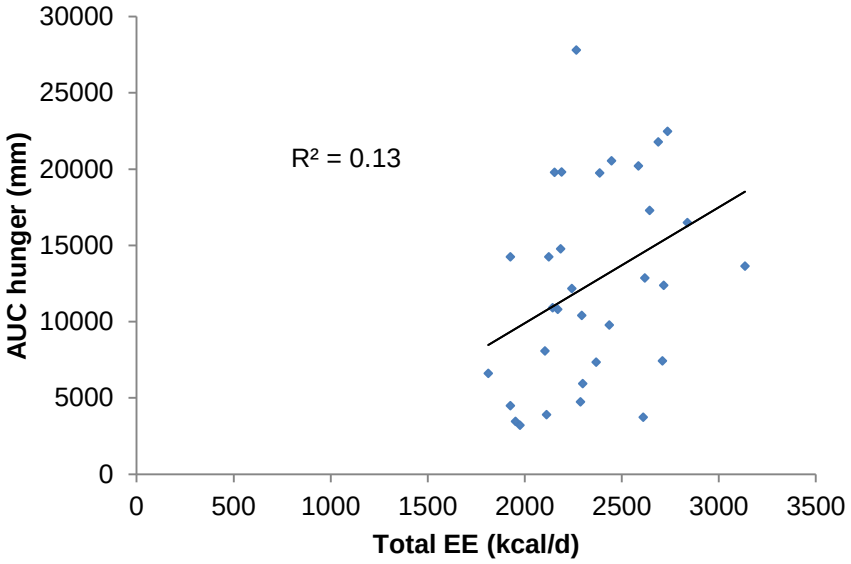


Figure 8.10 The association between total EE and AUC for hunger

Table 8.10 displays the correlations between components of EE and SQ for hunger, fullness, desire to eat and prospective food consumption in the four hours after consumption of a individually calibrated fixed breakfast. There were no significant correlations between measures of RMR, free-living total and activity EE, sedentary and active behaviours and SQ variables. However, FFM was negatively associated with SQ for desire to eat.

Table 8.10 Correlations between components of EE and SQ[5] for hunger, fullness, desire to eat and prospective food consumption in response to the fixed breakfast

	SQ hunger (mm/kcal)	SQ fullness (mm/kcal)	SQ desire to eat (mm/kcal)	SQ PFC (mm/kcal)
FFM (kg)	-.25	.33	**-.42***	-.29
RMR (kcal/d)	.04	.27	.11	.05
Activity EE (kcal/d)	.14	.04	-.08	.10
Total EE (kcal/d)	.03	.04	-.17	-.02
Light PA (min/d)	.31	.08	.18	.22
Moderate PA (min/d)	.11	.08	-.13	.10
Vigorous (min/d)	.12	.02	-09	.16
MVPA (min/d)	.12	.08	-.12	.11
Steps^{SWA}	.15	.21	-.04	.01
Steps^{AP}	.06	.16	-.09	-.08
Standing (min/d)	.09	.08	.09	.16
Stepping (min/d)	.12	.18	-.07	-.06
SED^{SWA} (min/d)	-.28	-.03	-.18	-.18
SED^{AP} (min/d)	-.14	-.12	-.20	-.10
SED^{INT} (min/d)	-.23	-.05	-.27	-.22

Data are Pearson correlation (r). *** p<.001; ** p<.01; * p<.05.

- **Total EE was negatively associated with fasting fullness and positively associated with AUC for hunger and desire to eat throughout the day**

8.3.3 Relationship between objectively measured probe day energy intake and subjective appetite sensations

Correlation analysis was performed to ascertain whether EI was related to subjective appetite sensations (see Table 8.11). Total and *ad libitum* EI were positively associated with AUC for hunger (see Figure 8.11) and desire to eat and negatively associated with AUC for fullness (see Figure 8.12). There was a negative correlation between *ad libitum* EI and SQ for fullness (see Figure 8.13) that appeared to be driven by snack box EI. Measures of EI during the probe day were not associated with any of the fasting appetite sensations nor were they associated with SQ for hunger, desire to

[5] SQ was calculated on the basis of the breakfast meal

eat or prospective food consumption and AUC prospective food consumption. Lunch EI was not associated with any of the subjective appetite variables.

Table 8.11 Correlations between EI and subjective appetite variables

	Total EI (kcal/d)	*Ad libitum* EI (kcal/d)	Lunch EI (kcal/d)	Dinner EI (kcal/d)	Snack box EI (kcal/d)
AUC hunger (mm x min)	**.41***	**.40***	.20	.29	**.40***
AUC fullness (mm x min)	**-.40***	**-.41***	-.28	**-.38***	-.23
AUC desire to eat (mm x min)	**.47****	**.45***	.16	**.37***	**.45****
AUC PFC (mm x min)	.34	.32	.19	.20	.33
Fasting hunger (mm)	.25	.24	.11	.28	.12
Fasting fullness (mm)	-.13	-.12	-.13	-.23	.12
Fasting desire to eat (mm)	.19	.18	.13	.24	.01
Fasting PFC (mm)	.15	.13	.22	.09	-.01
SQ hunger (mm/kcal)	-.08	-.08	-.08	.11	-.23
SQ fullness (mm/kcal)	-.34	**-.35***	-.25	-.14	**-.42***
SQ desire to eat (mm/kcal)	-.13	-.12	-.07	.05	-.28
SQ PFC (mm/kcal)	-.18	-.17	-.02	-.05	-.32

Data are Pearson correlation (r). *** p<.001; ** p<.01; * P<.05.

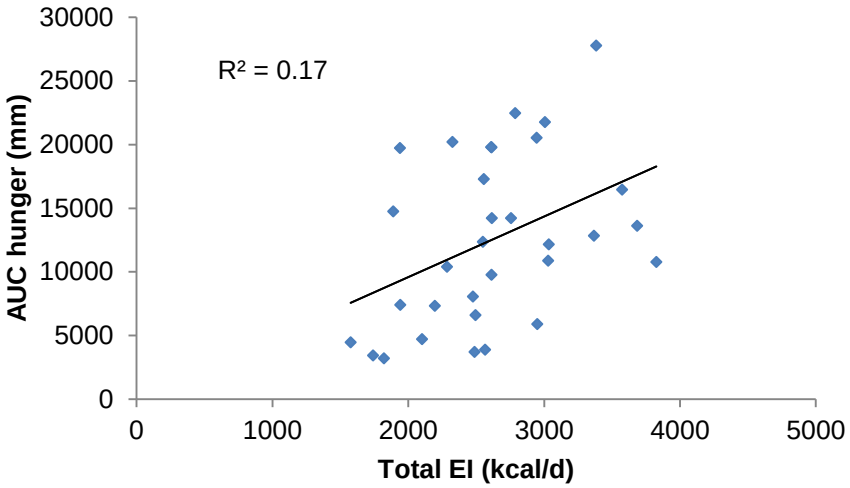


Figure 8.11 The association between AUC for hunger and total EI

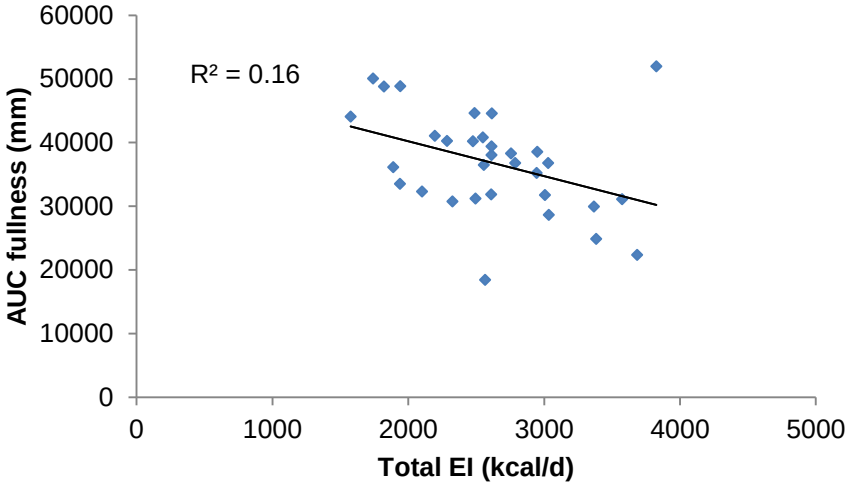


Figure 8.12 The association between AUC for fullness and total EI

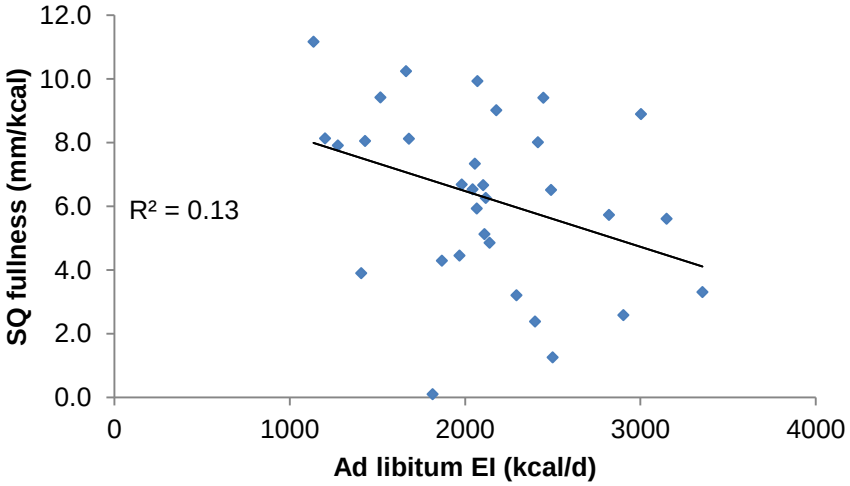


Figure 8.13 The association between SQ for fullness and *ad libitum* EI

- **Homeostatic appetite variables (EI and AUC for hunger) were positively related indicating coherence in the appetite system**

8.4 Discussion

This study investigated the relationship between behavioural and metabolic contributors to total EE and measures of EI and homeostatic appetite control in overweight and obese inactive women. It was hypothesised that total EE, RMR and FFM would be positively associated with EI and hunger reflected in appetite sensations during the probe days. There was a positive association between free-living total EE measured using the SWA and objectively measured probe day EI. Furthermore, total EE was associated with lower fasting fullness and higher levels of hunger and desire to eat throughout the day (AUC). These data support the work of Edholm et al. (1970) who reported a positive association between EI and EE in army cadets when data were averaged over the course of a week. Indeed, a similar association has been observed when individuals are categorised based on their PA status (Shook et al., 2015). In a recent systematic review, Beaulieu et al. (2016) investigated the relationship between standardised EI data from 10 cross-sectional studies and PA level and reported a j-shaped curve for EI as habitual PA level increased. This supports the findings of Mayer et al. (1956) over 60 years ago who observed a linear relationship between EI and EE in jute mill workers, however, the linear relationship was only observed above a certain level of PA. More recently Harrington et al. (2013) and Shook et al. (2015) have provided support for the uncoupling of EI to EE using objective measurement techniques to classify participants based on activity related EE and levels of MVPA, respectively. The positive correlation between EI and EE (total, *ad libitum* and snack box) in the current study suggests participants were performing enough PA to be in the 'regulated zone' of the j-shaped curve. Furthermore, participants performed a similar number of steps to the threshold for the 'optimum' level of PA (7116 steps/d) reported by Shook et al. (2015).

When total EE was divided in to metabolic (RMR, FFM) and behavioural (categories of PA, activity EE) components, RMR showed a positive relationship with EI. RMR is the largest component of total EE (50-70%) and, unlike PA EE, is stable across the day and between days and therefore generates a more constant and stable energetic demand (Shetty, 2005, Goran, 2000, Ravussin et al., 1982). The tonic energy demand arising from RMR was positively associated with total, *ad libitum* and dinner EI and AUC desire to eat throughout the day in the current sample of overweight and obese inactive women. A similar relationship has previously been reported in overweight and obese men and women in an inactive and active state. Caudwell et al. (2013a)

concluded participants with high RMRs showed higher levels of hunger across the day and greater food intake than did individuals with lower RMRs.

FFM, which has the largest influence on RMR and accounts for around 60-70% of the variability (but not FM which accounts for 5-7% of the variability in RMR (Johnstone et al., 2005)), was also positively associated with EI and negatively associated with SQ desire to eat throughout the morning in the current study. This relationship has previously been highlighted (Caudwell et al., 2013a, Weise et al., 2013, Blundell et al., 2012b) and it has been proposed that the relationship between FFM and EI might be driven by some signal arising from FFM which drives food intake, however evidence for such a mechanism is yet to be established (Blundell et al., 2012a). In the current study RMR and FFM showed similar associations with EI; both were positively associated with EI. It has been postulated that RMR acts as a mediator variable to reflect the influence of FFM on appetite and EI (Blundell et al., 2012b). Recently, Hopkins et al. (2016) demonstrated that both FFM and RMR predicted daily EI but when both were included in the regression model FFM did not independently predict EI. Furthermore, a mediation model using path analysis indicated that the effect of FFM on EI was fully mediated by RMR.

It would seem logical to assume that the positive association of RMR and FFM to EI and subjective appetite sensations might lead to weight gain by stimulating the drive to eat. However, the relationship between RMR, FFM and EI could be viewed as a homeostatic mechanism to prevent a negative energy balance by producing a drive to eat to ensure EI does not fall below the requirements of the body to maintain the functioning of the body's vital systems and to preserve the body's FFM (Blundell et al., 2012a). Indeed, the amount of energy actually consumed may depend on the energy density and palatability of the food being eaten (McCrory et al., 2000, Rolls, 2009). Caudwell et al. (2013a) reported a positive correlation between RMR and EI when participants consumed both high energy dense foods (~4.1 kcal/g) and low energy dense foods (~2.4 kcal/g); but importantly the high energy dense condition resulted in a 39% increase in EI (535 kcal) across the day. This is a clear example of passive overconsumption and highlights the importance of food choice in the current obesogenic environment (Swinburn et al., 2011).

In the current study, participants were in a positive energy balance consuming more energy on probe days (270 kcal) compared with the average daily EE calculated from the previous week. Although EI increased with EE in a linear fashion, participants did not accurately match EI to EE and if sustained would result in weight gain. In turn, further increases in weight (FM) might act as a disincentive to perform PA and also result in further appetite dysregulation. Since leptin resistance increases as adiposity increases this implies the inhibitory action of FM on EI would weaken with increased

fat (Considine et al., 1996, Blundell et al., 2015a) and could explain why the overweight and obese participants in the current study were in a positive energy balance. Leptin resistance would result in the blunted inhibition of the orexigenic drive arising from FFM and RMR. However, EI was averaged over two days, whereas EE was averaged over 5-7 days. It is possible that participants could have consumed fewer calories on the days where EI was not measured. Furthermore, participants eating behaviour in the laboratory may not have reflected their normal eating patterns. For example, exposure to higher energy dense foods than they would usually consume (i.e. tomato and herb risotto: 1.68 kcal/g) could have led to passive overconsumption (Blundell and MacDiarmid, 1997).

It was also hypothesised that time spent in MVPA would be positively associated with EI and hunger reflected in appetite sensations throughout the probe day, and time spent sedentary would also be positively associated with EI. The associations between time spent in different intensities of activity, objectively quantified 24 hour eating behaviour and subjective appetite sensations have not previously been examined. Studies suggest that SB is associated with unhealthy dietary patterns including higher EI and snacking on high energy dense fast foods (Pearson and Biddle, 2011, Hu et al., 2003, Scully et al., 2009, Chaput et al., 2011). In the current study, regardless of how SB was operationally defined and objectively measured (SED^{SWA}, SED^{AP} and SED^{INT}) there were no significant relationships with EI or subjective appetite sensations. This was surprising in light of previous work reporting an association between SB (TV viewing and screen-time) and EI. However, these studies predominantly use TV viewing as a proxy for SB and TV viewing specifically may distract attention from internal signals in response to food consumption and lead to an impaired satiety response (de Graaf and Kok, 2010) limiting the generalizability of TV viewing to SB *per se*. Study 1 in this thesis examined the relationship between objective measures of free-living SB and MVPA with trait measures of appetite dysregulation (TFEQ-D and BES). There was no relationship between sedentary time, and a negative association between MVPA time and appetite dysregulation, however this relationship was no longer apparent after controlling for body fat. The current study was designed to expand on findings from previous studies by examining the relationship between different intensities of PA (including SB) and objectively quantified measures of homeostatic appetite. Time spent in different intensities of activity (from sedentary to vigorous) was not systematically related to any of the homeostatic measures of appetite or EI. Indeed, the lack of a relationship between MVPA and measures of appetite sensations and EI could reflect the relatively small contribution of PA to total EE in this sample of inactive, overweight women and the large variability in volitional PA.

The measurement of EI in this study was positively associated with hunger and desire to eat and negatively associated with fullness throughout the day. Greater hunger and desire to eat was associated with greater EI whereas higher fullness throughout the day was associated with lower EI. This demonstrates that the method for measuring EI was valid as it was related to subjective appetite sensations. The probe day methodology used in the current study ensured adequate sensitivity to reveal the relationship between EI and EE. The meals provided were designed to reflect an eating pattern that were consistent with the normal eating habits of the participants. In addition, participants consumed breakfast, lunch and dinner in a purpose built feeding cubicle under scientifically controlled conditions that were free from disturbing or interfering stimuli. Consequently, the procedure allowed the volitional intake of participants to sensitively reflect the physiologic demand for energy that arose from individual differences in total EE, RMR and FFM. However, laboratory based measures of EI are not without limitations. Indeed, the measurement of EI under controlled conditions allow precise measurement of eating behaviour but generalisability to the free-living setting is limited (Gibbons et al., 2014). Alternatively, free-living measures of EI are externally valid but less precise. Methods to measure eating behaviour in the free-living environment are less reliable and often produce physiological implausible estimates of EI. For example, examination of NHANES data on EI collected over 39 years found underreporting may be as much as 800 kcal/d (Archer et al., 2013a). Hence, the preference to measure eating behaviour under controlled laboratory conditions.

This study examined the associations among free-living sedentary and active behaviours, EE and homeostatic appetite control using an experimental platform which enables the measurement of behavioural, physiological and metabolic components of energy balance. It was possible to identify a relationship between total EE, RMR, FFM and subjective appetite sensations and probe day EI. As the demand for energy increased (total EE and RMR) so too did total EI. When total EE was divided in to metabolic and behavioural component, only RMR and FFM were associated with EI and subjective appetite sensations. Such associations do not provide evidence of a causal relationship nor do they provide any mechanism. However, the results of this study, along with others, justify further research to identity mechanisms through which any energy demand reflected by RMR and total EE is translated into a drive to eat and subsequent eating behaviour.

8.5 Outcomes

- **The metabolic contributors to total EE (RMR and FFM) were positively associated with total EI (confirming previously identified relationships)**
- **Total EE was positively associated with total EI, *ad libitum* EI and snacking**
- **Total EE was negatively associated with fasting fullness and positively associated with AUC for hunger and desire to eat throughout the day**
- **Homeostatic appetite variable (EI and AUC for hunger) were positively related indicating coherence in the appetite system**
- **Behavioural components of total EE were not associated with any measures of homeostatic appetite control. Activity EE and time spent in different intensities of activity (from sedentary to vigorous) were not systematically related to any of the homeostatic measures of appetite**
- **These outcomes may only be true for overweight, inactive women with very low levels of vigorous PA**

Chapter 9
Study 5 - Effects of a 12-Week Aerobic Exercise Intervention on Body Composition, Appetite Control And Free-Living Sedentary and Active Behaviours in Overweight And Obese Inactive Women

This chapter will examine the effects of a12-week supervised aerobic exercise intervention on body composition, homeostatic appetite control and free-living sedentary and active behaviours in overweight and obese inactive women. Body composition, 24 hour eating behaviour and subjective appetite sensations were measured at baseline and post-intervention. Free-living physical activity and sedentary behaviour was assessed at baseline, week 1 and 10 of the exercise intervention and post-intervention. This study was designed to detect any effect of exercise-induced weight loss on compensatory changes in EI (appetite variables) and/or EE (free-living sedentary and active behaviours). Twenty-four participants completed the study.

9.1 Introduction

The current global obesity epidemic is well documented. A number of factors have been identified to explain the increase in obesity over the last 40 to 50 years including changes in diet (Vandevijvere et al., 2015, Crino et al., 2015, Swinburn et al., 2009) and PA related EE (Church et al., 2011, Archer et al., 2013b, Wen et al., 2006). As overweight and obesity levels continue to rise so too does the importance of identifying effective intervention strategies to prevent and reverse weight gain (Ng et al., 2014). Simplistically, weight gain and obesity are caused by a positive energy balance; if calories in exceed calories out weight gain will occur (Hall et al., 2011). Regular MVPA has been identified as an effective weight loss strategy, furthermore cardiovascular disease risk and body composition are improved even with modest weight loss (Shaw et al., 2006, King et al., 2009b, Manthou et al., 2010). Observational studies (including Study 1 in this thesis) have also found that individuals who are more physically active have less FM (Myers et al., 2016, Shook et al., 2015). Not only does exercise have a direct effect on energy balance by increasing EE, exercise has also been shown to have an indirect effect through its effect on EI and appetite (Blundell et al., 2015b).

It has been noted that weight loss observed with supervised and measured exercise interventions is highly variable between individuals and is often less than expected, based on the energy expended through exercise, suggesting some form of compensation (Thomas et al., 2012, King et al., 2008). The average weight loss observed with exercise interventions is between 30% (long-term interventions) and 85% (short term interventions) of that predicted (Ross and Janssen, 2001). This less than expected weight loss could be due to behavioural (reduced NEPA EE or increased EI) or metabolic (reduced RMR or more efficient exercise EE) compensation and would offset the negative energy balance accrued through the energy expended during exercise (King et al., 2007). In a recent review, Riou et al. (2015) reported compensation was approximately 18% on average for all studies included in the review (study duration 7 to 80 weeks), however, as the duration of the intervention increased so too did the degree of compensation and for long-term (~80 weeks) interventions compensation approached 84%. It is important to note that only four studies were conducted over >70 weeks and the authors were unable to determine whether the compensation was due to increased EI, reduced EE or a combination of both.

9.1.1 Changes in energy intake and appetite in response to increased exercise-induced energy expenditure

9.1.1.1 Acute exercise

EI is a major contributor to the behavioural determinants of body mass regulation. Therefore, changes in eating behaviour driven by changes in appetite could contribute to compensation in response to increased exercise-induced EE. These compensatory responses may differ depending on the intervention duration (acute vs. longer-term). It is often suggested that increases in EE through exercise are automatically compensated for by an increase in EI offsetting the negative energy balance created, rendering exercise futile for weight loss. The majority of studies investigating changes in EI in response to acute exercise suggest there is no compensation in hunger or food intake in the immediate hours after an exercise bout to restore energy balance (Schubert et al., 2013, Donnelly et al., 2014). In fact, when EI is adjusted to account for energy expended through exercise (relative EI), EI is lower after exercise compared with no exercise resulting in an acute energy deficit (Hagobian et al., 2012, King et al., 2013, Rocha et al., 2013, King et al., 2010b, Deighton et al., 2012). Indeed, there is evidence that an acute bout of moderate to high intensity aerobic exercise induces a transient decline in hunger (exercise-induced anorexia) perhaps mediated by the suppression of acylated ghrelin during and immediately after the exercise bout (King et al., 1994, Broom et al., 2007, Broom et al., 2017). The energy deficit observed

in the majority of acute studies, if maintained over a longer period, would have implications for weight management.

9.1.1.2 Exercise training

During longer term interventions it is plausible to expect some degree of compensation in EI in response to increased EE given the linear relationship between EI and EE (at moderate to high PA levels) observed by Mayer et al. (1956) and Edholm et al. (1970). However, it is difficult to quantify the degree of compensation in response to longer term exposure to increased exercise due to the limitations inherent with accurately measuring EI under free-living conditions (Dhurandhar et al., 2015). In keeping with the effects of acute exercise on appetite and eating behaviour, longer term interventions have often reported no change in hunger and EI (Donnelly et al., 2014). However, studies are open to a number of limitations, for example, exercise interventions are often unsupervised (are participants adhering to the intervention?), the exercise-induced energy deficit is low (is the energy deficit sufficient to induce weight loss and compensation?) and EI is self-reported (not sensitive to detect changes).

When change in EI has been measured objectively under laboratory conditions following longer term exercise exposure (up to 16 days) compensation has been estimated to be around 30% of the exercise-induced energy deficit (Blundell et al., 2003, Whybrow et al., 2008). This partial compensation suggests EI begins to track energy expended through exercise and raises the possibility that EI may continue to rise with longer term interventions (>16 days). Results from a 12-week aerobic exercise intervention study demonstrate large inter-individual variability in weight loss response (body mass change: -14.7 kg to +1.7 kg) and these differences can be attributed to changes in EI and appetite (King et al., 2008). Based on whether participants achieved the expected amount of weight loss calculated from the amount of energy expended though exercise, King et al. (2008) categorised participants as compensators (body mass change: -1.5 kg) or non-compensators (body mass change: -6.3 kg). Characterisation of the ‘compensators’ and ‘non-compensators’ behavioural responses to exercise revealed compensators increased their EI in response to exercise (+268.2 kcal) whereas non-compensators decreased their EI (-130 kcal). Furthermore, post-intervention subjective hunger was greater in the compensators compared with non-compensators whose hunger levels remained stable, however this did not reach statistical significance.

In a similar study, King et al. (2009a) demonstrated a ‘dual-process’ action of exercise on appetite characterised by an increase in fasting hunger coupled with improvements in meal-induced satiety. Although exercise increases the drive to eat in the fasted state, sensitivity to post-prandial satiety signals is enhanced because the same

amount of food (fixed breakfast) resulted in greater suppression of hunger immediately after consumption which was maintained until lunch. Similarly, Martins et al. (2010) reported that exercise-induced weight loss increased fasting hunger and acylated ghrelin. Furthermore, they reported an improved satiety response evidenced by an increase in late (90-180 minutes) post-prandial release of glucagon-like peptide-1 (GLP-1) and a significant increase in the suppression of acylated ghrelin. King et al. (2009a) performed further analyses by categorising participants based on whether their change in body composition was equal to or greater than (responders), or less than (non-responders) the expected change due to the exercise-induced EE. This revealed 'non-responders', but not 'responders', exhibited greater hunger levels throughout the day after the 12-week exercise intervention reflected in AUC for hunger. The increased hunger was accompanied by a significant increase in total EI.

9.1.2 Change in non-exercise physical activity in response to increased exercise-induced energy expenditure

Energy expended through PA also contributes to the behavioural component of energy balance, therefore it is possible that a reduction in PA outside of prescribed and structured exercise could compensate for the negative energy balance resulting from increased structured exercise (King et al., 2007). PA outside of structured exercise will be referred to as NEPA and reflects changes in PA outside of the exercise intervention[1]. This includes changes in NEPA represented by EE, steps per day, counts per minute or time spent in different intensities of activity. Whether structured exercise results in compensatory changes in NEPA throughout the rest of the day remains equivocal, partially due to the difficulty in accurately and reliably measuring free-living EE and time spent in different intensities of PA (Garland et al., 2011). Furthermore, there is evidence to suggest differences in exercise dose between studies could contribute to conflicting finding regarding compensation in NEPA (Church et al., 2009). The majority of studies that assess change in NEPA with structured exercise only report EE outcomes (total and activity EE) (Goran et al., 1994, Westerterp, 1998, Hollowell et al., 2009, Whybrow et al., 2008). Previously, NEPA has predominantly been measured using DLW. DLW allows for the determination of free-living EE using a non-invasive technique whereby on innocuous fluid containing stable isotopes ^{2}H and ^{18}O is ingested. The disappearance rate of ^{18}O relative to ^{2}H (measured in urine sample before and after drinking the DLW and again 1-2 weeks later) is a measure of CO_2 production rate and this can be converted to an estimate of EE (Buchowski, 2014). However, DLW is not without limitation and this method is

[1] NEPA includes non-exercise activity thermogenesis (NEAT) which refers to 'the thermogenesis that accompanies physical activities other than volitional exercise, such as the activities of daily living, fidgeting, spontaneous muscle contraction, and maintaining posture when not recumbent' (Levine et al., 1999)

expensive, requires specialist equipment and expertise, it is not possible to determine time spent in different intensities of activity and it only provides an average daily EE over a long period of time (e.g. 10-14 days). Activity monitors provide information on the pattern and intensity of activity (i.e. time spent sedentary or in MVPA) that cannot be achieved using the DLW technique, however few studies report NEPA as time spent in different intensities of activity. A recent systematic review and meta-analysis carried out on randomized controlled trials with exercise interventions lasting at least two weeks found that on average there was no statistically significant change in NEPA in response to exercise interventions (Fedewa et al., 2016). However, the exercise studies included were relatively small and were not specifically designed to detect changes in NEPA. Furthermore, the review was limited to EE, steps per day and counts per minute outside of the exercise sessions and did not examine changes in time spent in different intensities of PA and SB. An earlier systematic review that included cross-sectional, non-randomized and randomized trials also found no consistent evidence to support a compensatory reduction in NEPA in response to structured exercise (Washburn et al., 2014).

Turner et al. (2010) used synchronised HR monitoring and accelerometry to determine NEPA. The authors concluded there was no reduction in light or moderate intensity NEPA as a result of a six month aerobic exercise intervention. As with other behavioural compensatory responses to increased exercise EE, there appears to be large individual variability in responses to structured exercise. When NEPA was assessed using the SWA (Pro 2) in postmenopausal women, Di Blasio et al. (2012) reported a compensatory reduction in NEPA in half of the participants in their study in response to a 13-week walking intervention. Similarly, when participants were categorised based on whether they achieved the expected reduction in FM or not based on their exercise-induced EE, those who lost the expected amount of FM did not decrease their NEPA (activity EE outside of structured exercise measured using PA diaries and HR monitoring), whereas those who achieved less than expected FM loss did reduce their NEPA (Manthou et al., 2010). Furthermore, change in activity EE (excluding structured exercise EE) predicted 13% of the variance in change in FM as a result of the exercise-induced energy deficit. Herrmann et al. (2015) objectively quantified NEPA and compared participants who lost >5% of their baseline body mass (responders) as a result of a 10-month aerobic exercise intervention with those who lost <5% of their original body mass (non-responders). This revealed a significant difference in non-exercise EE (DLW) and NEPA (defined as time accumulate at >100 counts/minute measured using the Actigraph GT1M) with responders exhibiting an increase and non-responders decreasing non-exercise EE and NEPA. Similarly, Kozey-Keadle et al. (2014) found large individual variability in NEPA (time spent in MVPA measured using the AP) with nearly half of the participants decreasing NEPA

compared with baseline despite no change in NEPA when data were averaged across the whole sample.

As well as compensatory changes in PA, it has been hypothesised that SB could increase in response to structured exercise as a result of greater fatigue or, alternatively SB could be displaced by structured exercise and therefore decrease. Herrmann et al. (2015) found no difference in sedentary time during a 10-month exercise intervention. Similarly, when change in SB was examined before and during a marathon training regime there was no change in sedentary time despite a significant increase in vigorous PA (Swartz et al., 2016). Furthermore, Manthou et al. (2010) found no change in sedentary EE following an 8-week exercise intervention. As with NEPA, change in sedentary time is highly variable between participants with half of the participants in the study by Kozey-Keadle et al. (2014) increasing sedentary time in response to a 12-week exercise intervention. Further research is needed to better understand the effects of structured exercise on sedentary time.

Increased structured exercise affects energy balance directly through increased EE and indirectly by impacting on appetite control and therefore EI. There is growing evidence to demonstrate large individual variability in weight loss as a result of exercise interventions and this could be due to behavioural compensation in both eating behaviour (EI) and NEPA (EE). The aim of this study was to investigate the effects of a 12-weeks supervised and monitored aerobic exercise intervention on body composition, 24 hour eating behaviour, subjective appetite sensations and free-living sedentary and active behaviours in overweight and obese inactive (PA level 1.5) women.

9.1.3 Hypotheses

- The 12-week aerobic exercise intervention will lead to reduced body mass, FM and increased FFM
- There will be large individual variability in weight loss in response to the exercise intervention
- The 12-week aerobic exercise intervention will lead to increased EI and hunger
- There will be a compensatory decrease in NEPA and an increase in SB in response to the 12-week exercise intervention

9.2 Methods

9.2.1 Participants

Thirty-two overweight/obese and inactive participants (women) were recruited to take part in the study. Of those 32 participants, 24 women aged 33.1 years (SD = 11.7) with

a BMI of 27.9 kg/m^2 (SD = 2.7) completed the study. Participants were recruited from the University of Leeds, UK, and surrounding area using posters and email mailing lists for this medium-term exercise study. The recruitment email contained a link to an online survey to assess the eligibility of potential participants. The online questionnaire contained questions pertaining to the inclusion and exclusion criteria and also asked respondents to rate study foods on a 7-point Likert scale to assess the acceptability of the study foods. Based on the responses to the online survey, eligible participants were sent a Participant Information Sheet and invited to the HARU for an information and screening visit (see section 9.2.4). All participants provided written informed consent before taking part in the study. The study procedures and all study materials were reviewed and approved by the National Research Ethics Service Committee Yorkshire & the Humber; ref: 09/H1307/7; date: 02/04/15.

9.2.2 Inclusion and exclusion criteria

Details of specific inclusion and exclusion criteria are outlined in Study 4 (Chapter 8).

9.2.3 Design

This study was a repeated measures design with a 12-week supervised aerobic exercise intervention. Participants were required to exercise five times per week for 12 weeks. Each exercise session was individually calibrated to expend 500 kcal at 70% of their HR maximum (2500 kcal per week). This was calculated from information gathered using IC during the maximal fitness test (see 9.2.8.1). Compliance with the exercise intervention was monitored and tracked daily using HR monitors (S610, POLAR, Finland) to ensure the correct intensity and duration of exercise was achieved. Participants completed two probe days prior to the exercise intervention commencing and two on completion of the exercise intervention. Participants were provided with an individually fixed energy breakfast (25% of RMR), an *ad libitum* lunch, dinner and evening snack box to assess the effects of exercise on 24 hour eating behaviour and VAS were completed at intervals throughout the day to assess subjective appetite sensations. Health markers, body composition, psychometric eating behaviour traits and RMR were taken before and after the exercise intervention and free-living PA was measured at multiple time points throughout the 16 week study period (see Figure 9.1). Participants received payment of £240 on completion of the study to reimburse them for their time and expenses. Further details of the laboratory visits, study procedures and exercise intervention are provided below. Probe day procedures have been described in the previous chapter (Chapter 8) and will not be repeated here.

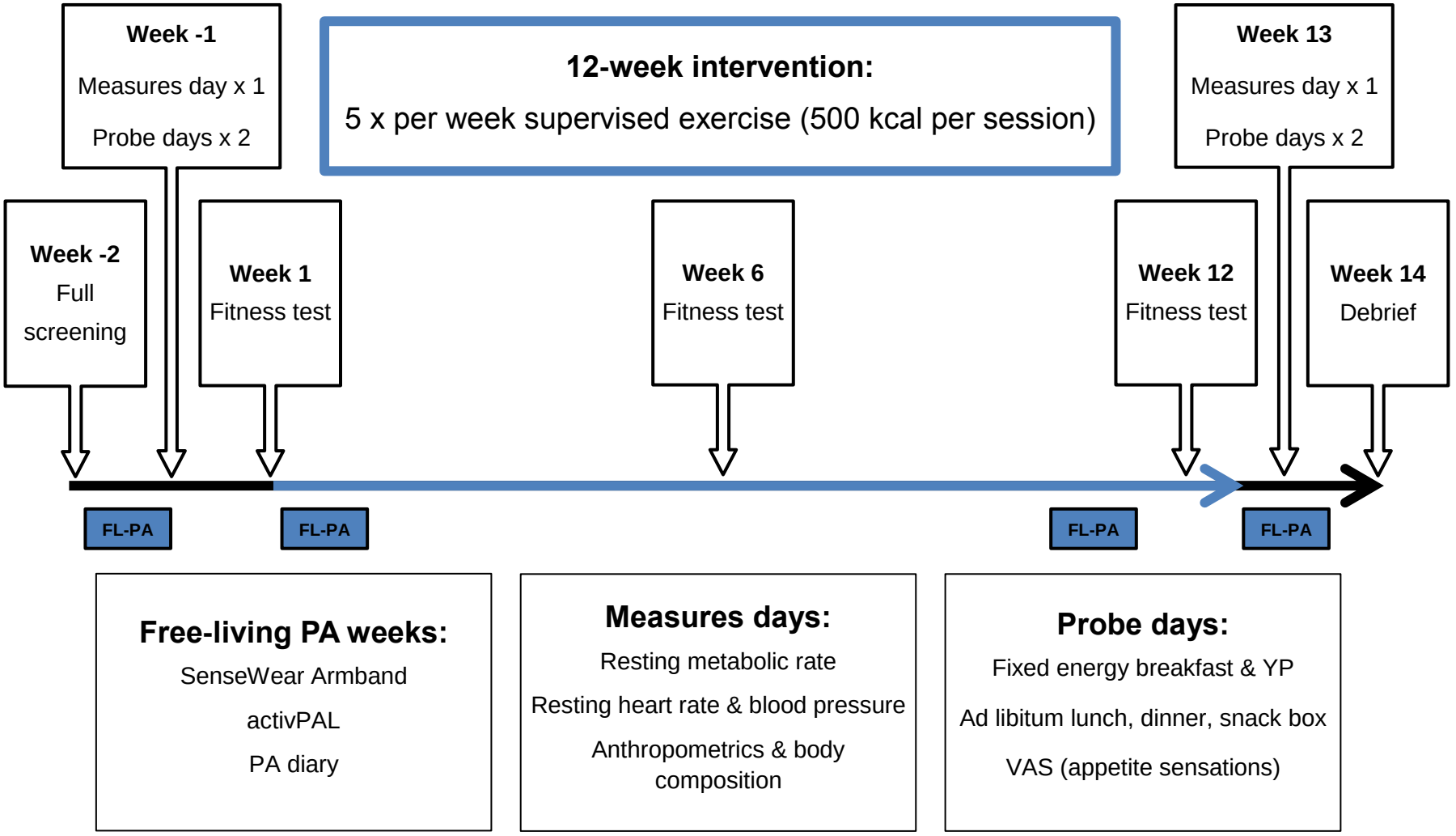


Figure 9.1 Overview of the medium-term exercise study procedures

9.2.4 Full screening

At week -2 (laboratory visit 1) potential participants attended the HARU and were provided with a hard copy of the Participant Information Sheet and received further information about the study. An informed consent form was signed before any study-specific procedures were undertaken and confidentiality and anonymity were assured. Further participant information was obtained as well as medical screening and stature and weight were measured to confirm eligibility. Participants who met the eligibility criteria for the study upon completion of the screening visit checks and were keen to take part in the study were informed that they were able to take part in the study and assigned a unique study identification code. Participants were given the SWA and AP to wear for the following seven days to measure free-living PA and SB and asked to complete a PA diary. Written and verbal instruction were provided on proper wear of the PA monitors and a return date was arranged. The study dates and times for their first measurement and probe days were arranged.

9.2.5 Measurement days

Participants completed two measurement days (laboratory visits 2 and 5); one at baseline in the week prior to commencing the exercise intervention and one post-intervention in the week after completion of the exercise intervention. Participants were fasted from 10:00 pm the previous night and had abstained from exercise and alcohol for at least 24 hours before both laboratory visits. The following measurements were taken: stature, weight, waist and hip circumference, body composition and RMR. BP and resting HR were taken immediately after the RMR measurement. Psychometric eating behaviour trait were assessed using the TFEQ and the BES. Finally, participants completed a $\dot{V}O_2$max treadmill fitness test. In some instances the fitness test was completed at the start of the first exercise session. Detailed information on the above measurements can be found in the general methods chapter (Chapter 4).

9.2.6 Probe days

Participants completed four probe days (laboratory visits 3, 4, 6 and 7); two pre-intervention in the week prior to commencing the exercise intervention and two post-intervention in the week after completion of the exercise intervention. Participants were fasted from 10:00 pm the previous night and had abstained from alcohol for at least 24 hours before the probe days. Participants were instructed not to exercise during the 24 hours prior to the pre-intervention probe days. However, for the post-intervention probe days participants were instructed to exercise for the prescribed duration and intensity (individually calibrated to expend 500 kcal) they had exercised during the exercise intervention so they were in a similar physiological state to the intervention period. All probe day meals were consumed in a private cubicle free from distractions

with the exception of the snack box which was taken home in the evening and any uneaten items returned the next day.

Probe day data were averaged across the two baseline probe days and the two post-intervention probe days.

9.2.6.1 Probe day procedures

Twenty-four hour eating behaviour and subjective appetite sensations were objectively measured during probe days. A detailed explanation of probe day procedures and foods provided can be found in Study 4 (Chapter 8).

9.2.7 Free-living physical activity and sedentary behaviour

Free-living PA and SB was objectively measured using the SWA and AP at baseline, the first and tenth week of the exercise intervention and post-intervention to assess whether a structured and supervised exercise intervention led to a decrease in free-living PA and an increase in SB. Data are presented with structured exercise included in the data (see section 9.3.6.1) and with structured exercise removed (see section 9.3.7). Participants also completed a PA diary to coincide with the PA monitoring period detailing the intensity, duration and type of activity performed along with details regarding removal of the activity monitors. Further details on the methodological platform to measure free-living PA and SB can be found in Chapter 6.

In brief, participants wore the SWA on the posterior surface of their upper non-dominant arm for a minimum of 22 hours per day for 7-8 days in order to obtain data for at least five 24 hour periods from midnight to midnight, including at least one weekend day. Participants were instructed to remove the SWA when showering, bathing or swimming. The SWA only record data when it is in contact with the skin and for the SWA data to be valid there had to be >22 hours of data per day and at least five 24 hour periods (midnight to midnight) including at least one weekend day.

The AP was placed in a nitrile sleeve and attached to the midline anterior aspect of the upper thigh on the non-dominant leg with a hypafix waterproof dressing. Participants were instructed to wear the AP at all times. If they removed the device they were asked to record the day, time and reason for removing in the activity diary provided. Compliance with the AP wear protocol was determined by cross-checking any prolonged periods of sitting/lying (>2 hours) with SWA data from the same period. If the SWA recorded PA during this period it would indicate the AP had been removed and that days data was excluded from analyses.

Data from the SWA and AP were combined to generate an integrated (INT) data SB variable which classified behaviour based on all three components of the widely accepted SB definition; awake, sitting/lying and <1.5 METs.

9.2.8 Exercise intervention

Participants were required to exercise five times per week at 70% of their age-predicted maximum heart rate (APMHR; 220 - age) for an individually calibrated duration calculated to expend 500 kcal per session (see section 9.2.8.1). Consequently, the total weekly EE was 2,500 kcal. To calculate how long each participant would have to exercise at 70% of their APMHR to elicit an EE of 500 kcal, a maximal fitness test was completed at baseline and 6 weeks (details of the fitness test procedure can be found in Chapter 4). Throughout the 12-week exercise intervention participants could choose from a selection of aerobic exercise equipment including a treadmill, rower, cross-trainer and stationary bike. All exercise sessions were supervised in the HARU, and participants were required to wear a HR monitor (Polar RS400, Polar, Kempele, Finland) to record the intensity and duration of exercise performed to measure compliance with the intervention.

9.2.8.1 Calculating exercise session duration

Information from the maximal aerobic fitness test was used to calculate how long each participant would need to exercise at 70% of their APMHR to expend 500 kcal. The duration of each exercise session was individually calculated based on the relationship between HR and VO_2/VCO_2. Standard stoichiometric equations were used with respiratory data (VO_2/VCO_2) from the fitness test to calculate the energy expended at 70% APMHR (Péronnet and Massicotte, 1991). Consequently, the duration of exercise required to expend 500 kcal at a given HR could be calculated. The ratio of CO_2 production and O_2 consumptions (respiratory exchange ratio; RER) differs between fat and carbohydrate metabolism. The chemical equations for oxidation of CHO and fat are:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 38\ ATP$$

$$RER = VCO_2/VO_2 = 6\ CO_2/6\ O_2 = 1.0$$

Oxidising 1 g of CHO uses 0.746 L of O_2 and provides 3.75 kcal (16 kJ).

$$C_{16}H_{32}O_2 + 23\ O_2 \rightarrow 16\ CO_2 + 16\ H_2O + 129\ ATP$$

$$RER = VCO_2/\ VO_2 = 16\ CO_2/23\ O_2 = 0.7$$

Oxidising 1 g of fat uses 2.012 L of O_2 and provides 9 kcal (39 kJ).

From respiratory data total fat and carbohydrate oxidation rates were calculated using the non-protein respiratory quotient (Péronnet and Massicotte, 1991). In extreme conditions (i.e. very prolonged exercise of several hours with no food intake) amino acid oxidation may reach 10 % of total substrate utilization. However, in most exercise conditions it seems reasonable to assume that protein oxidation is negligible. Because

the RQ of protein is in between that of carbohydrate and fat, the RER would not be affected much even if the contribution of protein was 5-10 %. The following equations assumes that the amount of protein oxidised is usually small and negligible (<1%) and therefore focus on fat and carbohydrate:

$$\text{CHO oxidation rate} = (4.585 * VCO_2) – (3.226 * VO_2)$$

$$\text{Fat oxidation} = (1.695 * VO_2) – (1.701 * VCO_2)$$

With VO_2 and VCO_2 in litres per minute and oxidation rate in grams per minute. The rates of substrate oxidation were calculated at each stage of the maximal fitness test. Oxidation rates were then converted from grams to kcal using the energy equivalents for carbohydrate (3.75 kcal) and fat (9 kcal). The target EE for each exercise session was then divided by the EE per minute at 70% of APMHR to give the duration of each exercise session. For example:

70% APMHR = 135 bpm

EE at 135 bpm = 8.4 kcal

500 kcal / 8.4 kcal = 59.5 minutes

The exercise prescription for this example would be 60 minutes (rounded to the nearest minute) of aerobic exercise at 135 bpm, five times per week. EE at 70% APMHR was recalculated during week six of the intervention.

9.2.9 Debrief

On completion of all study procedures, participants attended the HARU for a final time for a debrief session. Individualised feedback on their outcome measures (i.e. BMI, body composition, aerobic capacity, PA level) was provided and a payment form was completed.

9.2.10 Statistical analysis

Data are reported as mean ± SD throughout, unless otherwise stated. Statistical analysis was performed using IBM SPSS for Windows (Chicago, Illinois, Version 21) and significance was set at $p < .05$. All variables were checked for outliers and normality was assessed using the Shapiro-Wilk test. Change in anthropometrics, body composition, EI, subjective appetite sensations and health markers from baseline to post-intervention were assessed using paired sample t-tests. To examine changes in free-living PA in response to structured aerobic exercise, one-way repeated measures ANOVAs (Week) were performed. Change in subjective appetite sensations from baseline to post-intervention were assessed using two-way ANOVAs (Week*Time) with repeated measures. Where appropriate Greenhouse-Geisser probability levels were used to adjust for spherecity, while post-hoc comparisons using Bonferroni adjustments were used if statistical significance was detected. Simple linear

regression was also performed to identify whether differences in exercise-induced EE explained variation in body composition change between participants.

9.3 Results

9.3.1 Attrition rate

Figure 9.2 provides details of the recruitment process and reasons for exclusion and attrition from the study. In the nine months between June 2015 and February 2016, 254 individuals responded to the various recruitment methods. Of those 254 individuals, 89 were eligible for the study and invited to a full screening visit. Fifty-eight individuals completed the full screening visit and 36 agreed to take part in the study and signed informed consent. Four of the 36 participants dropped out before the first measures day visit and of the remaining 32 participants, 24 women complete the study.

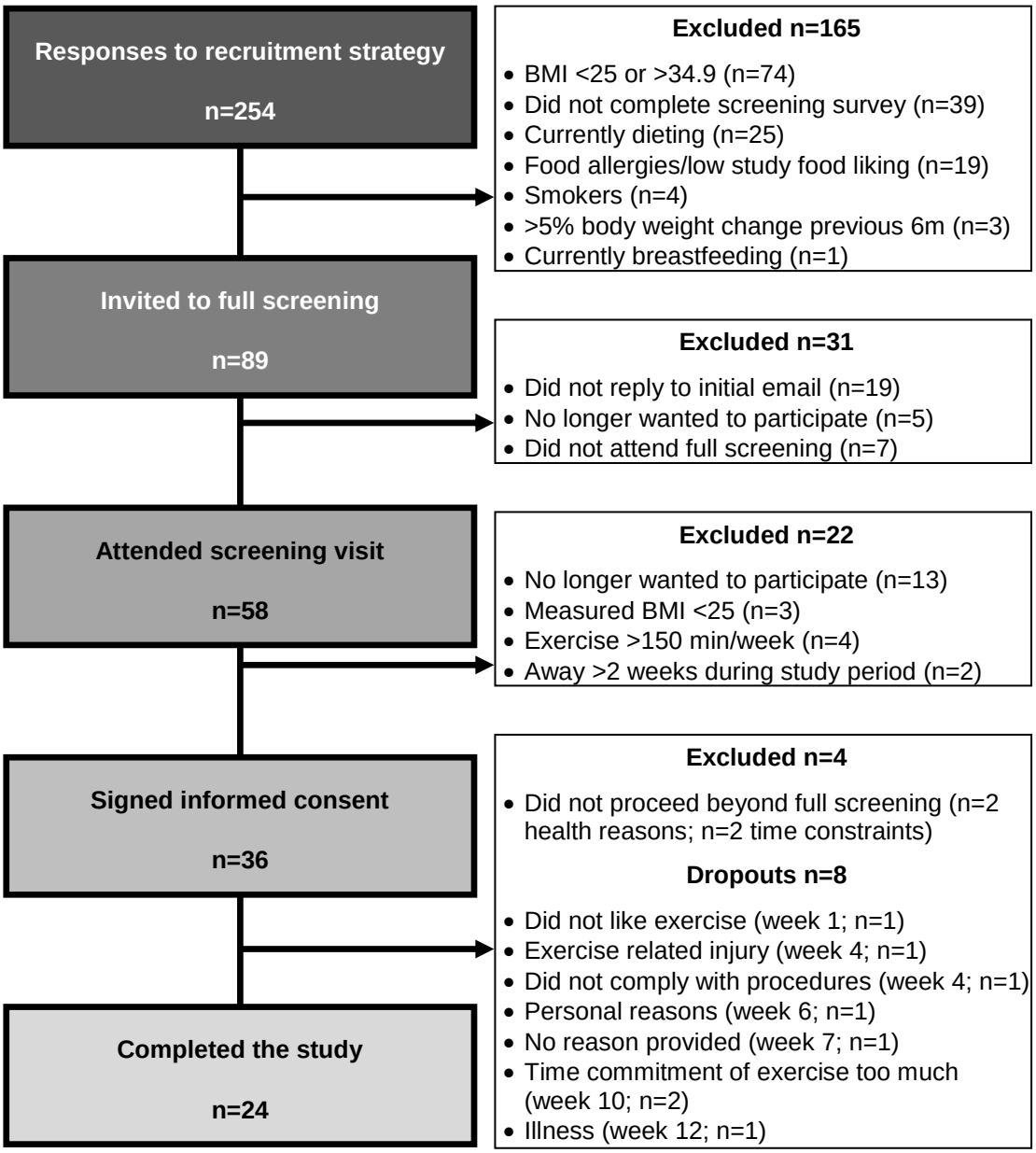


Figure 9.2 Flow chart of recruitment and attrition rate

9.3.2 Exercise intervention adherence

The target total EE over the 12-week exercise intervention was 29,000 kcal for each participant. The mean total exercise-induced EE was 28,792.29 kcal (SD = 872.96), which was 99.3% of the prescribed EE. On average participants exercised for 65.63 min/session (SD = 9.96) during weeks 1 to 6 and 55.79 min/session (SD = 8.26) during week 6 to 12 of the intervention. Average exercise session duration was significantly shorter during weeks 6 to 12 compared with 1 to 6 [$t(24) = 5.49$, $p < .001$]. The average HR was 77.42% (SD = 6.48) of APMHR during weeks 1 to 6 and 79.79% (SD = 5.77) of APMHR during weeks 6 to 12 [$t(24) = 2.40$, $p = .025$].

9.3.3 Change in body composition, anthropometrics and resting metabolism

Table 9.1 displays the change in anthropometric, body composition and RMR from baseline to post-intervention. Paired sample t-tests revealed there was a significant reduction in body mass [$t(23) = 2.18$, $p = .04$], BMI [$t(23) = 2.25$, $p = .035$], WC [$t(23) = 4.60$, $p < .001$], FM [$t(23) = 3.36$, $p = .003$] and % FM [$t(23) = 4.09$, $p < .001$]. There was a significant increase in FFM [$t(23) = 3.35$, $p = .003$]. Assuming 1 kg of body mass (70:30 fat/lean tissue) is equivalent to 7,700 kcal (Wishnofsky, 1958), the predicted sample average weight loss resulting from the exercise-induced energy deficit (28,792.29 kcal) was 3.74 kg.

Table 9.1 Anthropometrics, body composition and RMR at baseline and post-intervention (n=24). Data are mean (SD)

	Baseline	Post-intervention	Change	*p*
Body mass (kg)	76.50 (10.40)	75.68 (10.23)	-0.83 (1.85)	**p = .040**
BMI (kg/m^2)	27.94 (2.67)	27.63 (2.70)	-0.30 (0.66)	**p = .035**
WC (cm)	95.21 (9.89)	91.60 (9.03)	-3.62 (3.85)	**p < .001**
FM (kg)	30.28 (7.97)	28.78 (7.96)	-1.50 (2.18)	**p = .003**
FFM (kg)	46.23 (4.16)	46.90 (3.89)	0.67 (0.98)	**p = .003**
% FM	39.16 (5.19)	37.50 (5.46)	-1.66 (1.99)	**p < .001**
RMR IC (kcal/d)	1616.09 (201.98)	1668.85 (205.12)	52.76 (154.51)	p = .108
RMR WHO (kcal/d)	1560.07 (153.06)	1555.83 (152.33)	-4.24 (19.75)	p = .304

There was considerable variability in weight loss and body composition change between participants (see Figure 9.3). Seventeen participants lost weight, one participant remained the same and six participants gained weight following the 12-week supervised aerobic exercise intervention. Changes in body mass ranged from -4.3 kg to +3.1 kg. Of the 24 participants, 20 reduced their FM, one remained the same and three gained FM with changes ranging from -4.4 kg to +4.9 kg. Two participants had unfavourable changes in both FM (increased) and FFM (decreased). Total exercise-induced EE did not explain the variation in body mass change [$F(1, 22) = 1.259$, $p = .274$, $R^2 = .054$], FM change [$F(1, 22) = 2.418$, $p = .134$, $R^2 = .099$] or FFM change [$F(1, 22) = 1.475$, $p = .237$, $R^2 = .063$].

Figure 9.3 Individual variability in body mass, FM and FFM change from baseline to post-intervention following 12-weeks of supervised aerobic exercise (n=24)

- **Twelve weeks of supervised aerobic exercise resulted in reduced body mass, BMI, WC and FM and increased FFM**
- **There was large individual variability in weight loss between participants**

9.3.4 Energy intake

Objectively measured 24 hour EI at baseline and post-intervention is displayed in Table 9.2. Paired sample t-tests revealed participants total EI during post-intervention probe days was significantly higher compared with total EI during baseline probe days [$t(23) = 2.35$, $p = .028$]. Furthermore, *ad libitum* EI (lunch, dinner and snack box EI combined) [$t(23) = 2.31$, $p = .03$] and snack box EI [$t(23) = 2.09$, $p = .048$] were also higher post-intervention, see Figure 9.4. However, there was no significant difference in lunch or dinner EI between baseline and post-intervention probe days.

Table 9.2 EI at baseline and post-intervention (n=24). Data are mean (SD)

	Baseline	Post-intervention	Change	*p*
Total EI (kcal/d)	2603.26 (590.25)	2781.46 (582.27)	178.20 (371.64)	**p = .028**
***Ad libitum* EI (kcal/d)**	2106.53 (569.60)	2279.42 (558.74)	172.89 (366.50)	**p = .030**
Lunch EI (kcal/d)	798.90 (236.07)	799.00 (249.08)	0.10 (195.51)	p = .998
Dinner EI (kcal/d)	945.18 (300.19)	1009.58 (305.10)	64.40 (235.83)	p = .194
Snack box EI (kcal/d)	362.45 (240.05)	470.83 (260.93)	108.38 (254.68)	**p = .048**

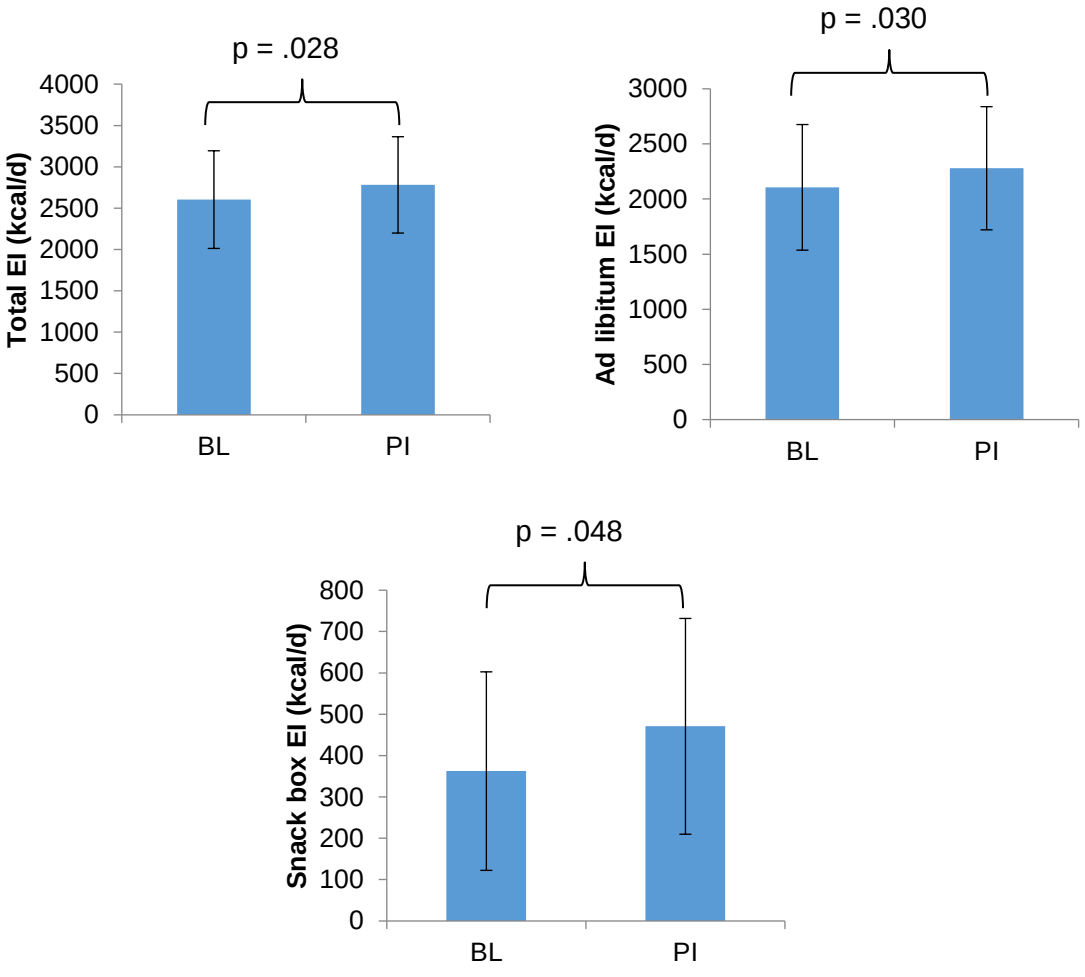


Figure 9.4 Change in total, *ad libitum* and snack box EI from baseline to post-intervention

There was considerable variability in total EI change from baseline to post-intervention between participants (see Figure 9.5). Ten participants decreased their EI, whereas 14

participants increased their EI. Change in total EI ranged from -581.5 kcal/d to +763.9 kcal/d.

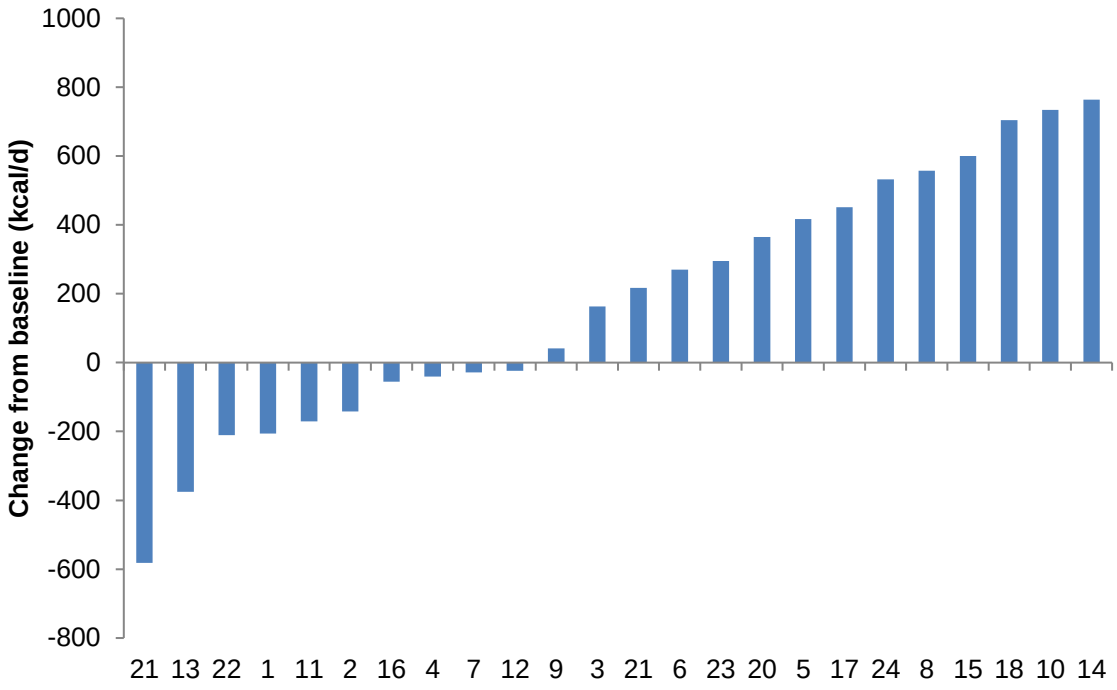


Figure 9.5 Individual variability in total EI change from baseline to post-intervention following 12-weeks of supervised aerobic exercise (n=24)

- **There was an increase in total EI throughout the day, *ad libitum* EI and snacking in response to the exercise intervention**
- **There was large individual variability in change in EI between participants**
- **The change in EI appears to be a compensatory response to the exercise intervention**

9.3.5 Subjective appetite sensations

9.3.5.1 VAS hunger ratings

There was no significant difference between baseline and post-intervention fasting hunger ratings [t(23) = 1.64, p = .12]. There was a main effect of week [F(1, 23) = 7.82, p = .01] with hunger being higher post-intervention (M = 25.58, SE = 2.21) compared with baseline (M = 21.68, SE = 1.97). Pairwise comparisons with Bonferroni adjustments revealed VAS hunger ratings were significantly higher during the post-intervention probe days compared with baseline immediately post-breakfast [t(23) = 2.08, p = .049], 15 minutes [t(23) = 2.65, p = .014], 30 minutes [t(23) = 2.63, p = .015], 90 minutes [t(23) = 2.20, p = .038], immediately post-lunch [t(23) = 2.33, p = .029],

immediately post-dinner [t(23) = 2.63, p = .015] and at 600 minutes [t(23) = 3.01, p = .006]. There was also a main effect of time [F(2.69, 61.95) = 66.99, p < .001) but no week*time interaction [F(6.12, 140.70) = 0.73, p = .63], see Figure 9.6.

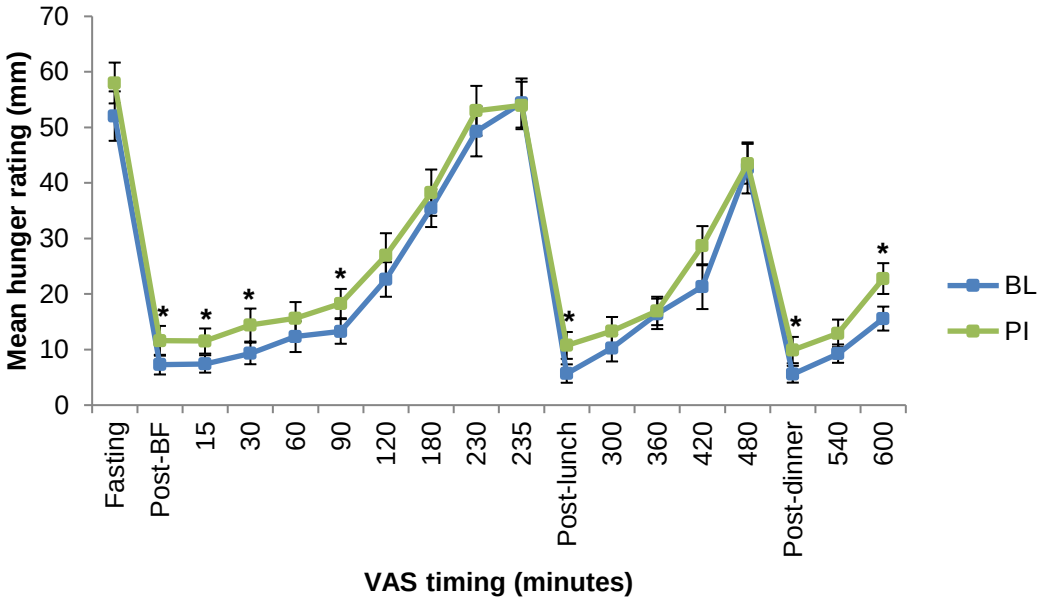


Figure 9.6 VAS hunger ratings during baseline and post-intervention probe days (error bars are standard error). * = p < .05, indicates significant difference between baseline and post-intervention

9.3.5.2 VAS fullness ratings

There was no significant difference between baseline and post-intervention fasting fullness ratings [t(23) = 1.03, p = .32]. There was a main effect of week [F(1, 23) = 5.55, p = .03], with fullness being lower post-intervention [M = 56.12, SE = 2.87] compared with baseline [M = 60.06, SE = 2.52]. Pairwise comparisons with Bonferroni adjustments revealed VAS fullness ratings were significantly lower during the post-intervention probe days compared with baseline at 30 minutes [t(23) = 2.17, p = .040] and 180 minutes [t(23) = 2.65, p = .014] post-breakfast, immediately post-lunch [t(23) = 2. 78, p = .011], immediately post-dinner [t(23) = 2.49, p = .021] and at 600 minutes [t(23) = 2.41, p = .024]. There was also a main effect of time [F(4.26, 97.99) = 75.28, p < .001) but no week*time interaction [F(7.54, 173.32) = 0.58, p = .78], see Figure 9.7.

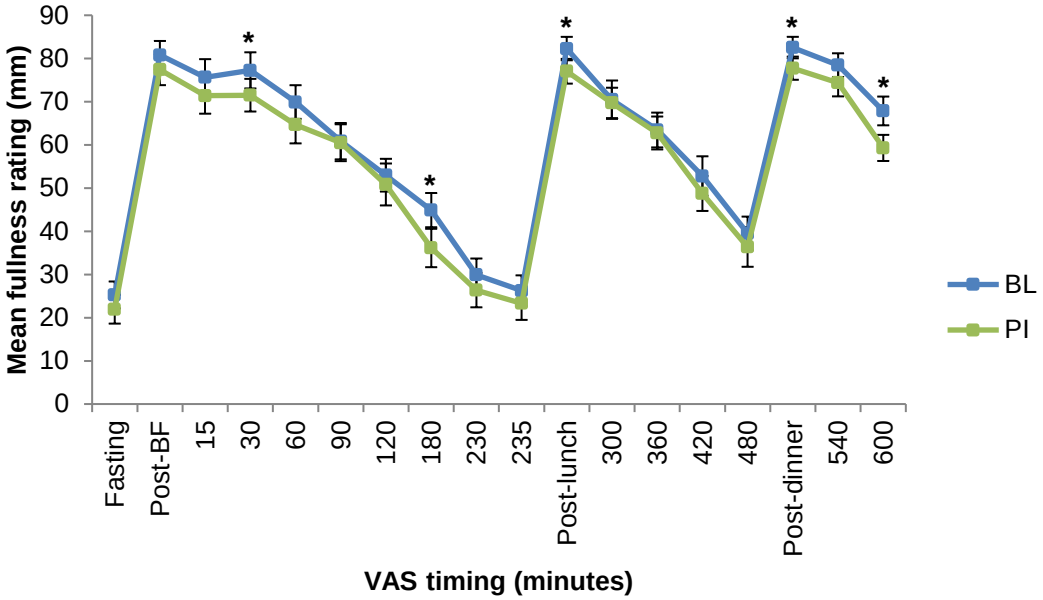


Figure 9.7 VAS fullness ratings during baseline and post-intervention probe days (error bars are standard error). * = p < .05, indicates significant difference between baseline and post-intervention

9.3.5.3 VAS desire to eat ratings

There was no significant difference between baseline and post-intervention fasting desire to eat ratings [t(23) = 1.23, p = .23]. There was no main effect of week [F(1, 23) = 2.18, p = .15]. There was a main effect of time [F(3.23, 74.31) = 59.05, p < .001] but no week*time interaction [F(5.18, 119.14) = 0.58, p = .72], see Figure 9.8.

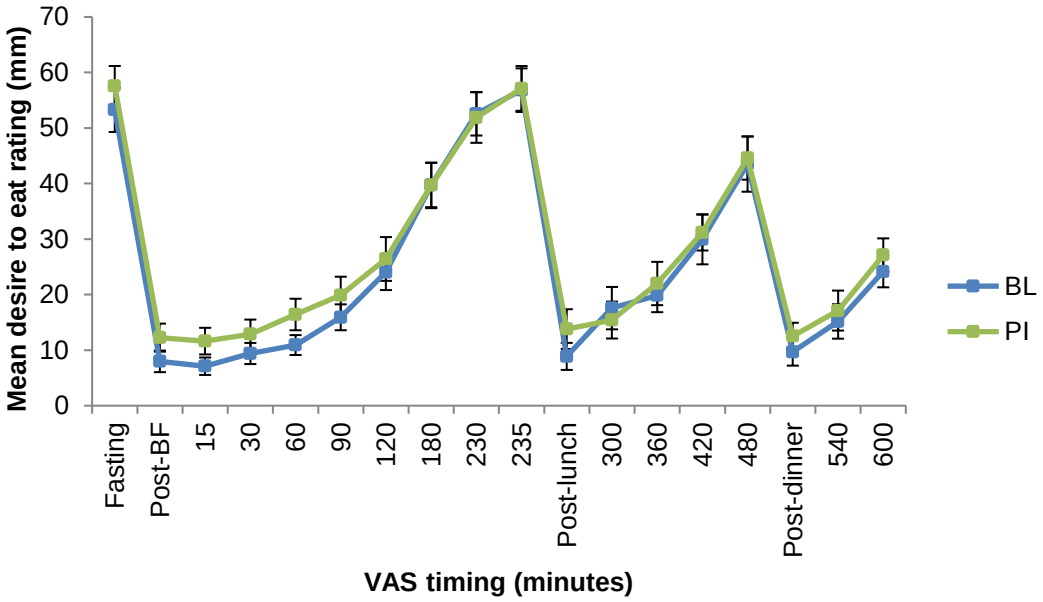


Figure 9.8 VAS desire to eat ratings during baseline and post-intervention probe days (error bars are standard error)

9.3.5.4 VAS prospective consumption

There was no significant difference between baseline and post-intervention fasting prospective consumption ratings [t(23) = 1.16, p = .26]. There was no main effect of week [F(1, 23) = 3.57, p = .07]. There was a main effect of time [F(3.54, 81.50) = 72.38, p < .001) but no week*time interaction [F(7.07, 162.70) = 0.54, p = .81], see Figure 9.9.

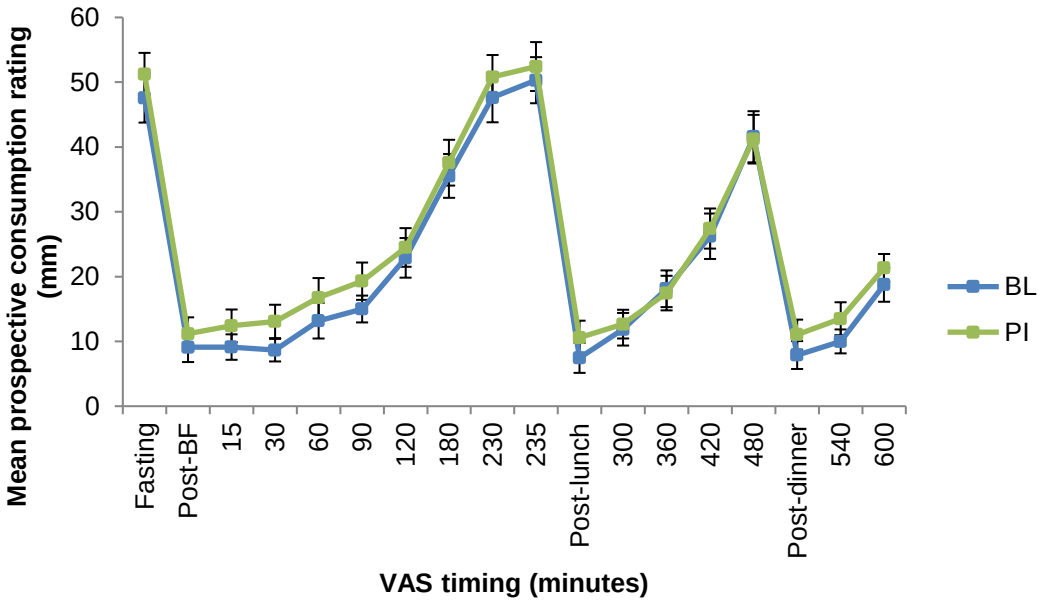


Figure 9.9 VAS prospective consumption ratings during baseline and post-intervention probe days (error bars are standard error)

9.3.5.5 Area under the curve and Satiety Quotient

Table 9.3 displays change in AUC and SQ for hunger, fullness, desire to eat and prospective consumption from baseline to post-intervention. Paired sample t-tests revealed there was a significant difference between baseline and post-intervention measures of AUC for hunger [t(23) = 2.61, p = .016] and AUC for fullness [t(23) = 2.18, p = .04], see Figure 9.10. There were no significant differences between AUC for desire to eat and prospective consumption or any of the SQ measures between baseline and post-intervention.

Table 9.3 AUC and SQ at baseline and post-intervention (n=24). Data are mean (SD)

	Baseline	Post-intervention	Change	*p*
AUC hunger (mm/min)	12055.42 (5983.86)	14307.08 (7002.00)	2251.67 (4219.84)	**p = .016**
AUC fullness (mm/min)	36656.67 (7978.98)	34328.96 (9009.61)	-2327.71 (5223.76)	**p = .040**
AUC desire to eat (mm/min)	14414.53 (7077.45)	15537.19 (7589.98)	1122.66 (5371.90)	p = .317
AUC PFC (mm/min)	12755.47 (6558.31)	13962.60 (6677.79)	1207.14 (4034.21)	p = .156
SQ hunger (mm/kcal)	5.76 (3.81)	6.25 (3.97)	0.49 (3.96)	p = .55
SQ fullness (mm/kcal)	6.48 (2.42)	6.35 (3.43)	-0.13 (3.44)	p = .86
SQ desire to eat (mm/kcal)	5.76 (3.73)	6.04 (3.82)	0.28 (3.83)	p = .72
SQ PFC (mm/kcal)	4.88 (3.25)	4.99 (2.92)	0.11 (3.01)	p = .86

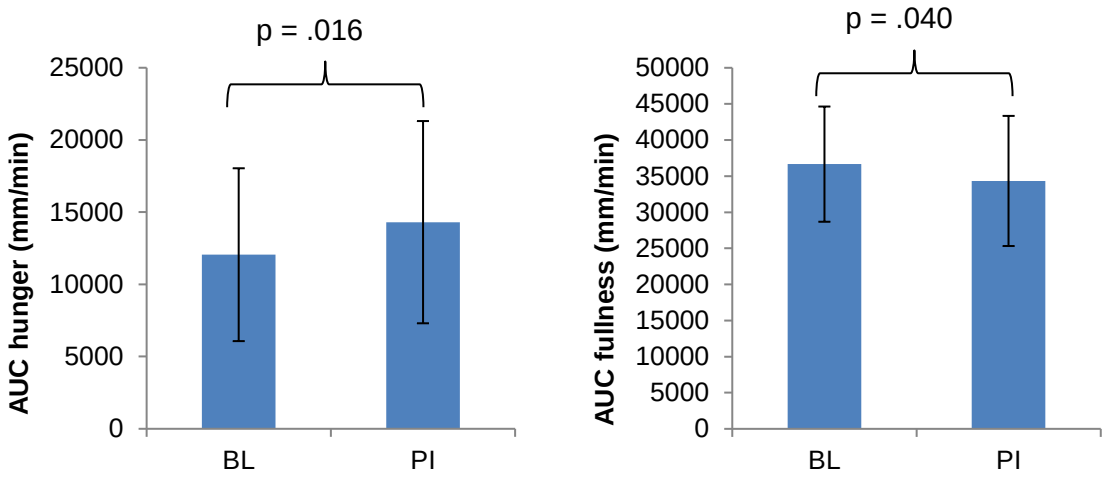


Figure 9.10 AUC for hunger and fullness throughout whole probe day at baseline and post-intervention

- **There was an increase in subjective hunger and a decrease in fullness throughout the day at the end of the exercise intervention compared with baseline**

9.3.6 Free-living physical activity and sedentary behaviour

Table 9.4 shows the amount of time spent in sedentary and active behaviours at the different time points before, during and after the 12-week exercise intervention. PA data presented in this section for week 1 and week 10 of the exercise intervention includes the structured exercise.

Table 9.4 Measures of sedentary and active behaviours before during and after the 12-week exercise intervention. Data are mean (SD)

Variable	BL	Week 1	Week 10	PI
Sleep (min/d)*	447.78 (72.92)	425.79 (55.62)	433.63 (66.88)	436.57 (71.55)
SED^{SWA} (min/d)*	716.99 (103.28)	689.95 (82.09)	688.20 (86.44)	724.99 (85.65)
SED^{AP} (min/d)**	623.38 (100.71)	591.62 (107.25)	585.67 (109.88)	620.99 (107.16)
SED^{INT} (min/d)**	566.01 (105.68)	530.06 (107.46)	531.49 (109.12)	573.58 (109.65)
Light PA (min/d)*	167.33 (71.64)	156.75 (55.46)	169.08 (68.78)	167.38 (61.82)
Mod PA (min/d)*	81.51 (45.42)	115.06 (52.61)	111.14 (42.62)	82.41 (35.96)
Vig PA (min/d)*	4.29 (4.41)	20.93 (13.61)	17.28 (18.04)	6.06 (7.13)
MVPA (min/d)*	85.78 (47.02)	135.99 (51.57)	128.41 (43.73)	88.48 (38.09)
Standing (min/d)**	241.69 (79.16)	257.52 (94.92)	252.31 (24.55)	247.18 (108.89)
Stepping (min/d)**	95.92 (28.33)	144.35 (30.31)	132.72 (35.23)	104.30 (36.48)
Steps^{SWA} (per/d)*	8410.10 (3021.01)	12964.65 (2232.89)	11892.07 (2985.16)	8509.39 (2855.27)
Steps^{AP} (per/d)**	8331.69 (2828.97)	14383.99 (2850.40)	12651.58 (3614.07)	9246.80 (3417.62)
Total EE (kcal/d)*	2349.41 (336.16)	2678.00 (296.66)	2593.84 (313.52)	2374.21 (314.70)
activity EE (kcal/d)	856.08 (367.92)	1199.79 (306.70)	1139.63 (331.09)	864.39 (297.54)

* n=23; ** n=17

9.3.6.1 Sedentary time and physical activity

A repeated measures ANOVA revealed that there was a significant difference in mean SED^{SWA} between the different time points [F(3, 66) = 3.32, p = .03]. Post hoc tests revealed that there was a significant difference in SED^{SWA} between the first week of exercise and the week following the completion of the exercise intervention [p = .02]. When sedentary time was measured using SED^{AP} [F(3, 48) = 2.40, p = .08] and SED^{INT} [F(3, 48) = 2.64, p = .06], sedentary time did not differ significantly at any of the time points (see Figure 9.11).

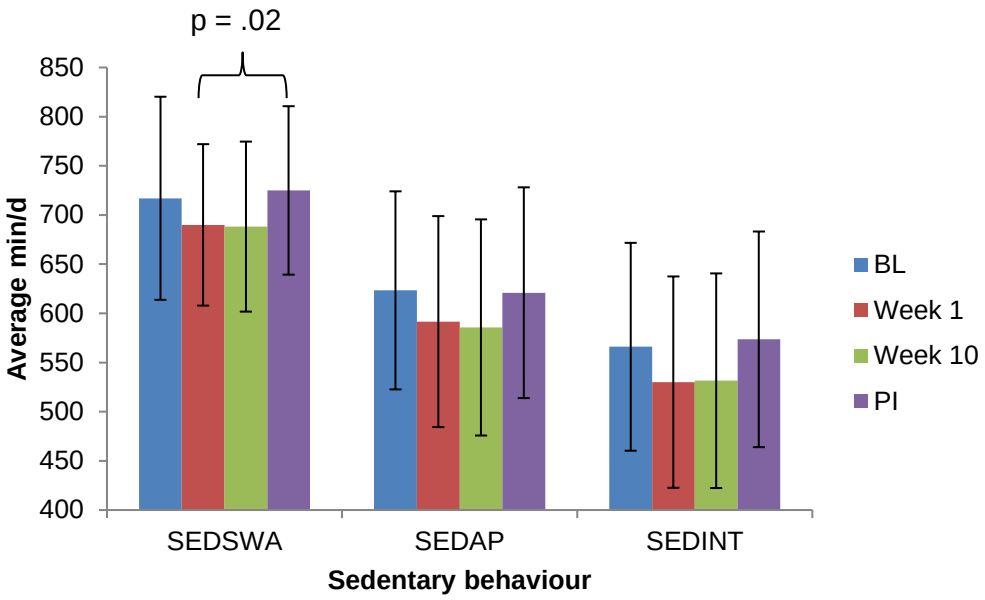


Figure 9.11 Change in sedentary time during the 12-week exercise intervention measured using the SWA (n=23), AP (n=17) and integrated method (n=17)

A repeated measures ANOVA revealed that there was no significant difference in time spent in light PA between the different time points [F(3, 66) = 0.47, p = .70]. However, there was a significant difference in mean moderate PA between the different time points [F(3, 66) = 9.51, p < .001]. Post hoc tests revealed that participants performed significantly more moderate PA during the first and tenth week of the exercise intervention compared with baseline and post-intervention [p < .05], see Figure 9.12. There was a significant difference in mean vigorous PA between the different time points [F(1.90, 41.89) = 14.92, p < .001]. Post hoc tests revealed that participants performed significantly more vigorous PA during the first week of the exercise intervention compared with baseline and post-intervention and there was a significant difference between BL and week 10 of the intervention [p < .05], see Figure 9.13. The amount of time spent in MVPA was also significantly different between the different

time points [F(3, 66) = 18.57, p < .001]. Post hoc tests revealed MVPA was significantly higher during the first and tenth week of the exercise intervention compared to baseline and post-intervention [p < .05], see Figure 9.14.

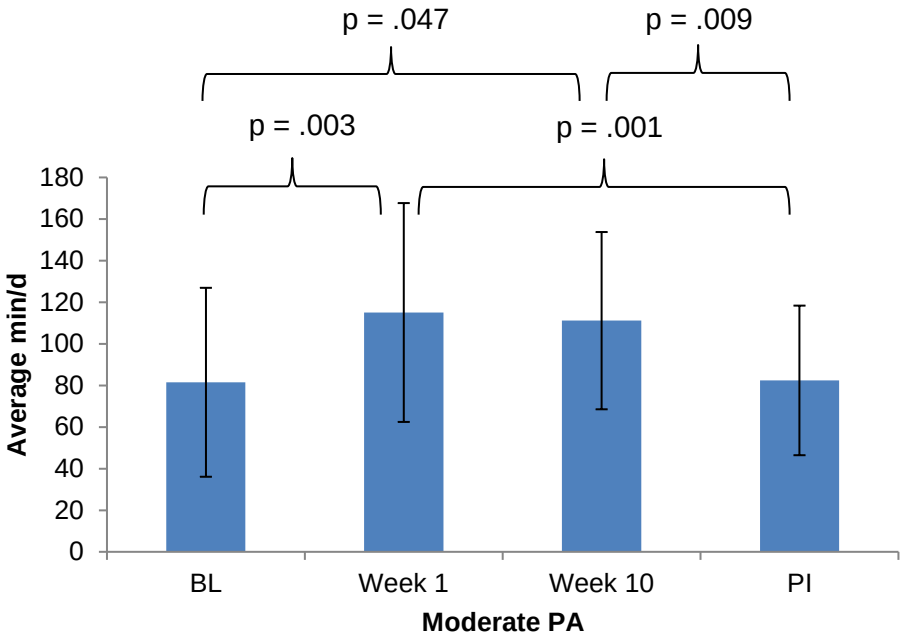


Figure 9.12 Change in time spent in moderate PA during the 12-week exercise intervention measured using the SWA (n=23)

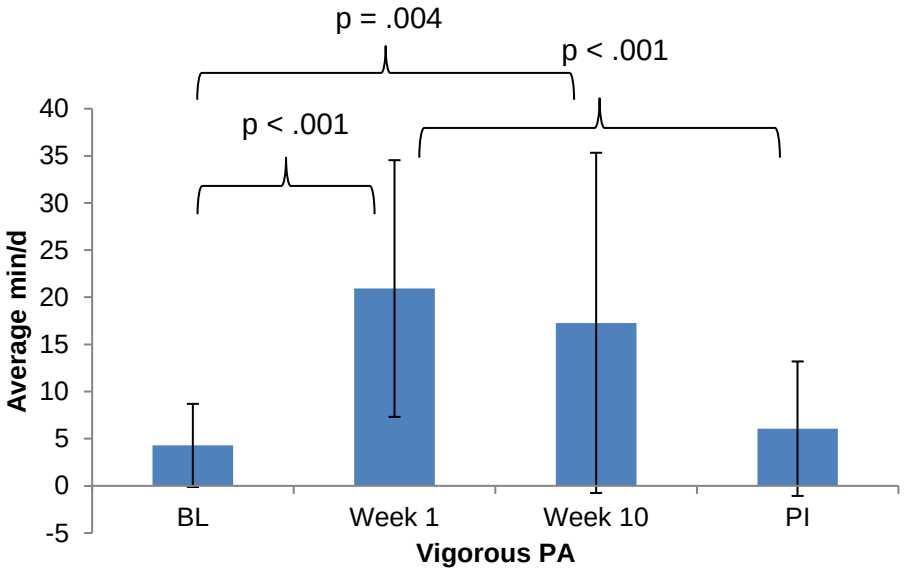


Figure 9.13 Change in time spent in vigorous PA during the 12-week exercise intervention measured using the SWA (n=23)

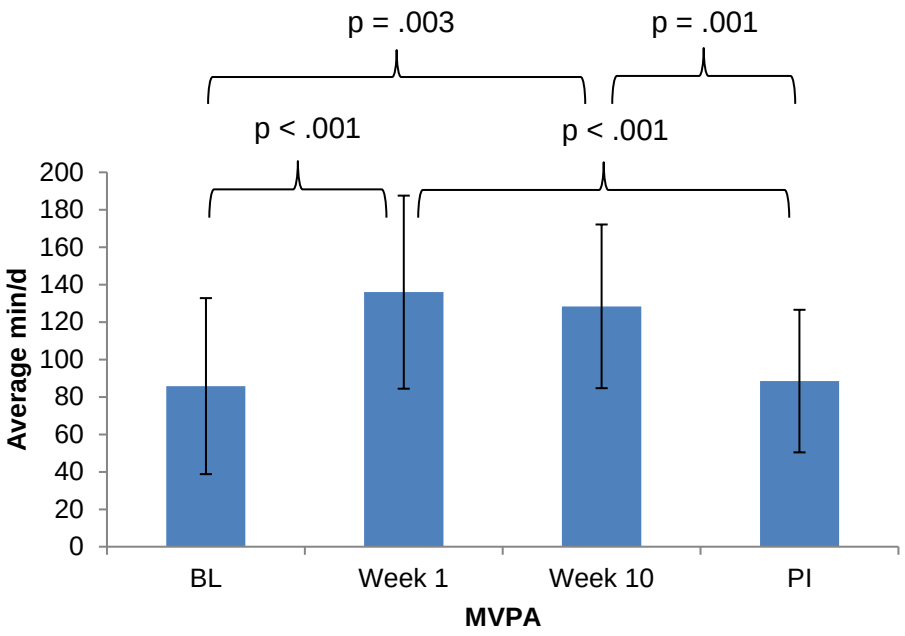


Figure 9.14 Change in time spent in MVPA during the 12-week exercise intervention measured using the SWA (n=23)

A repeated measures ANOVA revealed that there was no significant difference in time spent standing between the different time points [F(3, 48) = 0.36, p = .78]. However, there was a significant difference in time spent stepping between the different time points [F(3, 48) = 24.13, p < .001]. Post hoc tests revealed that participants spent significantly more time stepping during week 1 and 10 of the exercise intervention compare with baseline and post-intervention [p < .05], see Figure 9.15.

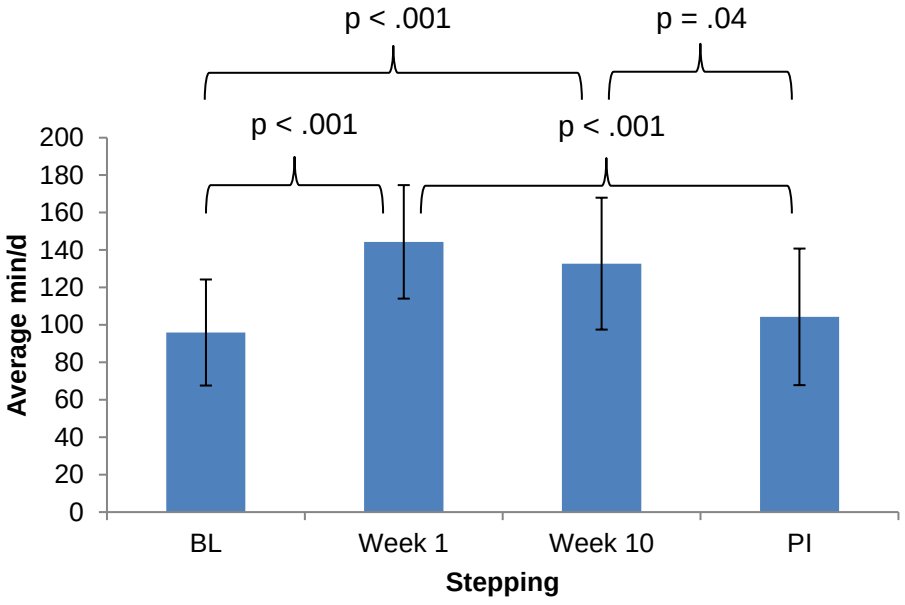


Figure 9.15 Change in time spent stepping during the 12-week exercise intervention measured using the AP (n=17)

9.3.6.2 Steps

A repeated measures ANOVA revealed that there was a significant difference in average steps per day between the different time points when measured using the SWA [F(3, 66) = 28.40, p < .001] and AP [F(1.99, 31.79) = 31.74, p < .001]. Post hoc tests revealed that the average number of steps per day was significantly higher during the first and tenth week of the exercise intervention compared with baseline and post-intervention when measured with the SWA and AP [p < .05], see Figure 9.16.

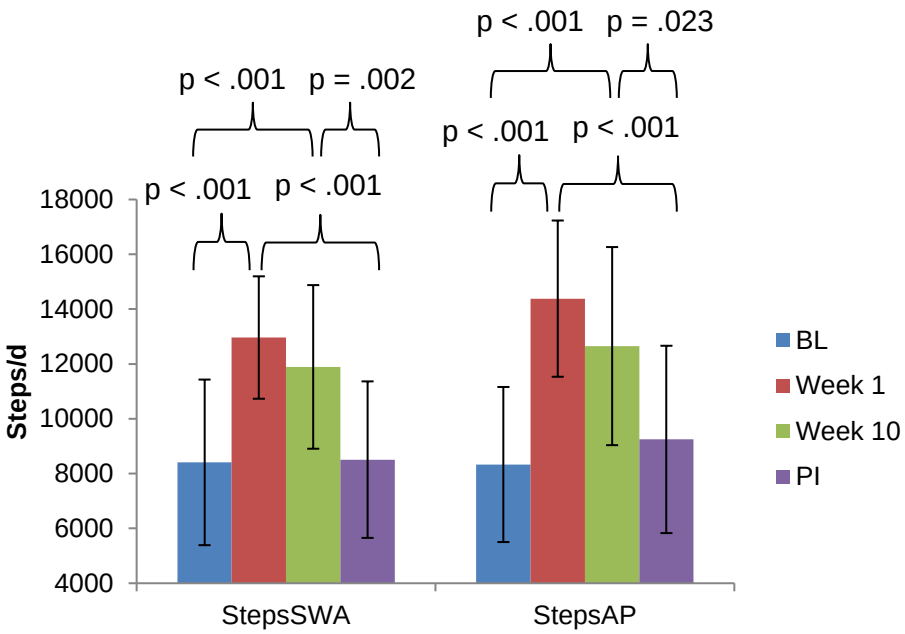


Figure 9.16 Change in steps per day during the 12-week exercise intervention measured using the SWA (n=23) and AP (n=17)

9.3.6.3 Total energy expenditure

A repeated measures ANOVA revealed that there was a significant difference in total EE between the different time points [F(3, 66) = 25.56, p < .001]. Post hoc tests revealed total EE was significantly higher during the first and tenth week of the exercise intervention compared with baseline and post-intervention [p < .05], see Figure 9.17.

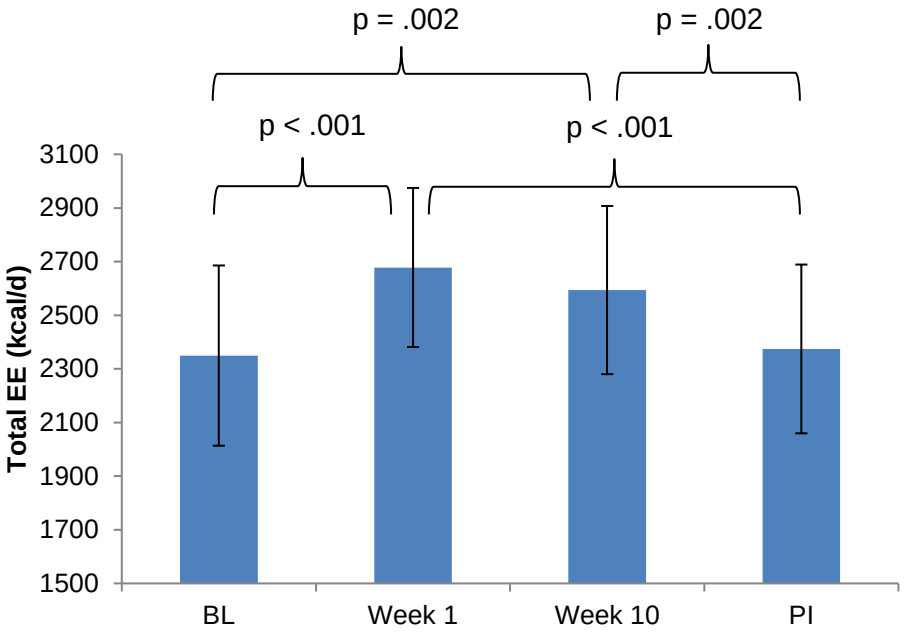


Figure 9.17 Change in total EE during the 12-week exercise intervention measured using the SWA (n=23)

9.3.6.4 Activity energy expenditure

A repeated measures ANOVA revealed that there was a significant difference in activity EE between the different time points [$F(3, 66) = 17.16$, $p < .001$]. Post hoc tests revealed activity EE was significantly higher during the first and tenth week of the exercise intervention compared with baseline and post-intervention [$p < .05$], see Figure 9.18. On average, participants completed 89.5% and 90.7% of their prescribed exercise during week 1 and 10 of the exercise intervention, respectively.

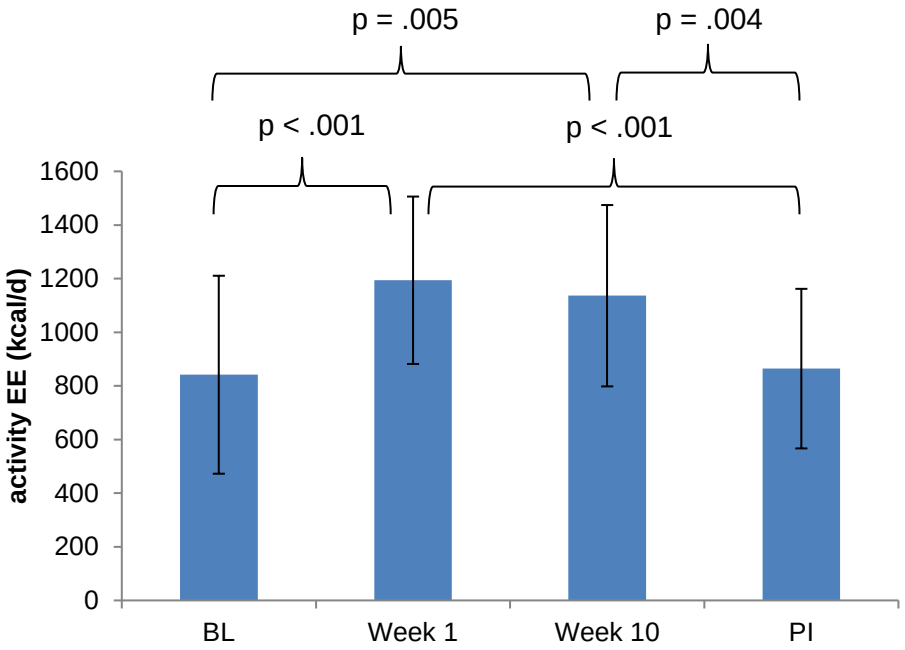


Figure 9.18 Change in activity EE during the 12-week exercise intervention measured using the SWA (n=23)

9.3.7 Change in non-exercise physical activity

Sleep, sedentary time, light PA and MVPA are collinear which means an increase in one category of activity would lead to a decrease in at least one other. The sum of the change in sleep, sedentary time and light PA between baseline and week 1 and baseline and week 10 was calculated to identify whether a reduction in these activities could account for the increase in MVPA. The sum of all the activity categories other than MVPA between baseline and week 1 was -59.61 min/d (SD = 43.89) and between baseline and week 10 was -41.19 min/d (SD = 51.70). Change in MVPA from baseline to week 1 was +50.20 min/d (SD = 37.96) and from baseline to week 10 was +42.63 min/d (SD = 49.87). Structured MVPA appears to displace sleep, SB and light PA but not NEPA MVPA.

Furthermore, when the weekly prescribed exercise duration was averaged over 7 days for week 1 (M = 47.30 min/d, SD = 6.96) and 10 (M = 40.16 min/d, SD = 5.83) of the exercise intervention and subtracted from time spent in MVPA per day during each of those time points the resulting NEPA MVPA was 88.69 min/d (SD = 51.24) and 88.26 min/d (SD = 43.15) for week 1 and 10 of the intervention, respectively. A repeated measures ANOVA revealed there was no significant difference between baseline, exercise intervention week 1 and 10 and post-intervention MVPA when structured exercise was removed from week 1 and 10 [$F(3, 66) = 0.05$, $p = .99$].

- **During the exercise intervention free-living PA and SB measurements there was a significant increase in activity EE (moderate and vigorous PA)**
- **During the exercise there was a decrease in the duration of sedentary time indicating that the structured exercise was displacing SB**
- **There was no evidence for a compensatory reduction in NEPA or a compensatory increase in SB as a result of increased structured exercise**

9.3.8 Health markers

Table 9.5 displays health markers at baseline and post-intervention. Paired sample t-tests revealed resting HR [t(23) = 2.58, p = .017] and fasting BG [t(21) = 2.14, p = .044] were significantly reduced post-intervention. However, systolic BP, diastolic BP and maximal aerobic capacity were not significantly different post-intervention compared with baseline.

Table 9.5 Health markers at baseline and post-intervention. Data are mean (SD)

	Baseline mean	Post-intervention	Change	*p*
Systolic BP (mmHg)*	117.04 (18.12)	114.23 (20.40)	-2.81 (12.74)	p = .291
Diastolic BP (mmHg)*	77.58 (12.09)	75.83 (13.73)	-1.75 (7.52)	p = .266
Resting HR (bpm)*	61.58 (8.09)	58.60 (7.65)	-2.98 (5.65)	**p = .017**
Fasting BG (mmol/L)**	4.65 (0.44)	4.44 (0.33)	-0.21 (0.47)	**p = .044**
$\dot{V}O_2$max (ml/kg/min)***	33.33 (5.04)	35.25 (6.61)	1.92 (4.56)	p = .083

* n=24; ** n=22; *** n=19

9.4 Discussion

This study investigated the effects of a 12-week supervised exercise intervention on body composition, 24 hour eating behaviour, subjective appetite sensations and free-living sedentary and active behaviours in overweight and obese inactive women.

A key finding was that participants lost weight on average as a result of the 12-week aerobic exercise intervention. This is a particularly pertinent finding as the efficacy of

exercise for weight loss has recently been questioned (Malhotra et al., 2015), despite systematic reviews supporting the role of exercise in weight reduction and management independent of diet (Shaw et al., 2006). It is important to note that adherence to the exercise intervention is often poor. Adherence is a major contributor to whether weight loss is achieved. The purpose of this study was to examine weight change when participants were known to have completed the exercise (supervised and measured) and not what factors influence adherence to an exercise intervention. The exercise intensity and volume used in the current study has previously been shown to result in a reduction in body mass (King et al., 2008, King et al., 2009a, Hopkins et al., 2014b, Hopkins et al., 2014a). Furthermore, weight loss (-1.6 ± 1.7 kg) has previously been reported in men undertaking a 12-week aerobic exercise intervention (Rocha et al., 2016).

9.4.1 Individual variability in weight loss

As in previous studies (King et al., 2008, Martins et al., 2010), the current study produced large individual variability between participants in weight loss ranging from -4.3 kg to +3.1 kg. The variability in weight loss was not explained by exercise-induced EE. Therefore, the variability in weight loss must be due to metabolic (reduced RMR) and/or behavioural (increase in EI or reduced NEPA) compensatory mechanisms (King et al., 2008, Garland et al., 2011, King et al., 2007).

Although participants failed to achieve the predicted weight loss, they exhibited favourable changes in body composition. On average FFM increased (M = 0.67, SD = 0.98 kg) and FM decreased (M = -1.50, SD = 2.18 kg). In contrast, studies that use energy restriction alone and energy restriction as an adjunct to exercise to create a negative energy balance often result in reduced FFM (and therefore reduced RMR) that account for around 15-35% of weight loss (Nicklas et al., 2009, Metzner et al., 2011). Studies have demonstrated that weight loss as a result of exercise does not lead to a reduction in RMR and in some instances RMR is elevated, perhaps due to the preservation or increase in FFM with increased exercise (Stiegler and Cunliffe, 2006). In the current study, RMR was higher post-intervention compared with baseline (M = 52.76 kcal/d, SD = 154.51), although the difference did not reach statistical significance.

Improvements in health markers even with modest or no weight loss has previously been highlighted. King et al. (2009b) demonstrated that exercise produces significant health benefits (i.e. improved body composition, aerobic capacity and BP) even when the weight loss resulting from exercise is less than expected. Despite weight loss in the current study not reaching the 5% threshold for clinical significance (Williamson et al., 2015), health benefits were apparent. In the current study, there was a significant

reduction in both resting HR and fasting BG post-intervention. These changes confirm the health benefit of exercise even when weight loss is modest.

9.4.2 Behavioural compensation in response to exercise

It has been suggested that the increase in EE resulting from exercise will be compensated for through increased EI or decreased NEPA to offset the negative energy balance, rendering exercise futile for weight loss (King et al., 2007, Melanson, 2017). The negative energy balance created by the exercise intervention in the current study was not fully compensated for as participants did in fact lose weight. However, partial compensation was evident as participants lost less weight than predicted when calculated based on the exercise-induced energy deficit. Less than expected weight loss has previously been attributed to poor adherence to the exercise intervention (King et al., 2007). However, a strength of the current study is that all exercise sessions were supervised in the laboratory and EE was measured. Participants completed 99.3% of the prescribed exercise. It is worth noting that the static Wishnofsky predictive equation for estimating weight loss is simplistic and does not account for adaptations in other components of energy balance as a result of an energy deficit (for example, increased EI, physiological reductions in resting EE, an increase in FFM or a decrease in NEPA) and could lead to overestimation of predicted weight loss (Thomas et al., 2014). Furthermore, the 1 kg of body mass is equivalent to 7,700 kcal rule (1 kg of body mass consists of 70% fat and 30% FFM) is based on short-term low calorie diets and is not directly applicable to the change in body composition induced by exercise. Indeed, in the current study, and others, there is in fact a significant increase in FFM. However, in addressing the issue of compensation – to either exercise or diet induced weight loss – it is important to have an estimate of the weight loss expected on the basis of the energy deficit incurred. This is not an easy calculation to make. The work of Hall et al. (2011) has provided algorithms for calculating the required energy reduction to achieve a specified weight loss goal. However, there is no algorithm for the reverse process, i.e. calculating weight loss for a known energy deficit. In addition in the case of the two scenarios being reported in this thesis it is not possible to quantify the precise energy deficit incurred. For the exercise study the exercise session - although rigorously measured occurred on only five days per week; the influence of non-exercise activities, or activity on non-exercise days (and on weeks when the SWA was not worn) is not known. For the dietary induced weight loss (see Study 6, Chapter 10) it is not possible to be sure about the degree of compliance achieved. What can be concluded is that, in both scenarios, a substantial energy deficit occurred and a weight loss was produced.

Therefore, in order to make progress in estimating compensation it has been assumed (for convenience) that 1 kg of body mass is equivalent to 7,700kcal of energy deficit

(as noted above). This was the rule of thumb that has been used for many years before Hall carried out systematic studies from objective data. It is now recognised that this formula is not accurate. However, it is used here as a rough guide to the relationship of energy deficit and weight loss whilst recognising that this is only an approximation.

9.4.3 Change in energy intake and subjective appetite sensations

The less than expected weight loss observed in the current study indicates some degree of compensation. It was hypothesised that EI would increase post-intervention in response to increased exercise as has previously been demonstrated (Whybrow et al., 2008, King et al., 2008, Woo, 1985). Indeed, there was a significant increase in total, *ad libitum* and snack box EI post-intervention. When calculated as a proportion of the energy expended per exercise session, the increase in EI represented compensation of 36%, which is similar to the 30% compensation observed by Whybrow et al. (2008). The participants in the Whybrow study were normal weight men and women and would be expected to compensate for a negative energy balance more readily as they have less of a 'buffer' (energy stored as FM) than overweight or obese individuals. The observation that participants in the current study compensated to a greater extent than lean individuals is likely due to the longer intervention period. It has previously been noted that body mass regulation is asymmetrical; a positive energy balance (and weight gain) is well tolerated whereas a negative energy balance (and weight loss) is strongly defended against (Blundell and Gillett, 2001). This study, together with previous research (Stubbs et al., 2004), provides further support for the asymmetry of body mass regulation evidenced by the compensatory increase in EI to defend against weight loss in response to a prolonged period of increased exercise-induced EE. A strength of this study is the objective measurement of 24 hour EI, however, it is acknowledged that using episodic test meal intake to infer changes in habitual intake has limitations (Hill et al., 1995). Rather, probe day measures of EI can be viewed as assays for eating behaviour and give an indication of compensatory appetite responses to perturbations in energy balance that are free from external influences (Gibbons et al., 2014). Similar test meals and probe day procedures to those reported in the current study have previously been shown to detect exercise-induced compensation in eating behaviour (King et al., 2008).

The increase in EI was accompanied by an increase in hunger throughout the day (mainly during the morning) and decreased fullness reflected in AUC for hunger and fullness. The results of the current study are similar to those observed in 'non-responders' in the study by King et al. (2009a) with respect to change in body mass (-0.9 kg), FM (-1.2 kg), EI (+164 kcal) and AUC for hunger and fullness. A possible explanation is that the majority of the participants in the current study are 'non-

responders'; they do not achieve the predicted change in body composition calculated from their exercise-induced EE. When the current sample are categorised as 'responders' and 'non-responders' using the method described by King et al. (2009a), two thirds are classified as 'non-responders'. Participants in the current study had a lower BMI at the start of the study which could explain why their weight loss response was less pronounced than that observed by King et al. (2009a). Furthermore, the study by King et al. (2009a) included men and men have been shown to exhibit a greater weight loss in response to exercise than women (Ballor and Keesey, 1991, Donnelly and Smith, 2005).

9.4.4 Change in non-exercise physical activity

Greater compensation in NEPA rather than changes in EI have previously been reported in response to increased exercise (Stubbs et al., 2002a, Stubbs et al., 2002b). There is no standardised approach for quantifying change in NEPA as a result of increased exercise, particularly changes in NEPA under free-living conditions. When accelerometer based activity monitors have been used to measure NEPA, studies often remove the structured exercise session, along with the warm up and cool down period, from free-living PA data collected during the exercise intervention and compare it with pre-intervention values (for example, Kozey-Keadle et al. (2014) and Herrmann et al. (2015)). However, the validity of this approach is questionable as pre-intervention data may not be comparable with data collected during the intervention period with structured exercise removed; there is no way of knowing how participants would have used the time that was removed (structured exercise session) had they not been exercising. For example, if a participant exercises for 60 minutes, five times per week and this time was removed from PA monitor data, the outcomes are no longer comparable with baseline PA monitor data. There would be ~24 hours of data per day at baseline and only ~23 hours during the intervention, giving less time to perform NEPA during the intervention. Furthermore, the time spent exercising could displace some NEPA MVPA performed at baseline and removal of that data would exaggerate a compensatory reduction in NEPA in response to exercise. Because of the limitations described above, data on change in NEPA in response to structured exercise should be interpreted with caution. Initially, baseline, week 1 and 10 of the exercise intervention and post-intervention PA and SB measures were compared without removing any structured exercise from week 1 and 10. Total compensation in NEPA would be apparent if, for example, MVPA did not increase during the exercise intervention. In the current study there was a significant increase in MVPA, steps, time spent stepping and total EE between BL and week 1 and BL and week 10 of the exercise intervention but no difference between baseline and post-intervention. Furthermore, there was a significant decrease in all of these variables back to baseline values when PA was measured post-intervention. This indicates that the structured

exercise completed during the intervention period was not fully compensated for by a reduction in NEPA. Furthermore, participants did not maintain the increased PA once the intervention ended. Post-interventions PA levels similar to baseline have previously been highlighted (Rocha et al., 2016, Turner et al., 2010, Hollowell et al., 2009, Church et al., 2009).

There was no evidence for a compensatory increase in SB. In fact, SB was lower in the weeks during the exercise intervention but only the difference between week 1 of the exercise intervention and post-intervention reached statistical significance when measured with the SWA. This suggests that sedentary time was partially displaced by time spent exercising. This is in contrast with previous research that suggests that interventions need to specifically target reductions in SB to change sedentary time (Kozey-Keadle et al., 2014). Indeed, the magnitude of the reduction in SB may have been greater with a specific component of the intervention to target reduced SB in the current study. Further examination of activity monitor data suggests structured exercise also displaces some sleep time and light PA, but the difference in sleep and light PA at the different time points throughout the intervention were not significant. The sum of the difference in sleep, SED^{SWA} and light PA between baseline and week 1 and baseline and week 10 was greater than the change in MVPA (in the opposite direction) at the same time points. Furthermore, when the prescribed exercise duration at week 1 and 10 was averaged over 7 days and subtracted from time spent in MVPA at each of those time points, the remaining NEPA MVPA was remarkably similar to baseline and post-intervention values (<3 minutes difference between all four time points). Taken together, these findings suggest that increasing MVPA through a structure exercise intervention displaces time spent sleeping, sedentary and in light PA but not NEPA MVPA. This is in agreement with previous studies (Church et al., 2009, Hollowell et al., 2009, Turner et al., 2010) and a recent systematic review that concluded no statistically or clinically significant mean change in NEPA occurs during exercise training (Fedewa et al., 2016).

This study investigated the effects of a supervised aerobic exercise intervention on body composition, 24 hour eating behaviour, subjective appetite sensations and free-living sedentary and active behaviours. These data show that taking overweight or obese women from an inactive to an active state through a 12-week supervised and structured exercise regimen results in weight loss and favourable changes in body composition. However, these changes were highly variable between individuals. There was a significant increase in EI during post-intervention probe days compared with baseline. Change in eating behaviour was accompanied by changes in subjective appetite sensations; hunger increased and fullness decreased throughout the day. There was no evidence for a compensatory decrease in NEPA or on increase in SB.

9.5 Outcomes

- **Twelve weeks of supervised aerobic exercise resulted in reduced body mass, BMI, WC and FM (adiposity) and increased FFM**
- **There was large individual variability in weight loss between participants**
- **There was an increase in total EI throughout the day, *ad libitum* EI and snacking in response to the exercise intervention**
- **There was also an increase in subjective hunger and a decrease in fullness throughout the day**
- **Increased structured exercise did not result in a compensatory reduction in NEPA or an increase in SB**

Chapter 10
Study 6 - Change in Free-Living Physical Activity and Sedentary Behaviour in Relation to Diet Induced Weight Loss

This chapter will investigate whether diet induced weight loss leads to compensatory changes in free-living sedentary and active behaviours in overweight and obese women. Furthermore, the study will identify whether changes in free-living sedentary and active behaviours, or in appetite variables (energy intake, subjective appetite sensations and eating behaviour traits) can explain the variability in diet induced weight loss.

10.1 Introduction

The previous chapter explored the effects of increased structured and supervised aerobic exercise on outcomes related to energy balance. The present chapter will examine the effect of diet induced weight loss on free-living sedentary and active behaviours. There is no agreement on the effects of diet induced weight loss on free-living PA and SB, as studies have not yet shown systematic alterations. Some studies have shown diet induced weight loss leads to a compensatory reduction in PA and related outcomes such as total and active EE, effectively closing the energy balance gap and limiting weight loss (Serra et al., 2014, Camps et al., 2013, Redman et al., 2009, Martin et al., 2007, Leibel et al., 1995). On the other hand, some studies have found no effect of diet induced weight loss on PA (Martin et al., 2011, Levine et al., 2005), and in some instances, PA has increased post-weight loss (Weinsier et al., 2000, Bonomi et al., 2013). These disparities between studies could be due to the different measurement techniques used to quantify PA, the setting within which PA is measured (free-living vs. respiratory chamber), the degree of weight loss achieved and the duration of the intervention.

Most studies investigating the effects of dietary induced weight loss on free-living PA have focused on total daily EE and activity EE (measured using DLW), rather than time spent in different intensities of activity. In general, these studies have shown a reduction in EE in response to diet induced weight loss (Redman et al., 2009, Camps et al., 2013, Martin et al., 2007, Bonomi et al., 2013). However, DLW studies should be interpreted with caution when inferring change in movement behaviour (PA and SB)

from change in EE. Reduced EE associated with diet induced weight loss does not necessarily reflect changes in behaviour (free-living PA and SB) (MacLean et al., 2011) and instead the lower EE could reflect physiological and metabolic changes, such as a decrease in metabolically active tissue (Stiegler and Cunliffe, 2006), enhanced metabolic efficiency of remaining metabolically active tissue (Redman et al., 2009, Leibel et al., 1995, Rosenbaum et al., 2003) and the reduced energy cost of movement (Levine et al., 2000, Schoeller and Jefford, 2002). It is important to quantify the extent to which changes in PA and SB contribute to the reduced EE post-weight loss to optimise weight loss and weight loss maintenance strategies.

Technological advancements allow the objective quantification of free-living PA and SB using accelerometer based devices to examine the effects of dietary induced weight loss on time spent in different intensities of activity from sedentary to vigorous. As energy expended through PA is the most variable component of total daily EE (Melanson, 2017) it is plausible that change in this component of EE contributes to the reduction in activity EE and total EE previously reported in DLW studies (Camps et al., 2013, Redman et al., 2009, Martin et al., 2007, Leibel et al., 1995). PA may decrease and SB increase to act as a biological survival mechanism, conserving energy in times of low energy availability or starvation (Taylor and Keys, 1950). Alternatively, there could be a spontaneous increase in PA and decrease in SB due to the reduced physiological stress of exercise in weight reduced individuals (Weinsier et al., 2000). Despite advances in activity monitoring devices, there are still relatively few studies that have investigated the effects of diet induced weight loss on PA and SB.

Bonomi et al. (2013) measured PA using an accelerometer and activity EE was predicted from a DLW validated predictive equation using accelerometer data. The authors reported an increase in walking and cycling following diet induced weight loss in overweight and obese subjects and a reduction in sedentary time (lying, sitting or standing). Interestingly, there was a decrease in activity EE (derived from the same accelerometer data as the walking and cycling outputs but using a different equation) that could be explained by the reduction in body mass, as the predictive equation was based on counts per day and body mass. This suggests that to preserve activity EE, weight reduced individuals will need to increase the amount of PA they perform to compensate for the reduced metabolic cost of PA at a lower body mass. Previous research has demonstrated the energy cost of PA is proportionate to body mass (Levine et al., 2000, Schoeller and Jefford, 2002).

In contrast with the results from Bonomi et al. (2013), Camps et al. (2013) found there was a significant reduction in PA following diet induced weight loss. In conjunction with DLW, Camps et al. (2013) also measured PA using an accelerometer. The authors reported a significant reduction in total activity counts from baseline to week 8

following the very-low-energy diet and there was also a significant decrease in DLW derived measures of active EE. After 44 weeks of weight maintenance, baseline activity counts and activity EE were restored. However, the authors did not explore whether there was a change in the distribution of activity across intensities. It is possible that higher intensity activities might be displaced by lower intensity activities to compensate for the diet induced negative energy balance, but this information is not available when accelerometer information is reported as average activity counts per day. A previous study found that reduced free-living PA (but not reduced RMR) post weight loss was associated with weight regain up to 12 months post-intervention (Wang et al., 2008b). This highlights the importance of maintaining PA during weight loss to help achieve long term weight loss maintenance.

Another study reported no change in free-living PA following diet induced weight loss. Martin et al. (2011) investigated the effects of calorie restriction (resulting in weight loss) on free-living PA levels in non-obese adults. Despite no reduction in accelerometer derived free-living PA there was a significant reduction in DLW measures of activity EE. This suggests that either the accelerometer was not sensitive enough to detect a reduction in PA or the reduction in activity EE was due to some other biological or behavioural adaptation (for example, greater movement efficiency or reduced fidgeting). Accelerometer data reflects movement behaviour, whereas activity EE measured using DLW and IC might be lower post-weight loss, not because movement behaviour has decreased, but because the energy cost of movement is lower due to the reduced body mass. This study highlights how inferring change in free-living PA from DLW measures of activity EE may not provide a clear picture of the behavioural adaptations to diet induced weight loss.

Previous research has identified large individual variability in weight change in response to diet interventions (Camps et al., 2013, Astrup et al., 1995, Sorbris et al., 1982, Mutch et al., 2007). Baseline weight, initial weight loss (Handjieva-Darlenska et al., 2010), genetic factors (Mutch et al., 2007), thyroid hormones (Sorbris et al., 1982), baseline 24 hour EE and fat oxidation (Astrup et al., 1995) have all been associated with diet induced weight loss. Part of the variability in weight loss could also be accounted for by compliance with the diet intervention (Heymsfield et al., 2007). However, even when compliance is high, compensatory responses could occur to undermine the diet induced negative energy balance. The behavioural mechanisms underlying the variability in weight loss have received little attention. Previous research in mice concluded baseline activity and change in activity significantly predicted diet induced weight loss (Vaanholt et al., 2012). However, research in human participants is lacking. Understanding the mechanisms underlying variability in diet induced weight loss is necessary to develop interventions tailored to individual needs.

Despite consistent evidence for a reduction in total and activity EE following diet induced weight loss when measured using DLW (Camps et al., 2013, Redman et al., 2009, Martin et al., 2007, Leibel et al., 1995, Martin et al., 2011, MacLean et al., 2011), this does not necessarily reflect a change in movement behaviours (reduced PA and increased SB). Instead, the reduced EE could be a result of metabolic changes that occur with weight loss such as reduced FFM or the reduced energy cost of movement. Studies employing accelerometers to measure change in PA and SB as a result of diet induced weight loss are less consistent (Bonomi et al., 2013, Camps et al., 2013, Martin et al., 2011). Variability in weight loss between individuals in response to diet interventions has been documented, however, the mechanisms underlying this variability are not well known. Therefore, the purpose of this study was to investigate the effects of diet induced weight loss on free-living sedentary and active behaviours and to explore whether individual variability in weight loss could be explained by compensatory changes in free-living PA and SB and appetite related variables.

10.1.1 Hypotheses

- Diet induced weight loss will lead to a compensatory reduction in free-living PA and an increase in SB
- Change in free-living PA and SB will predict individual variability in weight loss response to the diet intervention
- Change in objectively measured EI, subjective appetite sensations and eating behaviour traits will predict individual variability in weight loss response to the diet intervention

10.2 Methods

10.2.1 Participants

Ninety six participants in total were recruited to the study; 49 women were recruited to a low energy dense diet[1] (LED) group and 47 women were recruited to a calorie restrictive diet[2] (CR) group. Of those participants, 40 and 44 completed baseline measurements in the LED and CR groups, respectively. Overall, 77 women (36 LED group) aged 42.5 years (SD = 12.2) with a BMI of 33.2 kg/m^2 (SD = 3.6) completed all study procedures. Overweight and obese women participants (aged 18-65 years; body-mass index 28-45 kg/m^2) were recruited by advertisement from the University of Leeds and surrounding area (CR group) and local commercial weight loss groups within 3 miles of the University of Leeds (LED group). Recruitment strategies included

[1] Low energy dense diet is a commercial weight loss diet
[2] Calorie restrictive diet is the NHS Choices diet

advertisement via email distribution lists, the University of Leeds research participant databases, distributing posters on the University of Leeds campus and contacting participants used in previous studies in the HARU who gave permission to be contacted about future studies. The commercial weight loss consultants also distributed recruitment materials to new members. Interested participants contacted the University of Leeds to be screened for eligibility by telephone or email. Those who met inclusion criteria were sent the Participant Information Sheet and invited for an information and screening visit at the laboratory. During this visit, participants were given an overview of all study procedures and consent was obtained. Following consent, participants completed a screening questionnaire (including age, food preferences checklist and medical history) and had their stature and weight measured by an investigator to check study eligibility. Study recruitment and test sessions were completed between September 2014 and December 2015. The study procedures and all study materials were reviewed and approved by the School of Psychology Ethics Board (14-0090). The study was also registered on ClinicalTrials.gov from December 2013 (NCT02012426).

10.2.2 Inclusion criteria

- Provided written informed consent
- Healthy women
- Aged 18-65 years
- BMI between 28-45 kg/m^2
- Reporting an interest in weight loss and not actively participating in a commercial weight loss programme
- Not increased PA levels in the past 2-4 weeks
- Able to eat most everyday foods and fruits and vegetables

10.2.3 Exclusion criteria

- Significant health problems
- Taking any medication or supplements known to affect appetite or weight within the past month and/or during the study
- Pregnant, planning to become pregnant or breastfeeding
- History of anaphylaxis to food
- Known food allergies or food intolerance
- Smokers and those who have recently ceased smoking (within the last 3 months)
- Participants receiving systemic or local treatment likely to interfere with evaluation of the study parameters
- Those who have previously taken part in a commercial weight loss programme in the last 2 months

- Individuals who work in appetite or feeding related areas
- Unable to consume foods used in the study
- Individuals who have had bariatric surgery
- History of an eating disorder
- Presence of untreated hypothyroidism
- Insufficient English language skills to complete study questionnaires

10.2.4 Design

The study was a non-randomised, parallel group design examining the effects of dietary induced weight loss on free-living PA and SB. There was a two week run-in period (week -2 to 0) in which participants in both the LED and CR groups followed their respective weight loss programme without any study involvement. This run-in period was to prevent initial weight loss confounding study parameters and to allow typical weight loss without any study interference. Between weeks 0 and 12 participants visited the laboratory in the HARU at the University of Leeds for measurement days and probe days (Figure 10.1). Participants received payment of £250 on completion of the study to reimburse them for their time and expenses.

Figure 10.1 Overview of medium-term diet intervention study procedures. BL, baseline; PI, post-intervention; FL-PA, free-living PA and SB measurement; PD, probe day; Qs, questionnaires

10.2.5 Weight management programme

Participants either followed the LED diet or the CR diet for the 12-week intervention period (plus two weeks run-in period). Information on both diets can be found in Appendix B. A weekly questionnaire was completed to assess participants' experience of the weight loss programme and included a question about self-reported adherence to the intervention. Participants responded to 'How well have you managed to stick with the weight control programme?' on a VAS anchored at each end with 'Not at all well' and 'Very well'. For the purpose of this study, data was analysed with both groups combined and participants were then categorised based on their weight loss (see 10.2.10). There were no specific research questions pertaining to the type of diet used to induce weight loss.

10.2.6 Full screening

Potential participants attended the HARU and were provided with a tour of the research facilities and received detailed information about study procedures and measurements. Participants were given the opportunity to ask questions about the trial. An informed consent form was signed before any study procedures were undertaken and confidentiality and anonymity were assured. Participants' stature was measured with a stadiometer (The Leicester Height Measure, Seca Ltd., Birmingham, UK) and their weight was measured with electronic scales (Adam Equipment MSP 200 series Personal Weighed, Dynamic Scales, Inc., Terre Haute, USA) with heavy clothing and shoes removed to confirm BMI. Participants then completed a screening questionnaire to confirm eligibility. Once eligibility had been confirmed, participants were assigned a unique study identification code. Participants in the CR group were provided with a hard copy of the NHS Choices programme and were instructed to start following the plan immediately. Finally, the dates and times for their first measurement and probe days were arranged.

10.2.7 Measurement days

Participants completed two measurement days; one at baseline following the 2-week run-in period and one post-intervention. Participants were contacted 48 hours prior to their measures visit and were asked to be fasted from 10:00 pm the previous night and to abstain from exercise and alcohol for at least 24 hours before both laboratory visits. The following measurements were taken: Stature, weight, WC, body composition, RMR, BP, resting HR, fasting BG and psychometric eating behaviour trait questionnaires (TFEQ and BES).

Detailed information about the above measurements can be found in the general methods chapter (Chapter 4). On completion of the measures visit participants were provided with breakfast before leaving the research unit.

10.2.8 Probe days

10.2.8.1 Probe day procedures

Participants were contacted 48 hours prior to their probe day visit and instructed to be fasted from 10:00 pm the previous night and to abstain from exercise and alcohol for at least 24 hours. Participants completed two probe days at baseline and post-intervention that varied based on the energy density of foods provided. On the low energy dense day, participants were provided with foods with an energy density of 0.78 kcal/g on average and on the high energy dense day foods were 2.93 kcal/g. Extensive piloting was carried out prior to commencing the study to ensure the difference in energy density was large enough and palatability of the meals was similar between the two conditions. Probe days began between 7:30 am and 9:30 am. Breakfast (fixed), lunch (fixed) and dinner (*ad libitum*) were provided in the laboratory and participants took a snack box (*ad libitum*) home in the evening and returned the following day. Details of the meals provided during high and low energy dense probe days can be found in (Appendix B.3). Participants were free to leave the HARU between meals but were instructed not to consume food or drinks whilst away from the facility other than the 500 ml bottle of water (recording any refills) provided between breakfast and lunch and lunch and dinner. When participants returned to the HARU for lunch and dinner, the water bottle was weighed and participants reported how many times they had refilled it to calculate the amount of water consumed. Throughout the probe days, VAS were completed immediately before and after meals and at hourly intervals between meals to assess subjective appetite sensations. For the purpose of the current study, high energy dense and low energy dense probe day data was averaged to give a measure of average 24 hour EI, dinner EI, snack box EI and *ad libitum* EI at baseline and post-intervention. There were no specific hypotheses regarding the energy density of meals and it was concluded that averaging the high and low energy dense probe days was more appropriate for the purposes of this study and would provide a more robust measure of EI. EI data from high and low energy dense probe days has previously been averaged to provide a single measure of EI (Hopkins et al., 2014a). VAS responses were also averaged across the two baseline probe days and the two post-intervention probe days. Figure 10.2 provides an overview of probe day procedures.

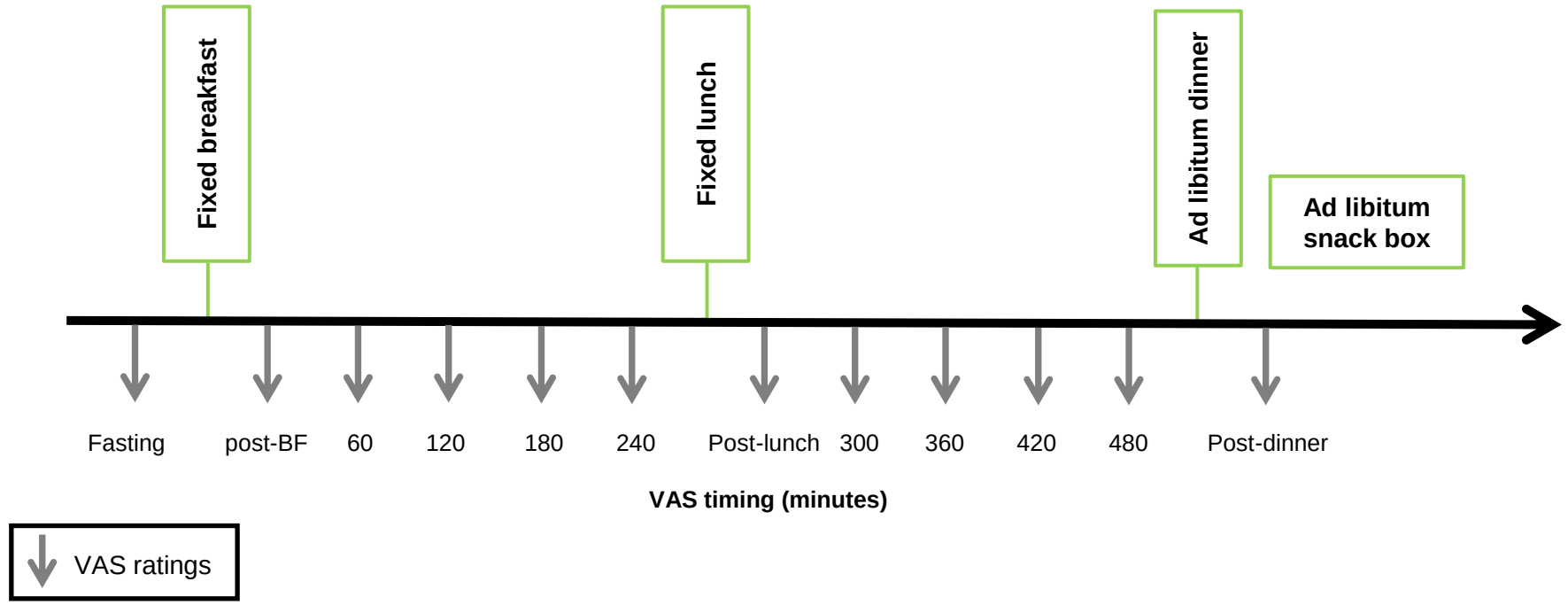


Figure 10.2 Diet intervention study probe day procedures

10.2.8.2 Subjective appetite sensations

The EARS-II device (Gibbons et al., 2011) was used throughout the probe days to assess subjective appetite sensations in response to food consumption. Participants answered the following questions: How HUNGRY do you feel now? How FULL do you feel now? How strong is your DESIRE TO EAT? How MUCH food could you eat now? VAS ratings were completed at 12 time points throughout the probe days. The first rating was completed 30 minutes after the participant arrived at the research unit on the morning of the probe day, immediately before breakfast was served. Ratings were completed before and after test meals and at hourly intervals in between. The EARS-II system was programmed to prompt the participants to complete VAS rating every hour whilst the participant was away from the laboratory between breakfast and lunch and between lunch and dinner. For further details on VAS subjective appetite sensations see the general methods chapter (Chapter 4).

10.2.8.3 Area under the curve

AUC was calculated for the whole day for hunger, fullness, desire to eat and prospective consumption using the trapezoid method (see Chapter 4). Fasting VAS ratings were excluded to remove differences in fasting levels of appetite sensations that might artificially alter the mean AUC. The following VAS rating were used to calculate AUC: 0 minutes (post-breakfast), +60 minutes, +120 minutes, +180 minutes, +240 minutes, +255 minutes (post-lunch), +300 minutes, +360 minutes, +420 minutes, +480 minutes, +500 minutes (post-dinner).

10.2.9 Free-living physical activity and sedentary behaviour

Free-living PA and SB was objectively measured at baseline (after the two week run-in period) and at post-intervention using the SWA to examine change in response to diet induced weight loss. Data from the AP was not included in the current study because APs were not available for use until July 2015, by which time the study had been running approximately 1 year. Participants were fitted with the SWA during the first probe day at baseline (probe day 1) and the first probe day at post-intervention (probe day 3). The first and last day of activity monitor data was removed as it was not a complete 24 hour period. Participants also completed a PA diary to coincide with the PA monitoring period detailing the intensity, duration and type of activity performed along with details regarding removal of the SWA. PA diaries were checked upon return and any spurious data was queried with the participant. Further details on the methodological platform to measure free-living PA and SB can be found in Chapter 6.

10.2.10 Classification of weight losers and gainers

To identify differences that could account for individual variability in weight loss, participants were grouped based on their diet induced weight change. There were 17

participants who gained weight following the 12-week intervention. These participants were classified as weight gainers. The sample size of the group classified as gainers was matched with participants who lost weight following the weight loss diet. The 17 participants who achieved the largest weight loss were classified as weight losers.

10.2.11 Statistical analysis

Data are reported as mean ± SD throughout, unless otherwise stated. Statistical analysis was performed using IBM SPSS for Windows (Chicago, Illinois, Version 21) and significance was set at $p < .05$. All variables were checked for outliers and normality was assessed using the Shapiro-Wilk test. Paired sample t-tests were performed to determine whether there was a significant change in body composition, anthropometrics, RMR, measures of free-living sedentary and active behaviours, EI, health markers and eating behaviour traits between baseline and post-intervention for the study sample as a whole. Pearson correlation analysis was performed to examine the association between change in body composition and change in free-living PA and SB. Participants were then categorised based on whether they gained or lost weight as a result of the intervention. The difference in baseline sample characteristics between the losers and gainers were assessed using independent sample t-tests. To examine the effect of weight loss (losers and gainers) on body composition, RMR, free-living PA and SB, EI and subjective appetite sensations and eating behaviour traits a series of 2 (week: baseline and post-intervention) x 2 (group: losers and gainers) mixed ANOVAs were performed. Where appropriate Greenhouse-Geisser probability levels were used to adjust for sphericity. All main effects and interaction effects were examined with Bonferroni post hoc tests. Finally, Pearson correlation analysis was performed to identify whether relationships among PA, SB, FM, FFM, RMR, total EE and EI reported previously in this thesis were reproducible.

10.3 Results

10.3.1 Attrition rate

Figure 10.3 provides details of the recruitment process and reasons for exclusion and attrition from the study. In total, 613 individuals responded to the various recruitment methods. Of the 291 LED group enquiries, 242 were excluded for various reasons and 49 were recruited to the study. A further 6 withdrew from the study and 7 were excluded resulting in a final sample of 36. The CR group had 322 responses and of those, 275 were excluded. Of the 47 participants who were recruited, 3 withdrew and 3 were excluded resulting in a final sample of 41. The total sample size was 77.

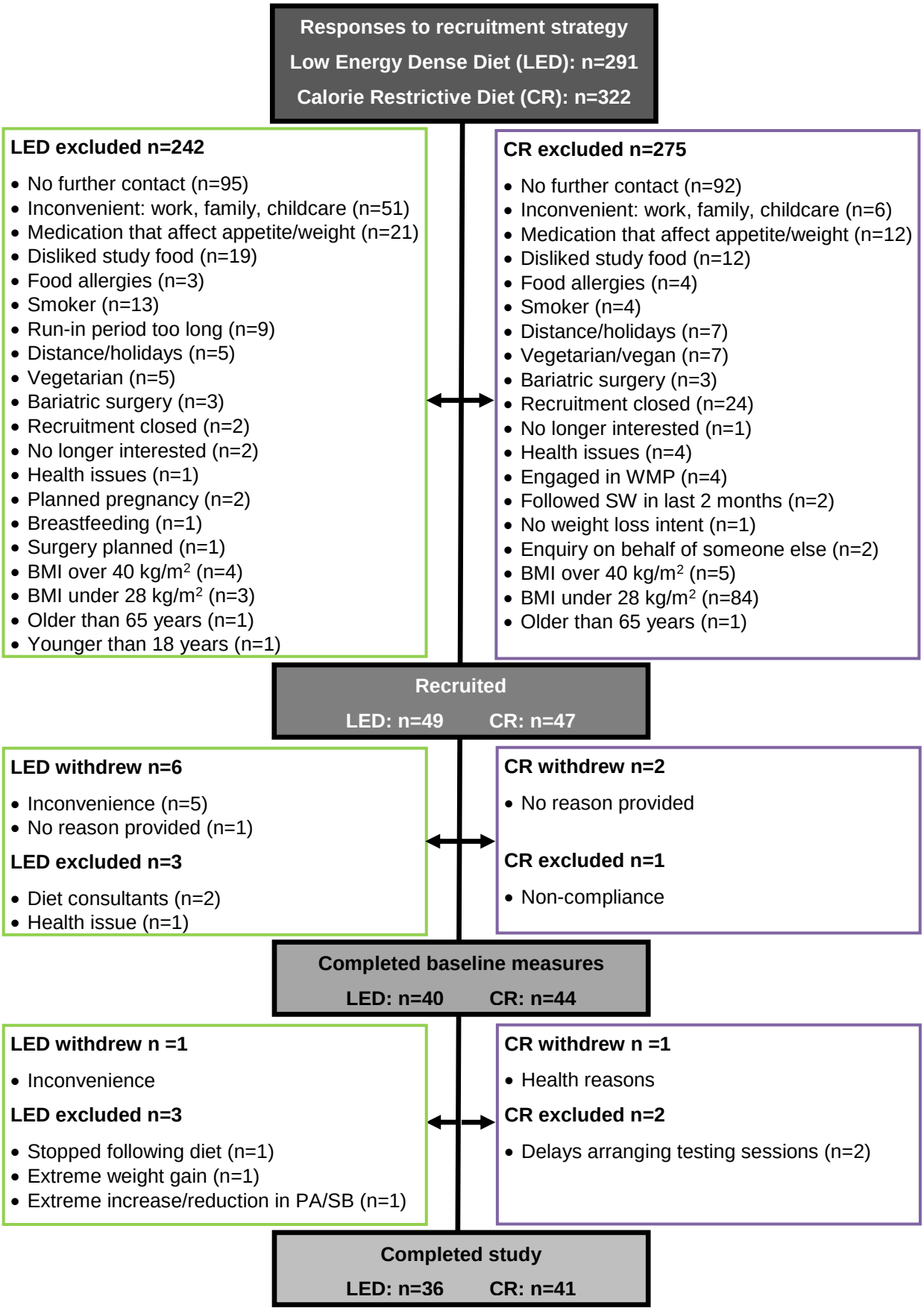


Figure 10.3 Flow chart of recruitment process and reasons for attrition and exclusion for the diet intervention study

10.3.2 Change in outcome measures from baseline to post-intervention

The average run-in period from beginning the weight loss diet to the first measures visit was 19.8 days (SD = 6.5) and the average weight loss during that time was 2.4 kg (SD = 1.43)[1]. This reported weight loss may have been exaggerated because body mass was taken with clothes on at the start of the weight loss diet and with minimal clothing using the BOD POD at the first measures visit. Comparison between baseline and post-intervention outcome measures for the study sample as a whole revealed there was a significant reduction in body mass, BMI, FM, WC, RMR, fasting BG, total EE and light PA. On the other hand, restraint significantly increased from baseline to post-intervention but no difference in any other outcome measures were observed (see Table 10.1 and Table 10.2). Average self-reported compliance (How WELL have you managed to stick with the weight control programme?) during the 12-week intervention was 47.92 mm (SD = 21.21).

Table 10.1 Change in anthropometric and physiological outcomes for whole study sample. Data are mean (SD) with p value from paired sample t-tests

	Baseline	Post-intervention	Change	*p*
Body mass (kg) ^	88.64 (12.54)	86.82 (12.95)	-1.82 (2.89)	**< .001**
BMI (kg/m^2) ^	33.21 (3.64)	32.53 (3.92)	-0.68 (1.10)	**< .001**
WC (cm)	108.51 (11.84)	105.63 (12.30)	-2.88 (4.72)	**< .001**
FM (kg) ^	41.15 (9.98)	39.32 (10.21)	-1.83 (2.71)	**< .001**
FFM (kg) ^	47.49 (5.52)	47.48 (5.47)	0.01 (1.04)	= .943
% FM ^	45.95 (5.93)	44.73 (6.13)	-1.22 (2.09)	**< .001**
RMR IC (kcal/d) †	1664.72 (234.93)	1594.67 (207.77)	-70.06 (203.55)	**= .006**
Fasting BG (mmol/L) **	4.87 (0.68)	4.65 (0.54)	-0.22 (0.73)	**= .010**
Systolic BP (mmHg)	118.74 (16.16)	118.74 (16.77)	0.00 (11.23)	= 1.00
Diastolic BP (mmHg)	80.95 (11.03)	80.69 (10.56)	-0.26 (9.07)	= .802
Resting HR (bpm) *	62.21 (8.35)	62.61 (8.63)	0.39 (7.16)	= .632

^ n=64; † n=69; ** n=75; * n=76.

[1] For the LED group weight was measured at their commercial weight loss centre and for the CR group weight was measured using an electronic scale in the HARU at the beginning of the run-in period. Both groups baseline weight was measured using the BOD POD at the end of the run-in period. Because the weighing scales used at the beginning and the end of the run-in period are inconsistent, weight change during this period was not included in further analyses.

Table 10.2 Change in behavioural and psychological outcomes for whole study sample. Data are mean (SD) with p value from paired sample t-tests

	Baseline	Post-intervention	Change	*p*
Total EE (kcal/d) ^	2566.87 (345.29)	2506.54 (326.29)	-60.33 (218.61)	**= .031**
Activity EE (kcal/d) ^	975.63 (422.67)	920.52 (409.27)	-55.11 (315.39)	= .167
Sleep (min/d) ^	419.69 (50.88)	427.32 (52.82)	7.63 (44.37)	= .174
Light PA (min/d) ^	192.61 (7.48)	174.24 (73.53)	-18.37 (60.81)	**= .019**
Moderate PA (min/d) ^	72.51 (47.71)	76.46 (51.29)	3.95 (32.91)	= .340
Vigorous (min/d) ^	1.84 (3.81)	2.75 (5.27)	0.91 (4.63)	= .121
MVPA (min/d) ^	74.35 (49.73)	79.21 (54.01)	4.85 (34.99)	= .271
Total PA (min/d) ^	266.97 (104.04)	253.45 (109.20)	-13.52 (82.23)	= .193
Steps^{SWA} ^	8179.57 (2991.24)	8385.96 (2994.97)	206.40 (2314.24)	= .478
SED^{SWA} (min/d) ^	727.44 (97.03)	735.71 (101.90)	8.27 (86.73)	= .448
PAL	1.57 (0.23)	1.62. (0.23)	0.05 (0.26)	= .180
Total EI (kcal/d) **	2374.45 (520.64)	2425.17 (629.42)	50.73 (442.68)	= .324
Dinner EI (kcal/d)	895.81 (300.59)	908.12 (338.18)	12.31 (207.63)	= .605
Snack box EI (kcal/d) **	497.92 (300.24)	545.03 (425.29)	47.11 (349.90)	= .247
Ad libitum* EI (kcal/d) *	1400.34 (496.58)	1455.34 (611.36)	55.00 (443.28)	= .286
AUC hunger (mm/min) ***	14614.00 (6856.72)	14953.99 (7441.16)	399.98 (5219.87)	= .577
AUC fullness (mm/min) ***	29397.65 (6683.97)	29503.80 (7461.05)	106.15 (5221.36)	= .862
AUC desire (mm/min) ***	15253.13 (7065.33)	15677.61 (7328.61)	424.48 (5585.82)	= .515
AUC PFC (mm/min) ***	13234.52 (6342.33)	14074.75 (6644.89)	840.23 (5019.02)	= .154
BES	15.30 (6.93)	14.71 (7.10)	-0.58 (5.84)	= .383
Restraint	9.10 (3.49)	10.48 (3.73)	1.38 (2.90)	**< .001**
Disinhibition	10.48 (3.73)	10.22 (3.01)	-0.25 (2.51)	= .392
Hunger	6.76 (3.24)	6.64 (3.58)	-0.13 (2.84)	= .689

^ n=64; *** n=74; ** n=75.

- **The dietary intervention induced significant reductions in body mass, BMI, WC, and FM**
- **Diet induced weight loss led to small reductions in total EE and RMR**
- **Diet induced weight loss did not lead to any changes in sedentary time or in MVPA**

10.3.3 Correlations between change in body composition and change in free-living physical activity and sedentary behaviour

To investigate whether dietary induced weight loss resulted in a compensatory reduction in free-living PA and an increase in SB, correlation analysis was performed. When data were checked for outliers a participants was identified whose increase in MVPA and decrease in SB was greater than three times the interquartile range. When the analysis included the participant who was an extreme outlier, significant relationships were apparent. There was a negative association between change in steps [$r(51) = -.35$, $p = .011$], change in MVPA [$r(51) = -.28$, $p = .043$] and FM change, see Figure 10.4 and Figure 10.5. The correlation between change in sedentary time and change in FM was also significant, but in the opposite direction [$r(51) = .28$, $p = .040$], see Figure 10.6. However, when the correlation analysis was repeated with the outlier removed, there were no significant associations (see Table 10.3). The outlier demonstrates the changes in PA and SB needed to produce large changes in FM. Large increases in PA and a concomitant reduction in sedentary time were associated with successful FM loss. Although other participants experienced the same degree of fat loss, their PA and SB did not change to the same extent as the outlier. This could indicate better compliance with the weight loss diet in those whose PA and SB did not change, whilst the participant who increased their PA and reduced their SB may not have complied with the weight loss diet as strictly, but achieved their weight loss through changing their PA and SB. These analyses also highlight the importance of checking the data for outliers and how outliers can affect the results of correlation analyses.

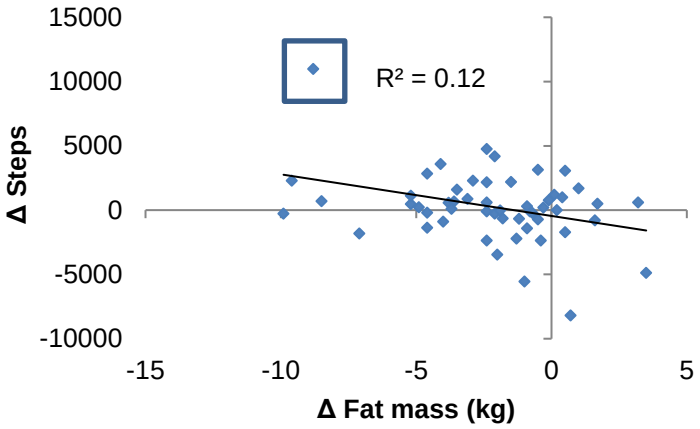


Figure 10.4 Correlation between change in FM and change in steps with outlier included in sample [p = .040]

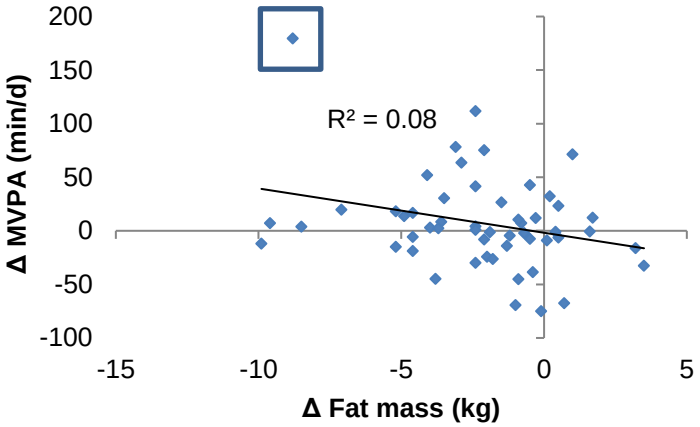


Figure 10.5 Correlation between change in FM and change in MVPA with outlier included in sample [p = .043]

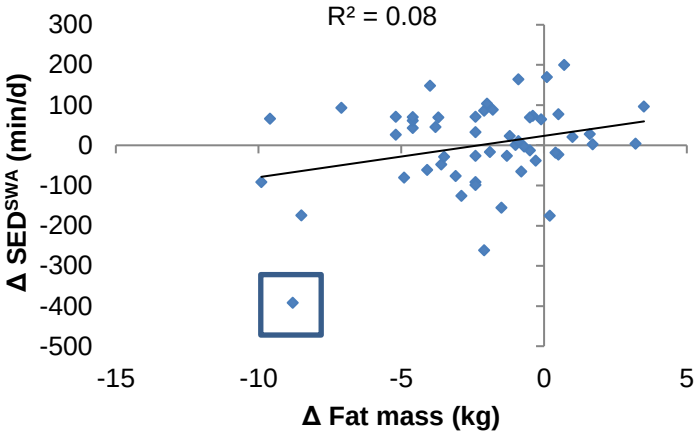


Figure 10.6 Correlation between change in FM and change in SB with outlier included in sample [p = .040]

Table 10.3 displays the correlations between change in body composition and change in free-living PA and SB between baseline and post-intervention with the outlier removed from the sample. There were no significant correlations. Figure 10.7 and Figure 10.8 show a negative relationship between change in steps, change in MVPA and change in FM, but the relationship was not statistically significant. On the other hand, Figure 10.9 shows a positive relationship between change in sedentary time and change in FM, but again the correlation did not reach statistical significance. There was a significant reduction in RMR from baseline to post-intervention and it was possible that this reduction could have been associated with change in body composition and anthropometrics. However, correlation analysis revealed change in RMR was not significantly associated with change in body mass [r(54) = .16, p = .243], FM [r(54) = .13, p = .336], FFM [r(54) = .10, p = .477] or WC [r(67) = .01, p = .969].

Table 10.3 Correlations between change in body composition and change in free-living PA and SB

	Δ Body mass (kg)	**Δ FM (kg)**	**Δ FFM (kg)**	**Δ WC (cm)**
Δ Total EE (kcal/d)	.25	.19	.21	.08
Δ Steps^{SWA}	-.20	-.22	.04	-.21
Δ Light PA (min/d)	-.03	-.04	.00	-.12
Δ Moderate PA (min/d)	-.06	-.12	.17	-.06
Δ Vigorous PA (min/d)	-.22	-.19	-.11	-.17
Δ MVPA (min/d)	-.08	-.14	.14	-.08
Δ Total PA (min/d)	-.06	-.08	.06	-.12
Δ SED^{SWA} (min/d)	.12	.15	-.06	.13

Data are Pearson Correlation (r). * p < .05; ** p < .01, ***p < .001

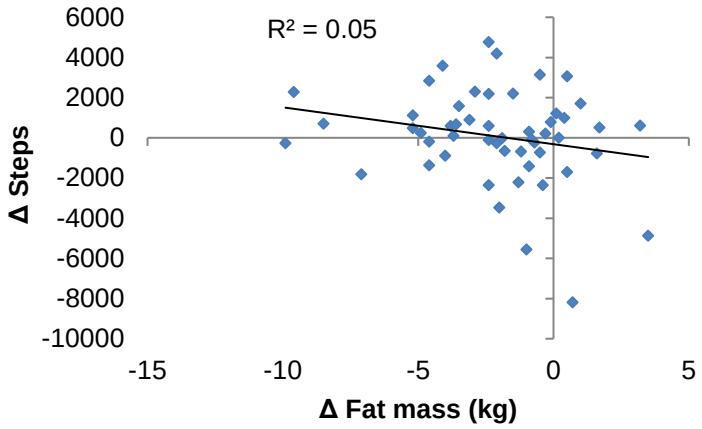


Figure 10.7 Correlation between change in FM and change in steps with outlier removed from sample (p = .112)

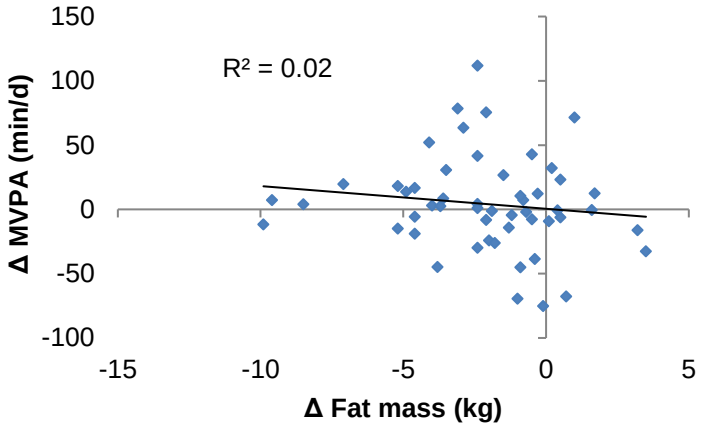


Figure 10.8 Correlation between change in FM and change in MVPA with outlier removed from sample (p = .337)

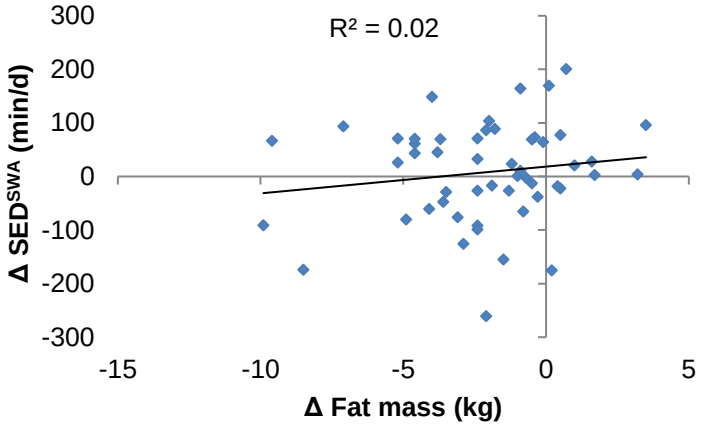


Figure 10.9 Correlation between change in FM and change in SB with outlier removed from sample (p = .280)

- **Change in FM was not significantly associated with changes in free-living PA and SB**

10.3.4 Individual variability: analysis of weight losers and gainers

There was considerable variability in weight loss between participants ranging from -10.1 kg to +4.8 kg as is illustrated in Figure 10.10. Linear regression analysis revealed that self-reported compliance with the diet intervention significantly predicted weight change [$F(1, 53) = 14.294$, $p < .001$, $R^2 = .212$]. To further explore what accounted for individual variability in weight loss response to a diet intervention, participants were categorised as losers (17 participants with the largest weight loss; see red box on left hand side of Figure 10.10) or gainers (17 participants who gained weight; see red box on right hand side of Figure 10.10).This resulted in 30 participants being excluded from analyses leaving two groups that exhibited a very different weight loss response to the diet. On average losers lost 5.51 kg (SD = 2.28) of body mass and gainers increased their body mass by 1.35 kg (SD = 1.14). Interestingly, the dietary intervention resulted in 26.6% of the sample gaining weight (17 out of 64). This is similar to the proportion of the sample in the exercise intervention study who gained weight (Chapter 9). Of the 24 participants in the exercise intervention, six gained weight (25.0%).

- **There was considerable individual variability in weight change among participants**

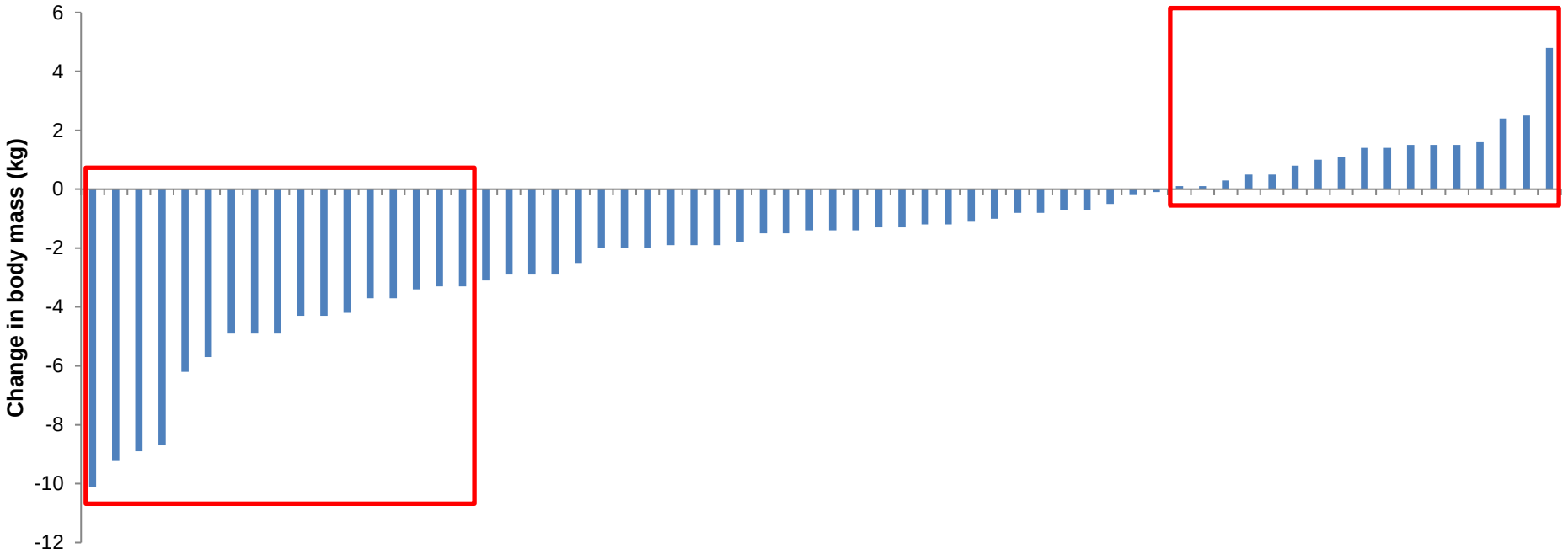


Figure 10.10 Individual variability in body mass change from baseline to post-intervention following the diet intervention. Each bar represents one participant (n=64). Left hand box highlights weight losers (n=17) and right hand box highlights weight gainers (n=17)

10.3.4.1 Baseline sample characteristics by group

Independent sample t-tests revealed there were no significant differences between groups at baseline for any of the anthropometric measurements, body composition, free-living PA and SB, RMR, health markers, appetite sensations or eating behaviour traits [$p > .05$]. However, the difference between groups at baseline for snack box EI was approaching significance [$t(31) = 2.03$, $p = .051$] with losers consuming fewer calories than gainers. See Table 10.4 and Table 10.5.

Table 10.4 Difference in anthropometric and physiological outcomes at baseline between losers (n=17) and gainers (n=17). Data are mean (SD) with p value from independent sample t-tests

	Losers	Gainers	*p*
Age (years)	42.24 (11.12)	43.47 (13.08)	= .769
Stature (m)	1.64 (0.07)	1.63 (0.07)	= .704
Body mass (kg)	89.68 (14.60)	91.16 (10.94)	= .739
BMI (kg/m^2)	33.08 (3.38)	34.24 (3.62)	= .342
WC (cm)	108.72 (11.92)	109.16 (11.89)	= .914
FM (kg)	41.80 (9.97)	43.37 (11.22)	= .669
FFM (kg)	47.88 (5.79)	47.81 (6.37)	= .975
% FM	46.16 (4.31)	47.02 (8.50)	= .713
RMR IC (kcal/d)	1615.88 (220.49)	1624.12 (235.40)	= .917
Fasting BG (mmol/L)	4.83 (0.53)	4.89 (0.71)	= .809
Systolic BP (mmHg)	117.82 (19.75)	121.24 (15.13)	= .576
Diastolic BP (mmHg)	83.35 (15.60)	82.65 (8.62)	= .871
Resting HR (bpm)	66.59 (10.85)	62.69 (9.12)	= .274

Table 10.5 Difference in behavioural and psychological outcomes at baseline between losers (n=17) and gainers (n=17). Data are mean (SD) with p value from independent sample t-tests

	Losers	Gainers	*p*
Sleep (min/d)	403.76 (43.36)	429.59 (45.21)	= .110
Total EE (kcal/d)	2612.42 (427.14)	2531.16 (258.60)	= .520
Light PA (min/d)	205.75 (92.52)	178.39 (69.43)	= .352
Moderate PA (min/d)	69.13 (39.72)	55.24 (38.65)	= .324
Vigorous (min/d)	2.25 (5.13)	0.93 (1.81)	= .337
MVPA (min/d)	71.39 (41.98)	56.16 (39.81)	= .301
Total PA (min/d)	277.13 (106.57)	234.5 (8.71)	= .215
PA level	1.62 (0.18)	1.57 (0.23)	= .502
Steps^{SWA}	8134.34 (3125.10)	7207.33 (3147.40)	= .410
SED^{SWA} (min/d)	734.25 (96.36)	748.43 (75.72)	= .250
Total EI (kcal/d)	2140.38 (631.55)	2436.09 (513.15)	= .152
Dinner EI (kcal/d)	769.03 (368.20)	940.53 (346.21)	= .171
Snack box EI (kcal/d)	374.47 (292.49)	603.44 (353.20)	= .051
***Ad libitum* EI (kcal/d)**	1188.59 (563.41)	1474.72 (519.58)	= .140
AUC hunger (mm/min)	12676.69 (7906.33)	12998.53 (6007.74)	= .895
AUC fullness (mm/min)	31108.49 (7184.80)	28104.23 (7301.86)	= .235
AUC desire (mm/min)	13914.19 (8013.26)	14844.78 (7364.58)	= .727
AUC PFC (mm/min)	12087.39 (7075.22)	12416.47 (5628.27)	= .882
BES	15.88 (7.61)	14.24 (6.51)	= .503
Restraint	9.47 (3.56)	9.65 (3.76)	= .889
Disinhibition	9.88 (3.84)	10.71 (2.78)	= .479
Hunger	7.00 (4.26)	6.35 (3.10)	= .616

10.3.4.2 Change in body composition, anthropometrics and resting metabolic rate

Change in body mass between groups is displayed in Figure 10.11A. There was a significant main effect of week [F(1, 32) = 45.23, p < .001] that showed a significant reduction in body mass on average from baseline (M = 90.42 kg, SD = 12.72) to post-intervention (M = 88.34 kg, SD = 13.64). Furthermore, there was a significant week x group interaction [F(1, 32) = 123.22, p < .001] that revealed losers lost weight (M = 5.51 kg, SD = 2.28) [p < .001] and gainers by definition gained weight (M = 1.35 kg, SD = 1.14) [p = .004]. There was no main effect of group [F(1,32) = 1.22, p = .278].

Change in BMI between groups is displayed in Figure 10.11B. There was a significant main effect of week [F(1, 32) = 38.77, p < .001] with BMI being lower at post-intervention (M = 32.88 kg/m^2, SD = 4.05) compared to baseline (M = 33.66 kg/m^2, SD = 3.50). There was a significant week x group interaction [F(1, 32) = 105.43, p < .001] that revealed losers BMI decreased (M = 2.06 kg/m^2, SD = 0.93) [p < .001] and gainers BMI increased (M = 0.51 kg/m^2, SD = 0.44) [p = .007]. At post-intervention, losers had a significantly lower BMI compared to gainers [p = .005]. The main effect of group was approaching significance [F(1,32) = 4.03, p = .053].

There was a significant main effect of week [F(1, 32) = 80.10, p < .001] that revealed average FM was lower post-intervention (M = 40.41 kg, SD = 11.07) compared to baseline (M = 42.59 kg, SD = 10.48). There was a significant interaction between week and group [F(1, 32) = 138.37, p < .001]. Post hoc tests revealed losers had a significant reduction in FM (M = 5.02 kg, SD = 2.46) [p < .001], whereas gainers slightly but non-significantly increased FM (M = 0.68 kg, SD = 1.54) [p = .181]. The main effect of group was not significant [F(1, 32) = 1.49, p = .231]. Change in FM between groups is displayed in Figure 10.11C.

There was a significant interaction between week and group [F(1,32) = 10.24, p = .003] that revealed a significant increase in FFM in gainers (M = 0.62 kg, SD 1.08) [p = .016] and a non-significant reduction in losers (M = 0.48 kg, SD = 0.92) [p = .057], see Figure 10.11D. However, there was no main effect of week [F(1, 32) = 0.17, p = .686] or group [F(1, 32) = 0.06, p = .811] on FFM.

Change WC between groups is displayed in Figure 10.11E. There was a significant main effect of week [F(1, 32) = 20.39, p < .001] that revealed average WC was higher at baseline (M = 108.94 cm, SD = 11.73) compared with post-intervention (M = 105.77 cm, SD = 12.33). There was also a significant week x group interaction [F(1, 32) = 16.17, p < .001] and post hoc test revealed losers had a significant reduction in WC (M = 5.99 cm, SD = 4.89) [p < .001] but the reduction was not significant for gainers (M = 0.34 cm, SD = 3.10) [p = .729]. There was no main effect of group [F(1, 32) = 16.17, p = .427].

There was no main effect of week [F(1, 29) = 0.11, p = .744] or group [F(1, 29) = 0.27, p = .608] on RMR and there was no week x group interaction [F(1, 29) = 2.56, p = .120].

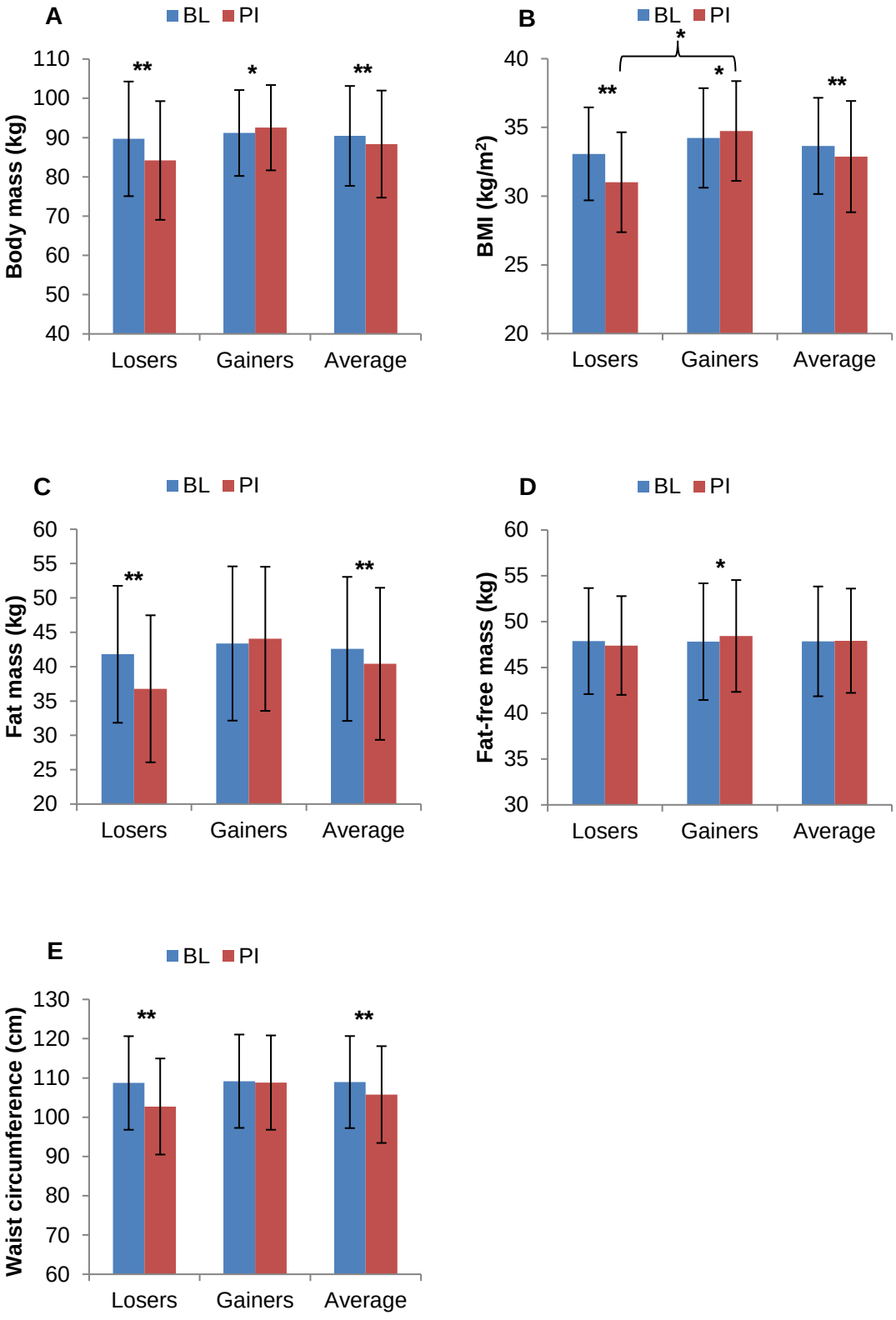


Figure 10.11 Change in body mass (A), BMI (B), FM (C), FFM (D) and WC (E) for losers (n=17) and gainers (n=17) between baseline and post-intervention. ** p < .01; * p < .05

10.3.4.3 Change in free-living physical activity and sedentary behaviour

The lack of significant correlations between change in body composition and change in free-living sedentary and active behaviours indicates that change in free-living activity does not contribute to the variability in diet induced weight loss in the current study. To confirm this, the two extreme groups of weight loss (losers and gainers) were examined for changes in sedentary and active behaviours using mixed ANOVAs. PA level was calculated by dividing total EE by measured RMR. There was no main effect of week [$F(1, 22) = 0.49$, $p = .493$] or group [$F(1, 22) = 0.36$, $p = .557$] and no week x group interaction [$F(1, 22) = 0.03$, $p = .856$] for PA level.

There was no main effect of week [$F(1, 25) = 0.28$, $p = .599$] or group [$F(1, 25) = 0.47$, $p = .499$] and no week x group interaction [$F(1, 25) = 2.08$, $p = .161$] for sleep.

There was a main effect of week [$F(1, 25) = 7.87$, $p = .010$] on total EE that revealed there was a significant decrease on average from baseline (M = 2558.46 kcal/d, SD = 353.95) to post-intervention (M = 2458.77 kcal/d, SD = 323.18). However, there was no main effect of group [$F(1, 25) = 0.38$, $p = .546$] and no week x group interaction [$F(1, 25) = 3.86$, $p = .061$] for total EE, see Figure 10.12A.

There was a main effect of week on light PA [$F(1, 25) = 6.69$, $p = .016$] that revealed participants performed more light PA at baseline (M = 190.99 min/d, SD = 83.48) compared to post-intervention (M = 161.73 min/d, SD = 68.65). There was no main effect of group [$F(1, 25) = 3.56$, $p = .071$] and there was no week x group interaction [$F(1, 25) = 0.01$, $p = .942$]. Figure 10.12B displays the change in light PA between baseline and post-intervention for the two groups.

There was no main effect of week [$F(1, 25) = 0.39$, $p = .536$] or group [$F(1, 25) = 1.30$, $p = .265$] and no week x group interaction [$F(1, 25) = 0.71$, $p = .408$] for moderate PA.

There was no main effect of week [$F(1, 25) = 1.28$, $p = .269$] or group [$F(1, 25) = 2.47$, $p = .129$] and no week x group interaction [$F(1, 25) = 1.89$, $p = .182$] for vigorous PA.

There was no main effect of week [$F(1, 25) = 0.12$, $p = .730$] or group [$F(1, 25) = 1.59$, $p = .219$] and no week x group interaction [$F(1, 25) = 1.18$, $p = .288$] for MVPA. However, there was a slight increase for losers and a slight decrease for gainers, see Figure 10.12C.

There was no main effect of week [$F(1, 25) = 0.08$, $p = .785$] or group [$F(1, 25) = 0.56$, $p = .463$] and no week x group interaction [$F(1, 25) = 2.22$, $p = .149$] for steps.

Figure 10.12D displays the change in sedentary time between baseline and post-intervention for the two groups. The main effect of week on SED^{SWA} was approaching significance [$F(1, 25) = 4.19$, $p = .051$]. On average, participants were more sedentary post-intervention (M = 775.88 min/d, SD = 81.46) compared to baseline (M = 746.09

min/d, SD = 89.76). The main effect of group was also approaching significance [F(1, 25) = 4.16, p = .052] with losers performing less SB on average (M = 736.12 min/d, SD = 18.29) than gainers (M = 792.07 min/d, SD = 20.44). There was no significant week x group interaction [F(1, 25) = 2.00, p = .170].

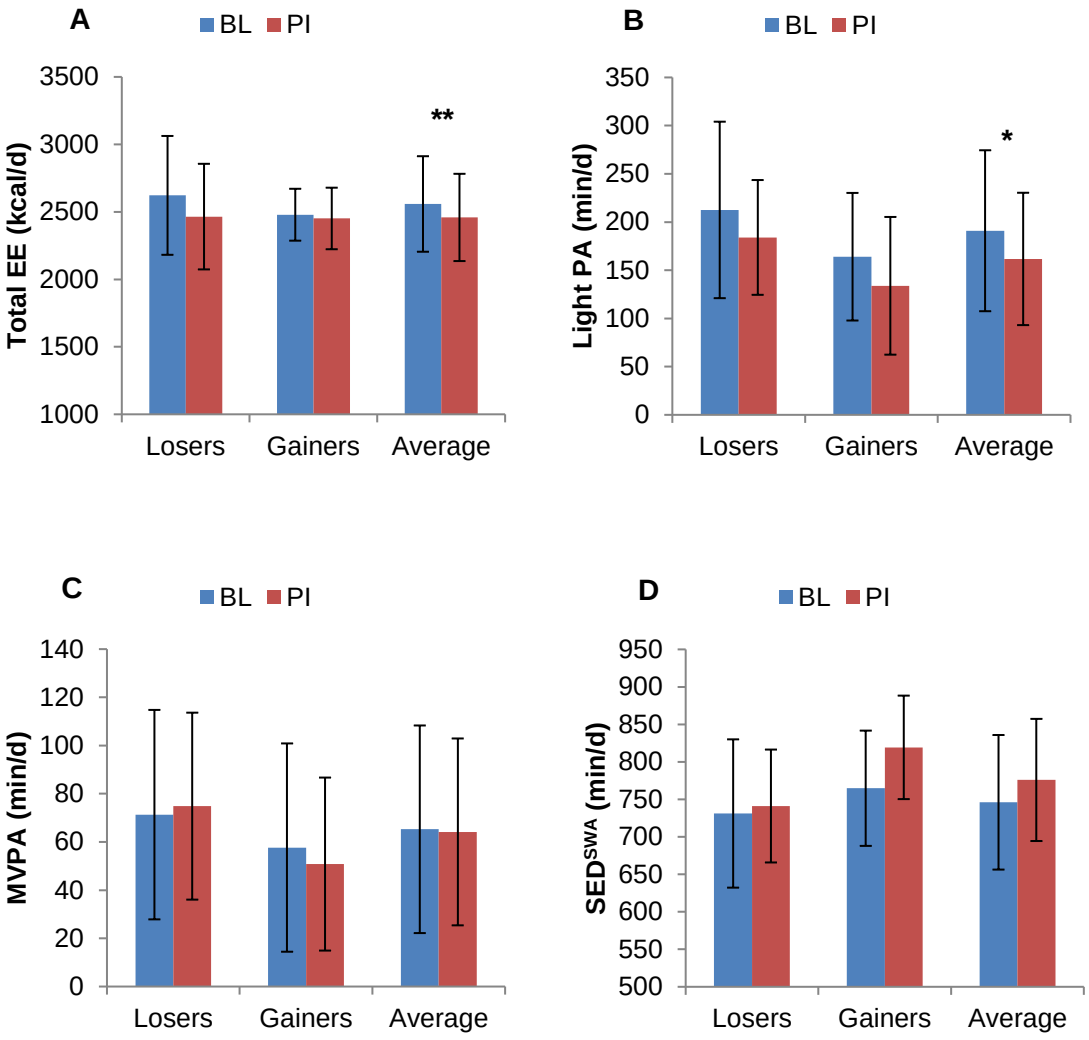


Figure 10.12 Change in total EE (A), light PA (B), MVPA (C) and sedentary time (D) for losers (n=15) and gainers (n=12) between baseline and post-intervention. ** p < .01; * p < .05

- **When the sample was stratified there was a clear difference between the weight losers (-5.51 kg) and the weight gainers (+1.35 kg)**
- **Across the weight loss period these two groups displayed no difference in SB or in PA**
- **Participants who gained weight in response to the diet intervention did not compensate by increasing SB or decreasing PA**

10.3.4.4 Change in energy intake and subjective appetite sensations

Change in eating behaviour between groups was examined to further explore what contributed to the variability in weight loss. There was a main effect of group on average snack box EI [F(1, 31) = 6.21, p = .018] that revealed losers (M = 358.87 kcal/d, SD = 289.86) consumed significantly fewer calories from the snack box than gainers (M = 643.02 kcal/d, SD = 446.46), see Figure 10.13A. However, there was no significant main effect of week [F(1, 31) = 0.13, p = .723] and no week x group interaction [F(1, 31) = 0.68, p = .416].

There was a significant week x group interaction [F(1, 32) = 5.14, p = .030] for dinner EI. Post hoc analysis revealed losers showed a non-significant decrease in dinner EI (M = 45.09 kcal/d, SD = 175.11) [p = .245] and gainers increased their dinner EI (M = 77.03 kcal/d, SD = 136.52) [p = .052], see Figure 10.13B. However, there was no main effect of week [F(1, 32) = 0.35, p = .557] or group [F(1, 32) = 0.98, p = .330].

There was a significant main effect of group [F(1, 31) = 4.67, p = .038] that revealed gainers (M = 1361.09 kcal/d, SD = 644.81) consumed significantly more calories during *ad libitum* meals (dinner and snack box EI combined) than losers (M = 1327.32 kcal/d, SD = 553.89), see Figure 10.13C. However, there was no main effect of week [F(1, 31) = 0.25, p = .619] and there was no week x group interaction [F(1, 31) = 2.35, p = .135] for *ad libitum* EI.

There was a significant main effect of group [F(1, 31) = 4.40, p = .044] that revealed gainers (M = 2312.48 kcal/d, SD = 670.53) consumed significantly more calories during the whole day than losers (M = 2283.76 kcal/d, SD = 587.74), see Figure 10.13D. However, there was no main effect of week [F(1, 31) = 0.19, p = .666] and there was no week x group interaction [F(1, 31) = 2.44, p = .128] for total EI.

There was a significant difference between groups on average over the 12-week intervention for self-reported diet compliance [t(30) = 3.31, p = .002] with losers (M = 61.43 mm, SD = 18.48) reporting higher compliance than gainers (M = 40.40 mm, SD = 17.47).

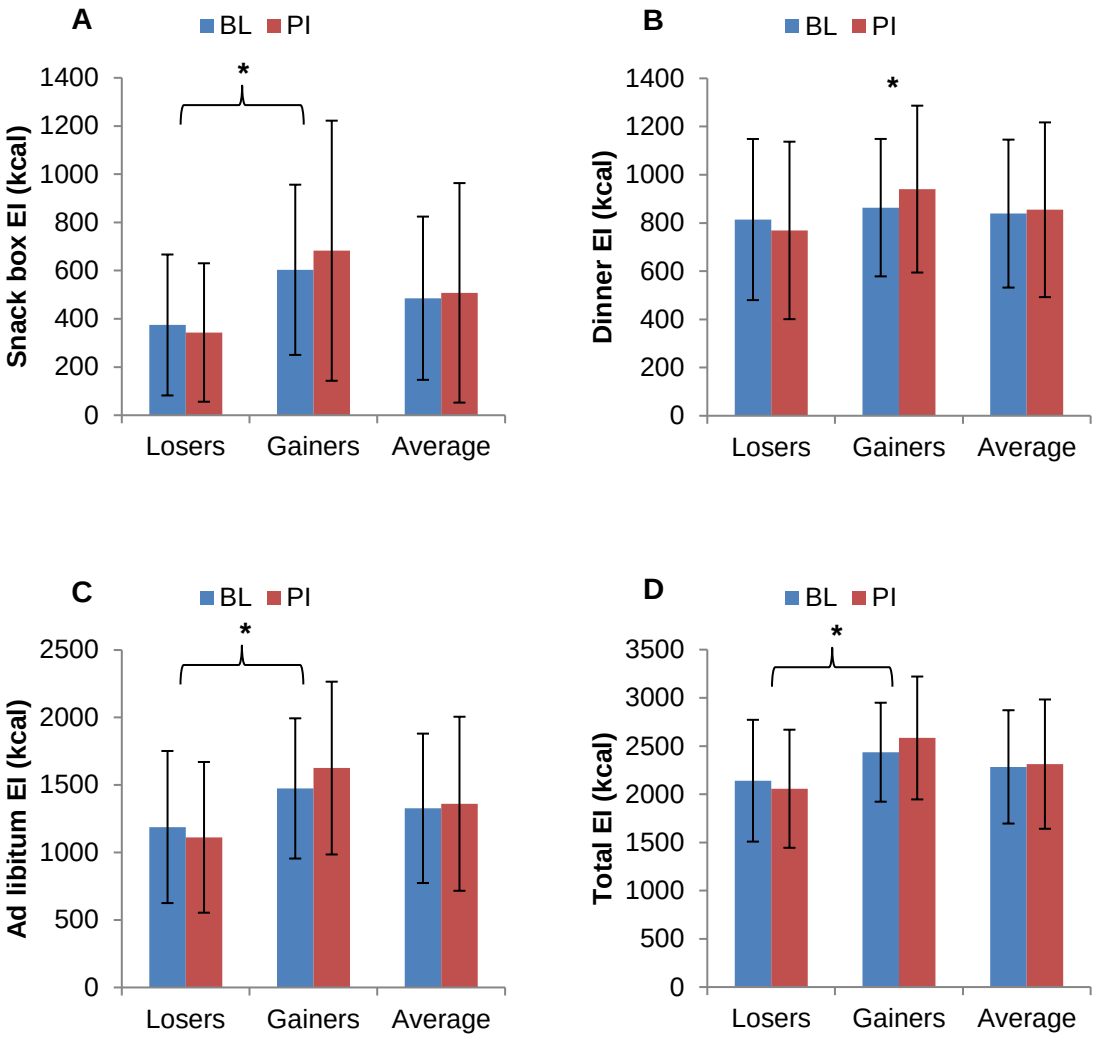


Figure 10.13 Change in snack box EI (A), dinner EI (B), *ad libitum* EI (C) and total EI (D) for losers (n=17) and gainers (n=16; n=17 for dinner EI) between baseline and post-intervention. ** = p < .01; * = p < .05

There was a significant main effect of week [F(1, 32) = 5.66, p = .023] on AUC for hunger and further examination revealed, on average, AUC for hunger increased from baseline (M = 12837.61 mm/min, SD = 6916.22) to post-intervention (M = 14640.13 mm/min, SD = 7586.92), see Figure 10.14A. However, there was no main effect of group [F(1, 32] = 0.05, p = .821] and there was no week x group interaction [F(1, 32) = 0.09, p = .764].

There was no main effect of week [F(1, 32) = 0.01, p = .936] or group [F(1, 32) = 1.73, p = .198] on AUC for fullness and there was no week x group interaction [F(1, 32) = 0.07, p = .787].

There was no main effect of week [F(1, 32) = 2.81, p = .104] or group [F(1, 32) = 0.18, p = .677] and there was no week x group interaction [F(1, 32) = 0.02, p = .891] for AUC desire to eat.

There was a significant main effect of week [F(1, 32) = 7.46, p = .010] that revealed AUC prospective food consumption was significantly higher on average post-intervention (M = 14060.57 mm/min, SD = 7124.92) compared to baseline (M = 12251.93 mm/min, SD = 6297.42), see Figure 10.14B. However, there was no significant main effect of group [F(1, 32) = 0.50, p = .485] and there was no week x group interaction [F(1, 32) = 3.49, p = .071].

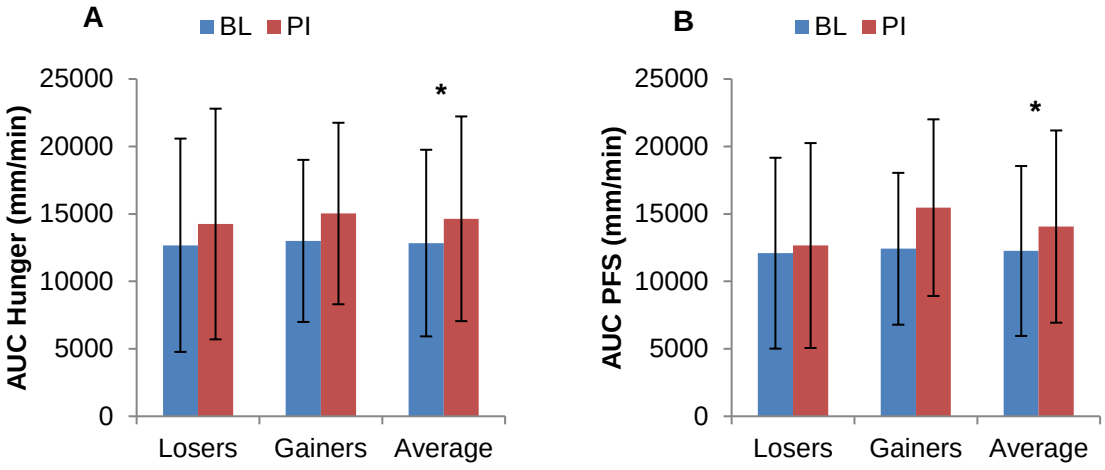


Figure 10.14 Change in AUC hunger (A) and prospective food consumption (B) for losers (n=17) and gainers (n=17) between baseline and post-intervention. ** = p < .01; * = p < .05

10.3.4.5 Change in eating behaviour traits

There was a significant interaction between week and group for BES score [F(1, 32) = 13.00, p = .002]. Simple contrasts revealed that losers had a significant reduction in BES score (M = 3.41, SD = 4.66) [p = .012], whilst gainers had a significant increase (M = 3.12, SD = 5.83) [p = .021], see Figure 10.15A. There was no main effect of week [F(1, 32) = 0.03, p = .872] or group [F(1, 32) = 0.53, p = .470].

There was no main effect of week [F(1, 32) = 9.19, p = .210] or group [F(1, 32) = 0.15, p = .704] and there was no week x group interaction [F(1, 32) = 1.15, p = .291] for TFEQ-R.

There was no main effect of week [F(1, 32) = 7.12, p = .095] or group [F(1, 32) = 0.15, p = .704] and there was no week x group interaction [F(1, 32) = 1.40, p = .246] for TFEQ-D.

There was a significant interaction between week and group for TFEQ-H [F(1, 32) = 11.80, p = .002]. Simple contrasts revealed that losers had a significant reduction in TFEQ-H (M = 1.76, SD = 2.70) [p = .012], whilst gainers had a significant increase (M = 1.59, SD = 2.98) [p = .016], see Figure 10.15B. There was no main effect of week [F(1, 32) = 0.03, p = .858] or group [F(1, 32) = 0.78, p = .385].

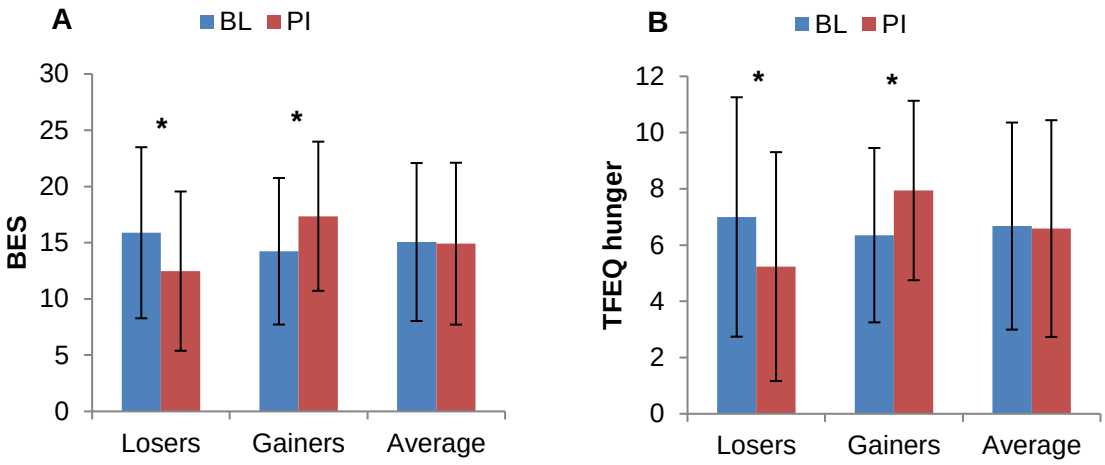


Figure 10.15 Change in BES score (A) and TFEQ-H (B) for losers (n=17) and gainers (n=17) between baseline and post-intervention. ** = p < .01; * = p < .05

- **Participants who gained or lost weight displayed differences in EI. This difference was apparent at baseline and was amplified over the course of the intervention**
- **Participants who gained weight showed a greater EI from the snack box**
- **In the weight gainers there was an increase in appetite variables (BES score and TFEQ-H) over the course of the intervention. Appetite control in the weight gainers appeared to deteriorate due to the intervention**
- **Appetite variables rather than free-living movement behaviours (PA and SB), seem to be responsible for the change in weight of those who gained weight**

10.3.5 Predictors of weight change

Correlation analysis was performed to identify whether baseline measures of PA and SB or appetite variables were related to body mass and FM change. There were no significant relationships between measures of free-living PA/SB or eating behaviour traits and change in body mass or FM [p > .05]. However, baseline snack box EI was associated with weight change [r(62) = .34, p = . 008] and FM change [r(62) = .35, p = .006] such that higher baseline snack box EI was associated with an increase in body mass and FM.

10.4 Discussion

This study investigated the effects of diet induced weight loss on free-living sedentary and active behaviours in overweight and obese women. Examination of body composition and anthropometric measurements revealed the diet intervention resulted in significant weight loss as well as a reduction in BMI, FM and WC. Furthermore, weight loss significantly impacted on health related outcomes with a significant decrease in fasting BG.

10.4.1 Change in free-living physical activity and sedentary behaviour

Diet induced weight loss did not lead to a compensatory reduction in free-living MVPA or an increase in SB. There was, however, a significant decrease in average light PA. Light PA was displaced with some lower intensity activities (SB) but also with higher intensity activity (MVPA). Using the average of the baseline and post-intervention weights for the study sample of 87 kg and the 1 MET = 1 kcal/kg/h rule, the 18 minute reduction in light PA (2 METs) would be equivalent to a reduction in EE of approximately 50 kcal. The increase in sedentary time (1.25 MET) would be equivalent to approximately 15 kcal, and the increase in MVPA (5 METs) would be equivalent to approximately 36 kcal. These estimates suggest the change in the distribution of PA across intensities from baseline to post-intervention would not have resulted in a reduction in activity EE and would be unlikely to impact on weight loss outcomes. A previous study reported there was no significant change in free-living movement behaviour as a result of diet induced weight loss when measured using an accelerometer (Martin et al., 2011). However, another study demonstrated there was a significant increase in time spent walking and cycling, and a significant reduction in time spent sedentary when measured using a triaxial accelerometer (Bonomi et al., 2013). A possible explanation for the spontaneous increase in PA and reduction in SB observed by Bonomi et al. (2013) could be due to reduced physiological stress of PA

in weight-reduced individuals (Weinsier et al., 2000). Weight-reduced individuals find being physically active easier, therefore they engage more in those behaviours. The current study resulted in a considerably lower average weight loss compared with previous studies that have demonstrated a significant increase in activity post-weight loss (Bonomi et al., 2013, Weinsier et al., 2000) and could explain the absence of a significant increase in PA or reduction in SB. For example, reduced physiological stress of exercise was reported in a study with an average weight loss of 12.8 kg, which is considerably greater than that observed in the current study (Weinsier et al., 2000). A limitation of the current study is that baseline measurement of PA and SB was completed after a two week run-in period, at which point participants had been following the weight loss diet for two weeks. It is possible that PA and SB had already changed in response to the initial weight loss when baseline measurements were taken, resulting in the difference between baseline and post-intervention measures being blunted. However, this is unlikely as there was minimal change in free-living PA and SB during the 12-week intervention when further weight loss occurred and the degree of weight loss achieved did not impact on change in PA and SB.

10.4.2 Change in total energy expenditure and resting metabolic rate

Previous studies have demonstrated a decline in PA related outcomes such as total and active EE in response to diet induced weight loss (Serra et al., 2014, Camps et al., 2013, Redman et al., 2009, Martin et al., 2007, Leibel et al., 1995). Therefore, in addition to examining the change in free-living movement behaviour, this study also investigated whether total EE and RMR (a major component of total EE) changed in response to diet induced weight loss. There was a significant reduction in both total EE and RMR, but this could not be attributed to a decrease in FFM as this did not differ significantly from baseline to post-intervention. FM accounts for 6% of the variance in RMR (Johnstone et al., 2005) therefore it is possible that the reduction in RMR could be attributed to the significant loss of FM, however change in RMR was not significantly associated with change in FM. Several other mechanisms have been proposed that could explain the reduction in RMR observed following weight loss including neuroendocrine disturbances, such as alterations in leptin level (Doucet et al., 2000) and alterations in sympathetic nervous system activity (Rosenbaum and Leibel, 2014, Rosenbaum et al., 2000). However, it was beyond the scope of this study to explore the underlying physiological processes leading to the observed reduction in RMR. It is likely that the change in RMR accounted for the change in total EE, as RMR contributes heavily (50-70%) to total EE (Shetty, 2005, Goran, 2000, Ravussin et al., 1982). There was also a significant reduction in light PA that could have contributed to the reduction in total EE, but this is unlikely because light PA was displaced with SB and MVPA that would have resulted in the equivalent EE as discussed earlier in this

section. EE was measured using the SWA, rather than DLW, which factors body mass in to the predictive equation. It is possible that the lower total EE is simply reflecting the lower post-weight loss body mass.

10.4.3 Predictors of individual variability in diet induced weight loss

There was considerable individual variability in weight change between participants (-10.1 kg to +4.8 kg). Large individual variability in weight loss response to exercise has previously been reported (King et al., 2008). Furthermore, classic genetic studies by Bouchard et al. (1990) demonstrated large variability in weight gain between sets of twins in response to overfeeding. However, the variability in diet induced weight loss between participants is not well characterised. To examine whether change in free-living PA and SB contributed to this individual variability in weight loss in response to a diet intervention, participants were categorised as losers and gainers and change in outcome measures were compared between groups. Change in free-living MVPA and SB did not differ between those who lost weight and those who gained weight. Furthermore, baseline measures of free-living PA and SB did not explain the variation in weight loss as has previously been demonstrated (Camps et al., 2013). However, there was a non-significant increase in MVPA in those who achieved an average weight loss of 6.1% of baseline weight. This small adjustment in MVPA (reverse compensation) could be an autonomic response as a result of the reduced physiological exertion of PA at a lower body mass or a volitional response reflecting a conscious effort to increase PA. On the other hand, although the interaction between week and group was not significant for sedentary time, gainers increased their SB by 54.5 min/d compared to a 10.0 min/d increase in losers. The change in SB in the gainers could represent a compensatory mechanism to reduce the energy deficit induced by the diet. Variability in diet induced weight loss was not explained by changes in free-living PA and SB in the current study. Further research using objective PA monitors to quantify different intensities of PA and SB will shed light on the behavioural compensatory responses to diet induced weight loss.

To further explore mechanisms contributing to the variability in weight loss, changes in appetite related outcomes were compared between groups. Appetite variables, rather than PA variables, seem to be responsible for the change in weight of the gainers. Those who gained weight experienced changes in dinner EI, BES score and TFEQ-H in the direction that would oppose weight loss. Gainers consumed significantly more calories from the snack box on average compared with losers. Increased EI and hunger (AUC) have previously been associated with less than expected weight loss following an exercise intervention (King et al., 2008, King et al., 2009a). These data suggest that behavioural adjustments occur (increased EI and drive to eat) to oppose weight loss regardless of how the energy deficit is created (diet or exercise). Baseline

snack box EI significantly predicted weight and FM change such that higher baseline snack box EI was associated with an increase in body mass and FM. Previous research has linked snack intake to weight gain (Bes-Rastrollo et al., 2010) and baseline snack intake in the current study could be a marker of weight gain or could reflect a lack of compliance with the diet intervention. Indeed, gainers had significantly lower self-report diet compliance rates on average over the 12-week intervention compared with losers. Snack intake may represent an intervention target to improve the effectiveness of diet interventions for weight loss and future research should examine the effects of snacking quality and frequency, for example, on diet induced weight loss.

Previous research has suggested different eating behaviour traits are in fact capturing the same underlying dimension, uncontrolled eating (Vainik et al., 2015). Results from this study suggest losers eating behaviour became more controlled (reduced BES score and TFEQ-H) and gainers lost control (increased EI, BES score and TFEQ-H) as a result of the diet intervention. As with an exercise-induced negative energy balance, acute (Hubert et al., 1998, Deighton et al., 2014) and longer-term (Sumithran et al., 2011) diet interventions have also been shown to result in compensatory responses in subjective hunger and objectively measured EI. Furthermore, energy restriction also results in a significant reduction in fasting leptin, peptide YY (PYY) and insulin concentrations and an increase in fasting ghrelin (Mars et al., 2005, Keim et al., 1998, King et al., 2011). These changes could be driving the change in EI and eating behaviour traits observed in those who gained weight in the current study.

A limitation of the current study is that compliance with the weight loss diets was based on a single question and was self-reported away from the laboratory in an uncontrolled environment. Therefore, it is not possible to form strong conclusions about how adherence to the diet interventions affected weight loss. Unsurprisingly, higher compliance has previously been associated with better weight loss outcomes (Wright et al., 2010). In the current study participants who lost weight had significantly higher self-reported compliance rates to the diet compared with those who gained weight. This suggests that diet compliance contributed to the variability in diet induced weight loss observed in this study. However, this does not detract from the importance of other factors identified in this study that were related to weight loss success, such as, eating behaviour and psychometric eating behaviour traits.

This study investigated the effects of diet induced weight loss on free-living PA and SB. Diet induced weight loss did not lead to a compensatory reduction in MVPA or increase in SB, but there was a decrease in light PA. There was also a significant reduction in total EE and RMR. It is possible that diet induced weight loss could be optimised by encouraging participants to increase their daily PA levels to maintain pre

weight loss total EE. Change in body mass was highly variable between participants and this was related to changes in appetite variables rather that changes in PA and SB. The psychological, physiological and behavioural mechanisms driving the individual variability in diet induced weight loss require further exploration. Results from the current study suggest changes in appetite variables related to uncontrolled eating (EI, BES score and TFEQ-H) and changes in energy metabolism (total EE and RMR), rather than changes in free-living PA and SB, contribute to the observed variability in diet induced weight loss.

10.5 Outcomes

- **The diet intervention produced significant reductions in body mass, BMI, WC and FM (adiposity)**
- **Diet induced weight loss did not lead to any compensatory reduction in free-living MVPA or an increase in SB**
- **The diet intervention induced a slight but significant reduction in total EE and RMR which would work against the diet-induced negative energy balance and tend to offset weight loss**
- **Change in FM due to diet intervention was not associated with change in free-living PA or SB**
- **There was considerable individual variability in weight loss between participants**
- **Appetite variables (snack box EI, BES score and TFEQ-H), rather than free-living movement behaviours (PA and SB), seem to be responsible for the change in weight of those who gained weight**

Chapter 11
General Discussion

11.1 Thesis overview

In order to introduce the discussion it is appropriate to briefly go over the ideas that formed the origin of this thesis. This thesis was inspired by a revival of the work of Edholm and Mayer conducted over 60 years ago who identified a relationship between EI and EE (Blundell, 2011). Edholm et al. (1955) found no relationship between EI and EE within one day, but there was a linear relationship when EI and EE were averaged over the course of several days (Edholm, 1977). However, the contemporaneous work of Mayer et al. (1956) suggested this relationship only operates above a certain level of PA, and that below that level EI and EE become dissociated such that there is an increase in EI that exceeds EE. More recently, experimental studies have examined the effects of exercise on appetite control and energy balance. Increased structured exercise that is supervised and mandatory results in improved satiety signalling and body composition (King et al., 2009a, Broom et al., 2009). However, being less active is associated with increased adiposity and EI is not down regulated (Shook et al., 2015, Stubbs et al., 2004). It has been suggested that sensitivity to appetite signals is reduced in those who perform little PA and large amounts of SB, perhaps due to the accumulation of FM and leptin resistance (Blundell et al., 2015a, Blundell, 2011). However, the mechanisms underlying the apparent uncoupling of EI to EE at low levels of PA require further investigation. Finally, weight loss resulting from exercise interventions and diet interventions is often less than expected and there is large individual variability (King et al., 2008, Thomas et al., 2012, Camps et al., 2013). These observations indicate there are compensatory responses to perturbations in energy balance to defend against weight loss. Change in NEPA (Camps et al., 2013, Fedewa et al., 2016), eating behaviour and appetite sensations (King et al., 2009a), or RMR (King et al., 2007) could contribute to the individual variability in weight loss, however, the behavioural and metabolic compensatory responses to perturbation in energy balance are not fully understood.

Work conducted in the HARU at the University of Leeds over the last 25 years has led to the development of a framework to study appetite control and energy balance (Figure 4.1). This framework formed the basis for the experimental studies reported here. As a direct result of early work conducted in this thesis, it was possible to integrate an additional component of energy balance within the Leeds multilevel platform; the measurement of free-living PA and SB using state-of-the-art motion sensing devices. A strength of this psychobiological approach is the simultaneous

measurement of physiological, behavioural and psychological parameters related to appetite control and energy balance that would otherwise be studied in isolation. This has exposed relationships among variables associated with appetite control and energy balance across diverse scientific domains.

This thesis is comprised of both observational studies (to examine how individuals behave under habitual circumstances; Studies 1, 2, 3 and 4) and experimental studies (to explore how individuals respond to perturbations in energy balance through exercise or diet; Studies 5 and 6). One important goal of this thesis was to develop a platform to quantify free-living sedentary and active behaviours in order to investigate the associations among free-living sedentary and active behaviours, adiposity and appetite control. The primary aim was to establish the relationship between SB, MVPA, and adiposity and appetite control. The secondary aim was to evaluate how PA and SB may change after exercise induced or diet induced weight loss. To achieve this the research platform was embedded within medium term intervention studies investigating the effects of i) supervised exercise and ii) dietary manipulation on energy balance, appetite control and free-living sedentary and active behaviours. This thesis has examined the relationship between objectively measured sedentary and active behaviours, adiposity and appetite control within an energy balance framework. This work has informed the conceptualisation of a theoretical framework to describe the relationship between free-living sedentary and active behaviours and appetite control. The aim of this general discussion is to summarise the key findings throughout this thesis and relate them to what is already known in the field of appetite control and energy balance.

11.2 Relationship between free-living sedentary behaviour, physical activity and adiposity

A fundamental component of this thesis was to develop a methodological platform to objectively quantify free-living sedentary and active behaviours using validated motion sensing devices. This methodological platform is described in detail in Study 2 (Chapter 6) and was embedded within all of the experimental studies in this thesis. The objective measurement of free-living PA and SB, along with body composition and anthropometric measurements revealed that MVPA, performed under normal daily living conditions, was negatively associated with multiple indices of adiposity. On the other hand, SB was positively associated with adiposity. FFM was not associated with any of the measures of free-living PA or SB. Interestingly, when the correlation between MVPA and adiposity was controlled for SB, the relationship remained significant. However, when the relationship between SB and adiposity was computed whilst controlling for MVPA, the association was reduced to a non-significant level.

Previous research has consistently reported that MVPA is beneficially associated with weight status and this is true for a number of different indices of adiposity, including BMI (Maher et al., 2013), WC (Healy et al., 2008a) and body fat (Shook et al., 2015). Furthermore, experimental studies have demonstrated that increased exercise results in weight loss (Jakicic et al., 2003, Donnelly et al., 2003) and this is supported by a Cochrane systematic review (Shaw et al., 2006). However, the relationship between SB and obesity is less clear with some studies reporting no association with indices of adiposity (Maher et al., 2013) and others reporting a positive relationship (Healy et al., 2011b). Interestingly, these dissimilar conclusions were drawn from the same study; NHANES 2003-2006. Both studies used the same accelerometer derived information about SB, however the measure of adiposity differed; Maher et al. (2013) reported BMI whereas Healy et al. (2011b) reported WC. These studies demonstrate the impact that measurement method can have on the reported relationship between SB and obesity. This will be discussed further in section 11.3.2. This indicates that relationships with adiposity require an objective measurement of body composition (independent of WC and BMI).

The results from Study 1 indicate that SB is positively associated with adiposity. However, this positive association apparently can be offset by performing adequate volumes of MVPA; when controlling for MVPA in the analyses the correlation between SB and adiposity was nullified. This suggests that the absence of MVPA is more important than the presence of SB in the accumulation of FM over time. This has implications for public health policies and government guidelines on PA and SB. Recommendations to displace sedentary time with light PA may not be sufficient for weight management, and to accrue any benefit, PA must be at least moderate intensity in line with current PA guidelines. It is acknowledged that the correlation analysis from which these conclusions were drawn are not proof of causality, but they do not rule out causality. The observed relationship between PA, SB and adiposity is likely to be bidirectional. Therefore, low levels of PA and high SB will favour a positive energy balance and lead to increased FM. In turn, more FM (as a result of low activity or high EI) will serve as a disincentive to perform PA and a tendency to engage in more SB resulting in further increases in FM. Further studies should be conducted to explore the causal relationship between sedentary and active behaviours and adiposity.

11.3 Relationship between free-living sedentary behaviour, physical activity and appetite dysregulation

Control over EI is traditionally regarded as being independent from the energy expended through PA, however studies have demonstrated that these behaviours can

interact. Those individuals who are habitually physically active have been shown to have more sensitive control over appetite (Long et al., 2002, Beaulieu et al., 2016) and becoming more active improves satiety signalling (King et al., 2009a). However, becoming less physically active does not down regulate EI (Stubbs et al., 2004). It has also been proposed that SB promotes overconsumption (Chaput et al., 2011), although the evidence underpinning these relationships tend to rely on self-report proxy measures of SB, such as TV viewing. A more recent experimental study found no effects of breaking up prolonged sitting (5 hours) with light or moderate PA (2 min every 20 min) on subjective appetite sensations, gut hormones or absolute energy intake (Bailey et al., 2015). However, the longer-term effects of such an intervention to break up sedentary time are unknown. In addition to the direct effect of free -living PA and SB on EE (and therefore adiposity), it is also possible that PA and SB have an indirect effect on energy balance by influencing appetite control and EI. A further aim of Study 1 (Chapter 5) was to establish the relationship between free-living sedentary and active behaviours and markers of appetite dysregulation (TFEQ-D and BES) thought to influence the tendency to overconsume. There was no relationship between these markers and sedentary time, but MVPA was negatively associated with trait measures of appetite dysregulation (TFEQ-D and BES) indicative of loss of control over eating or 'opportunistic eating' (Bryant et al., 2008a). However, this relationship was no longer significant after controlling for body fat. Individuals with high TFEQ-D scores have previously been shown to be less physically active and have higher body fat than those with low TFEQ-D scores (Lawson et al., 1995). However, another study reported no relationship between trait measures of dysregulated eating (TFEQ-D and BES) and questionnaire measures of free-living PA (Finlayson et al., 2012). It is important to note that the participants in the study by Finlayson et al. (2012) were normal weight and there was no association between PA level and adiposity. A further finding of Study 1 was the positive relationship between multiple indices of adiposity and questionnaire measures of appetite dysregulation (TFEQ-D and BES) confirming previous findings (Dykes et al., 2004, Hays et al., 2002, Provencher et al., 2003, Finlayson et al., 2012, Bellisle et al., 2004). Taken together, the results from Study 1 suggest that any relationship between PA and trait measures of appetite dysregulation is mediated indirectly through adiposity (see Figure 11.1). Furthermore, it is possible that trait measures of appetite dysregulation are both a consequence and cause of obesity, analogous to the relationship between PA and obesity previously described. Dysregulated appetite control could lead to increased EI and weight gain. Alternatively, greater FM could lead to dysregulated appetite control and further weight gain.

11.3.1 Relating free-living sedentary and active behaviours to adiposity and appetite dysregulation

Figure 11.1 is a diagrammatical representation of the relationship between free-living sedentary and active behaviour, adiposity and eating behaviour traits indicative of uncontrolled eating based on the findings from Study 1. PA and SB have a direct effect on adiposity by influencing EE. Low levels of PA and high SB will favour a positive energy balance and lead to increased FM. In turn, more FM will serve as a disincentive to perform PA and a tendency to be more sedentary resulting in further accumulation of FM. PA and SB may also impact on EI indirectly through their effects on adiposity. Adiposity is related to trait measures of appetite dysregulation (TFEQ-D and BES) indicative of loss of control over eating. As with the relationship between PA, SB and adiposity, it is proposed that the relationship between questionnaire measures of appetite dysregulation and adiposity is bidirectional. Dysregulated appetite control would be expected to lead to increased EI and weight gain. In turn, greater FM would lead to dysregulated appetite control and further weight gain. Section 11.4.2 explores the relationship between different intensities of free-living PA (including SB) and EI. Briefly, activity EE and time spent in different categories of activity, from sedentary to vigorous, were not systematically related to objectively measured EI. However, this does not rule out the possibility of a direct relationship between time spent in different categories of free-living activity and eating behaviour in more active individuals where the energy requirement arising from PA is more substantial.

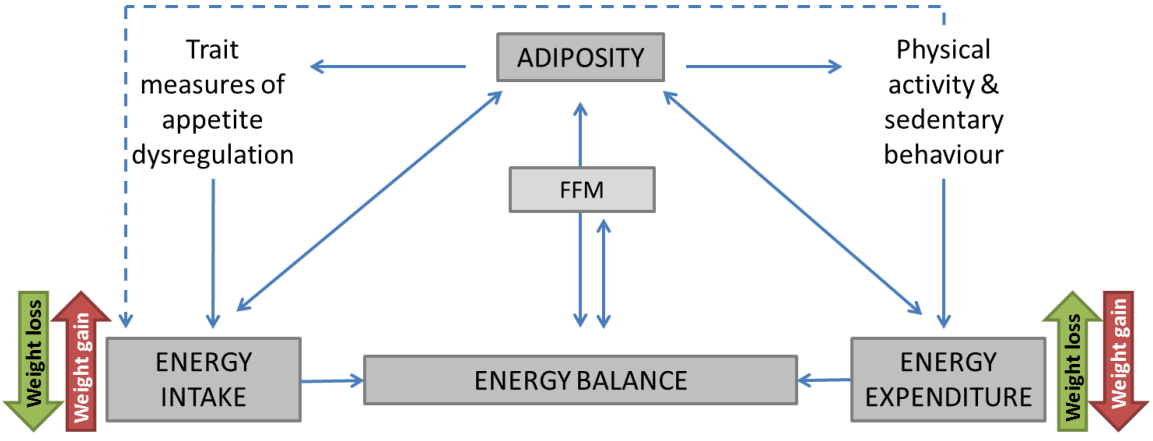


Figure 11.1 This diagram depicts the arrangement of variables involved in the original studies in this thesis. Namely the association of SB and MVPA with adiposity and appetite dysregulation. This diagram forms the basis for examining the interaction between PA (EE) and EI (food intake behaviour). Subsequent studies were superimposed on this basic platform.

11.3.2 A note on the measurement of sedentary behaviour

The measurement of free-living PA and SB is notoriously difficult (see Chapter 2). Over the past 10-15 years the use of objective measurement devices has expanded rapidly. However, large scale epidemiological studies still implement questionnaire based measures because they are cheaper to administer and less of a burden to participants. The inconsistencies in measurement method, particularly to quantify free-living SB, is problematic. Not only because many questionnaires often assess specific sedentary behaviours (for example, TV viewing) that are not representative of total sedentary time, but also because questionnaire measures are open to recall bias and have only slight to moderate reliability and validity (Atkin et al., 2012). The impact of SB measurement is exemplified in the NHANES 2003-2006 study by Maher et al. (2013) in which TV viewing was positively associated with risk of obesity, however, objectively measured total sedentary time was not.

Objective measures of SB are not without limitations, for example, hip worn accelerometers may classify standing as sedentary, whilst thigh worn accelerometers may classify seated activities of greater than 1.5 METs as sedentary. Study 2 in this thesis describes the methodological platform developed during the early stages of this PhD to integrate information from two validated activity monitors. The integration procedure resulted in three measures of SB, defined by i) an activity intensity of <1.5 METs, ii) a seated or reclining posture and iii) a combination of activity intensity and posture (all SB measures excluded sleep). Study 3 in this thesis examined whether the association between free-living SB and body composition (identified in Study 1) differed depending on the way SB was operationally defined and measured. The study revealed that only the measure of SB based on low activity intensity (<1.5 METs) was associated with indices of adiposity. Time spent sitting/reclining and time spent sitting/reclining with an activity intensity of <1.5 METs was not related to indices of obesity. This indicates that posture is a less important component of SB compared to activity intensity in relation to energy balance outcomes such as adiposity. The current leading definition of SB has both a postural and activity intensity component but there is no single measurement device that is able to measure these parameters simultaneously. It is a priority to develop such a device to clarify which components of SB are driving the negative association with multiple health outcomes. Although Study 3 in this study demonstrates that posture alone is not sufficient for the accumulation of FM, this does not rule out the possibility that the postural element of SB is important for other physiological health outcomes. Future research will shed light on whether the operational definition of SB used impacts on the relationship with other health outcomes.

11.4 Relating energy intake to energy expenditure

Study 4 revealed that total daily EI was positively associated with total EE. This is in agreement with previous research in army recruits that demonstrated EI was related to EE, such that higher expenditure was associated with higher intake when averaged over several days (Edholm et al., 1970). Furthermore, total EE was negatively associated with fasting fullness and positively associated with AUC for hunger and desire to eat. These subjective appetite sensations represent a drive to eat and were related to eating behaviour in the direction that would be expected (for example, AUC hunger was positively associated with total EI). There was no rise in EI at lower levels of EE in Study 4, as has previously been reported (Mayer et al., 1956, Shook et al., 2015). A potential explanation for this is that although participants had low levels of PA in comparison to government recommendations, they performed enough activity to be in the 'regulated zone' of appetite control (Blundell, 2011). Indeed, Shook et al. (2015) concluded that a moderate level of PA, equivalent to 7116 steps/d, corresponded to favourable regulation of EI and appetite. Participants in Study 4 performed a similar number of steps on average (7888 steps/d). Total EE consists of both metabolic (FFM and RMR) and behavioural (time spent in categories of activity and activity EE) contributors and it is possible that these separate elements of total daily EE may have a different relationship with EI.

11.4.1 Effects of metabolic contributors to energy expenditure on energy intake

In order to test the functionality and validity of the experimental platform, relationships previously established between metabolic variables and EI were examined. FFM and RMR were both positively correlated with total EI, confirming previous findings (Blundell et al., 2012b, Caudwell et al., 2013a, Weise et al., 2014). Furthermore, RMR was positively associated with desire to eat throughout the day and FFM was negatively associated with a measure of satiety (SQ desire to eat). A relationship between RMR and subjective appetite sensations has previously been identified (Caudwell et al., 2013a). Contemporary models of appetite control primarily describe the tonic and episodic inhibitory signals that modulate a constant and recurring excitatory drive to eat (Blundell and Gillett, 2001). Until recently, the source of this excitatory drive to eat was poorly defined (Halford and Blundell, 2000) but was proposed to arise from RMR (Blundell et al., 2001). Evidence has accumulated over the past five years strongly implicating FFM and RMR as the source of the excitatory drive. It has been proposed that FFM, as the main determinant of RMR, is a physiological source of hunger that drives EI at a level that is proportionate to basal energy needs. This tonic signal of energy demand would help match EI to basic energy requirements to ensure the maintenance and execution of key biological and

behavioural processes. Hopkins et al. (2016) explored the relationship between body composition, EE (RMR) and EI using path analysis and found that the effect of FFM on EI was fully mediated by RMR.

In agreement with previous research, FM was not associated with EI in Study 4 (Blundell et al., 2012b). This finding would seem to oppose the traditional adipocentric model of appetite control. However, the lack of a relationship between FM and EI should not be taken to imply that FM does not play a role in appetite control. Indeed, a negative relationship between FM and EI has previously been reported (Weise et al., 2014), supporting the inhibitory role of FM in appetite control. The observation that FFM is positively and FM negatively related to EI underlines the importance of measuring body composition as opposed to body mass or BMI. Data from Study 4 strengthens the evidence for the role of FFM and RMR as tonic drivers of EI that reflect the body's basal energy requirements (Blundell et al., 2012a). A priority for future research is to identify the mechanism that translates the basal energy demand arising from RMR into a motivational drive to eat.

The relationship between FFM, RMR and EI has important implications for body mass regulation. As FM increases (due to an imbalance between EI and EE), so too does FFM to support the greater body mass. This increase in FFM would result in a greater RMR, in turn, generating a greater metabolic demand for energy. On the other hand, a consequence of the increased FM could be reduced sensitivity to satiety signals (perhaps due to leptin and insulin resistance). The combination of the increased tonic drive to eat coupled with a blunted effect of inhibitory hormones would facilitate further weight gain and make weight loss/maintenance more difficult. It should be noted that the present findings in this thesis are based on overweight and obese individuals. Given that the accumulation of adipose tissue is associated with leptin and insulin resistance, the strength of the inhibitory influence on appetite and eating behaviour exerted by FM may differ between lean and overweight/obese individuals. Indeed, it is becoming recognition that the relationship between PA/SB and EI may differ between lean and obese individuals. This means that the PA/SB-EI relationship depends on how much adipose tissue there is in the body.

11.4.2 Effects of behavioural contributors to total energy expenditure on energy intake

Study 4 was the first study to investigate the associations between time spent in different intensities of activity measured using validated objective activity monitors, objectively quantified 24 hour eating behaviour and subjective appetite sensations. The study revealed that although total EE was related to EI, the behavioural contributors to total EE were not associated with homeostatic measures of appetite control. Specifically, activity EE and time spent in different categories of activity, from

sedentary to vigorous, were not systematically related to objectively measured EI (see Figure 11.1) or subjective appetite sensations. However, participants in Study 4 were relatively inactive and therefore, activity EE did not contribute greatly to overall EE. Indeed, the lack of a relationship between PA, subjective appetite sensations and EI could reflect the relatively small contribution of PA to total EE. The additional energy requirement above RMR in low active individuals, such as the participants in Study 4, is small in comparison to individuals who are more physically active. If the energy demand arising from PA was more substantial it might result in a motivational drive to eat similar to that associated with RMR. Furthermore, there is large variability in volitional PA between days and this could explain why there was no detectable relationship between activity and homeostatic measures of appetite control. This does not rule out the possibility of a relationship between free-living PA and EI in more active individuals. Study 5 in this thesis, along with other experimental studies, demonstrated that obligatory exercise leads to a partial compensatory increase in EI. The increase in EI has been shown to account for approximately 30% of the energy expended through exercise (Whybrow et al., 2008).This compensatory increase in EI could be a result of several factors including, deliberately seeking food as a reward for completing the exercise and overestimating the amount of calories expended during exercise. An alternative explanation is that the increased EE associated with exercise results in an increased drive to eat. Previous research has demonstrated that taking individuals from an inactive to an active state through 12-weeks of supervised exercise causes an increased drive to eat in the fasted state and in some individuals hunger is elevated throughout the day and EI is greater (King et al., 2009a, King et al., 2008). It is possible that there is a relationship between free-living activity EE, time spent in different intensities of PA and EI in those who are habitually more physically active (and therefore have a greater and more consistent energy demand arising from behavioural contributors to EE). The relationship between measures of homeostatic appetite control and eating behaviour and free-living PA requires further investigation across a range of PA levels. What characterises the effect of behavioural contributors to EE (activity EE) on EI and appetite control is its variability.

11.5 Effects of a negative energy balance induced by an exercise or diet intervention on body composition

The efficacy of exercise for weight loss has recently been questioned, despite a Cochrane review supporting the role of exercise for weight reduction and management independent of diet (Shaw et al., 2006). Study 5 demonstrated that exercise does lead to weight loss directly refuting the claims of Malhotra et al. (2015) that *'physical activity does not promote weight loss'*. The 12-week diet intervention (Study 6) also led to a significant reduction in body mass. The magnitude of the average weight loss was

greater in the diet intervention (1.82 kg) compared with the exercise intervention (0.83 kg), however, exercise resulted in an increase in FFM that was not seen in the diet intervention. Increased FFM is beneficial for energy balance and weight loss/maintenance, this is discussed in more detail in section 11.7.3.

11.6 Individual variability in weight loss

An important message from this thesis, and related works, concerns the individual variability in EI and body mass in response to challenges that induce negative energy balances. Until recently, the variability in weight loss response to weight loss interventions was not well documented. Studies focussed on reporting the group mean and this led to the assumption that all individuals respond in the same way to the same energy deficit (through diet or exercise). This approach concealed the variability in weight loss between individuals. There was considerable individual variability in weight loss in response to both the exercise intervention (-4.3 kg to +3.1 kg) and diet intervention (-10.1 kg to +4.8 kg) in this thesis. Large individual variability in weight change in response to exercise (King et al., 2008) and diet (Camps et al., 2013) interventions has previously been reported. Approximately 25% of the participants in Study 5 (exercise intervention) and Study 6 (diet intervention) gained weight. It is important to note that out of the six participants who gained weight in the exercise intervention, four experienced a reduction or no change in FM and their weight gain was due to an increase in FFM. This demonstrates the importance of examining changes in body composition to fully understand the effects of exercise on body mass regulation. Furthermore, exercise has been shown to have a beneficial effect on health related outcomes, such as BP and WC, in the presence of less than expected weight loss (King et al., 2009b). A strength of the exercise intervention was that exercise was supervised and measured and the variability in weight loss could not be explained by differences in measured exercise-induced EE. On the other hand, the self-reported measure of compliance in the diet intervention was shown to significantly predict weight change and indicates that variability in weight loss outcomes was at least partially explained by adherence to the diet.

11.7 Compensatory responses to perturbation in energy balance that defend against weight loss

The metabolic and behavioural adaptations to diet and exercise were investigated to help understand the mechanisms that drive the individual variability in weight loss. The compensatory responses to exercise induced weight loss have been the topic of several review articles (King et al., 2007, Melanson et al., 2013). However, studies investigating the compensatory responses to diet induced weight loss have not been

summarised. Increased post-prandial ghrelin and subjective hunger and decreased satiety hormones such as peptide YY (PYY) following diet induced weight loss have been reported as well as reduced fasting leptin levels (Sumithran et al., 2011). The compensatory adaptations to exercise can be categorised as either behavioural (change in eating behaviour or free-living PA) or metabolic (change in RMR, skeletal muscle energy efficiency or the energy cost of PA). Whilst the majority of the behavioural adaptations are (theoretically) under the individual's volitional control, the metabolic adaptations are automatic. A better understanding of the compensatory mechanisms that offset exercise and diet induced energy deficits will aid the development of effective tools to identify individuals who are resistant to weight loss and tailor interventions accordingly. Studies 5 and 6 in this thesis investigated the compensatory responses of overweight and obese women undergoing an exercise regime that caused increased EE and a weight loss diet that caused reduced EI. Specifically, the change in free-living PA, SB and NEPA, eating behaviour and appetite sensations, and metabolism were examined.

11.7.1 Free-living physical activity, sedentary behaviour and non-exercise physical activity

Free-living PA and SB were measured four times during the exercise intervention using validated PA monitors: Once in the week before the exercise commenced, the first and tenth week of the exercise, and the week immediately after the exercise intervention. As would be expected, there was a significant increase in PA (total EE, MVPA and steps) during the exercise intervention when the structured exercise was included in the PA monitor data. PA levels reverted back to baseline values after the exercise intervention. There was no consistent evidence for a reduction in NEPA when structured exercise was removed from free-living PA data. In other words, performing structured MVPA does not subtract from the MVPA that is carried out as part of a normal daily routine. When the structured exercise was deducted from MVPA measured using the activity monitors during week one and ten (during the intervention), there was no significant difference in MVPA between the four measurement periods. Indeed, average NEPA MVPA was remarkably similar with each of the four measurement periods falling within 3 minutes of each other (85.78 min/d to 88.69 min/d). The literature on the effects of structured exercise on NEPA is inconsistent partly due to the difficulty in accurately and reliably measuring free-living EE and time spent in different intensities of PA (Garland et al., 2011). The findings of Study 5 are in agreement with a recent systematic review of randomized controlled trials with exercise interventions lasting at least two weeks that found on average there was no statistically significant change in NEPA in response to exercise interventions (Fedewa et al., 2016). In addition, there was no compensatory increase in SB in response to the exercise intervention. This finding is consistent with previous research

(Swartz et al., 2016, Herrmann et al., 2015). In Study 5 there was some evidence that SB was displaced by the structured exercise as SB was lower during the intervention, but only the difference in SB (measured using the SWA) between week one of the intervention and the week immediately after the intervention was statistically significant. Importantly, the direction of the change in SB would favour a negative energy balance as opposed to reducing the energy deficit induced by the exercise.

There was no change in free-living MVPA or SB in response to the diet intervention. However, there was a significant reduction in light PA. Light PA was displaced with some lower intensity activity (SB) but also with higher intensity activity (MVPA). Using predictive equations based on body mass and the calorie equivalent of one MET it was possible to calculate how many fewer calories would have been expended due to the reduction in light PA and how many calories would have been expended due to the change in SB and MVPA. These calculations revealed that the change in the distribution of PA across intensities from baseline to post-intervention would not have resulted in a reduction in EE and would be unlikely to impact on weight loss outcomes. Furthermore, change in free-living PA and SB did not predict weight change and there were no differences in PA and SB between those who lost weight and those who gained weight following the diet intervention.

11.7.2 Eating behaviour and subjective appetite sensations

This is discussed here because appetite variables are often volatile measures and therefore may contribute to individual variability in response to interventions that perturb energy balance.

EI is a major contributor to the behavioural determinants of body mass regulation. Therefore, changes in eating behaviour (food intake) driven by changes in appetite mechanisms could contribute to energy compensation in response to increased exercise-induced EE thereby compromising weight loss. There is a belief, particularly in the popular press, that becoming more active will lead to an automatic compensatory response in eating behaviour that offsets the negative energy balance created by structured exercise, rendering exercise futile for weight loss. Evidence suggests that acute exercise does not lead to an increase in EI (Schubert et al., 2013, Donnelly et al., 2014), but when exercise is continued over several days EI begins to track total EE (Blundell et al., 2003, Whybrow et al., 2008). However, the compensatory increase in EI in response to longer term exercise is somewhat inconsistent and a factor contributing to this could be the difficulty in accurately measuring free-living EI. Study 5 in this thesis investigated the effects of a 12-week supervised exercise intervention on eating behaviour and subjective appetite sensations. Twenty-four hour EI was measured carefully and precisely during intensive probe days in the laboratory, subjective appetite sensations were measured before

and after meals and repeated between meals, and EE was measured using state-of-the-art indirect calorimetry, HR monitoring and accelerometry. There was a significant increase in total EI throughout the day, *ad libitum* EI and snacking in response to the exercise intervention. This increase in EI did not fully compensate for the energy expended through exercise as participants in the study lost weight (on average). However, as participants lost less weight than was predicted from the energy expended through exercise, partial compensation was apparent. The average increase in EI (178 kcal) equated to 35.6% of the prescribed EE per exercise session (500 kcal), similar to the 30% reported by (Whybrow et al., 2008). There was also an increase in subjective hunger and a decrease in fullness throughout the day reflected in AUC for hunger and fullness. Interestingly, these changes in subjective appetite sensation are similar to those observed in individuals categorised as 'non-responders' (did not achieve the predicted weight loss) in the study by King et al. (2009a). Indeed, when predicted weight loss was compared to actual weight loss in Study 5, only one participant would be categorised as a 'non-compensators' (based on body mass change; King et al. (2008)) and only one third would be categorised as 'responders' (based on body composition change; King et al. (2009a)). A lower initial BMI in the participants in Study 5 could explain why their weight loss response was less pronounced than that observed by King et al. (2009a). Furthermore, the study by King et al. (2009a) included men, and men have been shown to exhibit a greater weight loss in response to exercise than women (Ballor and Keesey, 1991, Donnelly and Smith, 2005). Although this is not a universal finding (Caudwell et al., 2013c).

Study 6 examined the effects of a 12-weeks diet intervention on eating behaviour and subjective appetite sensations and found there was no change in EI or appetite sensations. This is in contrast with previous research that demonstrated diet-induced weight loss in response to a 10-week very-low-calorie diet resulted in a significant increase in subjective hunger, perhaps mediated by increased levels of the orexigenic appetite hormone ghrelin and decreased anorexigenic hormones such as leptin and PYY (Sumithran et al., 2011). These changes persisted one year after initial weight loss despite partial weight regain. The contrasting findings may be due to the magnitude of the weight loss achieved or the type of dietary intervention implemented. In the study by Sumithran et al. (2011), the energy restriction was more severe and weight loss (13.5 kg) was considerably greater than in Study 6 (1.82 kg). When participants in Study 6 were categorised as weight losers (-5.51 kg) and weight gainers (+1.35 kg) appetite variables seemed to be responsible for the change in weight of the gainers. Those who gained weight consumed more calories on average (across baseline and post-intervention probe days) from the snack box, *ad libitum* EI and total EI throughout the day. Furthermore, gainers showed an increase in the eating behaviour traits TFEQ-H and BES. Baseline snack box EI significantly predicted

weight change such that higher baseline snack box EI was associated with an increase in body mass. It is possible that baseline snack intake could be a marker of weight gain or could reflect poor compliance with the diet intervention. Indeed, snacking has previously been associated with weight gain (Bes-Rastrollo et al., 2010). Furthermore, gainers self-reported significantly lower compliance with the diet compared to losers.

Taken together, the results from Studies 5 and 6 in this thesis suggest that changes in appetite related variables such as eating behaviour and subjective appetite sensations contribute to the variability in weight loss in response to exercise and diet interventions. On the other hand, there was no evidence to support a reduction in NEPA or an increase in SB in response to structured exercise and the change in free-living PA and SB did not predict diet-induced weight loss. For whatever reason, the biological system finds it easier to compensate for a negative energy balance through increased EI rather than reduced EE (NEPA).

11.7.3 Metabolic responses

RMR accounts for the largest proportion of total daily EE at approximately 50-70% (Goran, 2000, Shetty, 2005) and FFM accounts for 60-70% of the variance in RMR (Johnstone et al., 2005). Therefore, RMR and FFM are important contributors to energy balance and any change in these outcomes has important implications for weight loss. The metabolic responses to exercise and diet were examined in Studies 5 and 6, respectively. There was no significant difference in RMR between baseline and post-intervention in the exercise study, however, there was a significant increase in FFM. In contrast, following diet-induced weight loss there was a significant reduction in RMR with no change in FFM. These data are in agreement with previous findings that demonstrate exercise does not lead to a reduction in RMR and in some cases RMR is elevated, perhaps due to the preservation or increase in FFM associated with increased exercise. The opposite is true for diet-induced weight loss. It is well documented that weight loss induced by diet leads to a decline in RMR due to reduced body mass, which is based on FM, and additionally, FFM (Stiegler and Cunliffe, 2006). Rather than elevate RMR, the increase in FFM in Study 5 offset a decline in RMR that might accompany a significant reduction in FM. Furthermore, in Study 6, the decline in RMR could not be attributed to FFM as there was no significant difference post-intervention. Instead the decline in RMR was likely to be a result of the significant reduction in FM which accounts for around 6% of the variance in RMR (Johnstone et al., 2005). The reduction in RMR can be viewed as a compensatory mechanism to defend against further weight loss. To achieve further diet-induced weight loss, EI would need to be reduced further to account for the lower total EE. Alternatively, increased PA alongside the diet intervention would lead to an increase in total EE to

offset the lower RMR and could also lead to increased FFM, which in turn would prevent a decline in RMR. Data from Study 5 demonstrates that exercise-induced weight loss has favourable effects on metabolic contributors to total EE. Although RMR did not increase, exercise prevented the decline in RMR observed in the diet intervention due to increased FFM which offset the reduction in RMR associated with reduced FM.

11.8 Methodological strengths and limitations

The methodological platform, embedded within all of the studies in this thesis, was carefully designed to measure a range of variables related to appetite control and energy balance. This approach disclosed the interplay between variables in different scientific domains in relation to appetite control and energy balance, for example, body composition (physiology), RMR (metabolism) and EI (behaviour). A method was developed at the outset of this programme of work to measure free-living PA and SB using state-of-the-art activity monitors. This behavioural component of energy balance has not been studied previously within this research framework and is a strength of the current work. A second strength of this thesis was the objective measurement of 24 hour EI measured during probe days under laboratory conditions using test meals. This method permitted the precise measurement of volitional food intake in the absence of distraction and environmental cues. Whilst the internal validity of laboratory studies is high, they lack ecological validity (Blundell et al., 2010). It is acknowledged that using episodic test meal intake to infer changes in habitual intake has limitations (Hill et al., 1995). For example, exposure to higher energy dense foods could lead to passive overconsumption (Blundell and MacDiarmid, 1997). Rather than reflecting EI in the natural environment, probe day measures of EI can be viewed as assays for eating behaviour and give an indication of compensatory appetite responses to perturbations in energy balance that are free from external influences (Gibbons et al., 2014). Similar test meals and probe day procedures to those reported in this thesis have previously been shown to detect changes in eating behaviour (King et al., 2008).

Despite the strengths of the methodological framework that formed the basis of this thesis, it is not without limitations. It must be acknowledged that EI was not measured under free-living conditions. There are well documented limitations associated with measuring EI in the natural environment and it is for this reason free-living EI was not measured (Schoeller et al., 2013). Furthermore, it was not possible to control for the menstrual cycle (or oral contraceptive use) of women participants in the studies in this thesis. Due to the intensive testing schedule at baseline and post-intervention for both of the intervention studies, it was not possible to standardise the menstrual cycle stage across participants. Timing of the post-intervention laboratory measurement visits,

particularly in the exercise study, was extremely important. All participants had to expend a standardised number of calories during the 12-week intervention, immediately followed by post-intervention measurements to ensure participants were in the same physiological state as they had been during the intervention. The timings of these measurements would have been compromised if measures and probe days were timed around the menstrual cycle. Since there does not seem to be any discernible differences between sexes in the appetite and eating behaviour response to acute and longer term exercise interventions (Thackray et al., 2016, Caudwell et al., 2013c), it is unlikely that the menstrual cycle had a major impact on the study outcomes. Finally, the lack of a control group in both the exercise and diet intervention studies is a limitation. Both studies were EU funded projects with strict budgets and timescales that did not permit the inclusion of a control group. However, both diet and exercise are powerful stimuli and it is unlikely that the changes in body mass (and body composition) seen in both studies occurred by chance. The inclusion of a control group would have provided a benchmark to compare individual variability in weight loss against to identify whether the variability was due to random variation or to true individual differences (Atkinson and Batterham, 2015).

11.9 Mayer curve re-visited

The main figure in the article by Mayer et al. (1956) has formed a background for the work in this thesis. However, it is usual for most authors to refer only to the lower panel of Figure 11.2 which shows the relationship between the intensity of physical work (and by implication EE) and dietary intake (by implication EI). The lower panel has been adapted to include further interpretation based on more contemporary research (see Figure 2.1). The upper panel of Figure 11.2 is often overlooked; this shows a relationship between physical work and body mass. This feature is also central to the work in this thesis.

Change in body mass obviously means changes in FM and FFM. Although Mayer did not have access to body composition measures in Calcutta in the 1950s, it can be inferred that the body mass curve is mainly a reflection of FM, with the most inactive individuals exhibiting a greater FM than the more active individuals. This relationship between FM and PA/SB has been demonstrated in this thesis (Studies 1 and 3) and in other recent works. There is now considerable evidence to show an association between FM (adiposity) and appetite – reflected in appetite dysregulation traits (TFEQ-D, BES; Study 1) or variables associated with daily EI (Blundell et al., 2015b).There are also relationships between PA and EI – especially MVPA (Study 5 (Shook et al., 2015, Beaulieu et al., 2016)).

These findings indicate that there is a close set of relationships among PA/SB, appetite variables, and FM. These relationships have formed the basis for the work in this thesis. The findings, that have demonstrated an association between PA and adiposity (Study 1), total EE and EI (Studies 4 and 5) and adiposity and appetite (Study 1), resonate with the initial proposals of Mayer embodied in the celebrated curve (upper and lower panels in Figure 11.2).

There is now a need to further explore the nature of these relationships, and especially how the PA-EI association may be modulated (and possibly mediated) by the amount of fat (adipose tissue) in the body. It raises the possibility that the relationship between PA and EI will depend on the degree of fatness a person possesses.

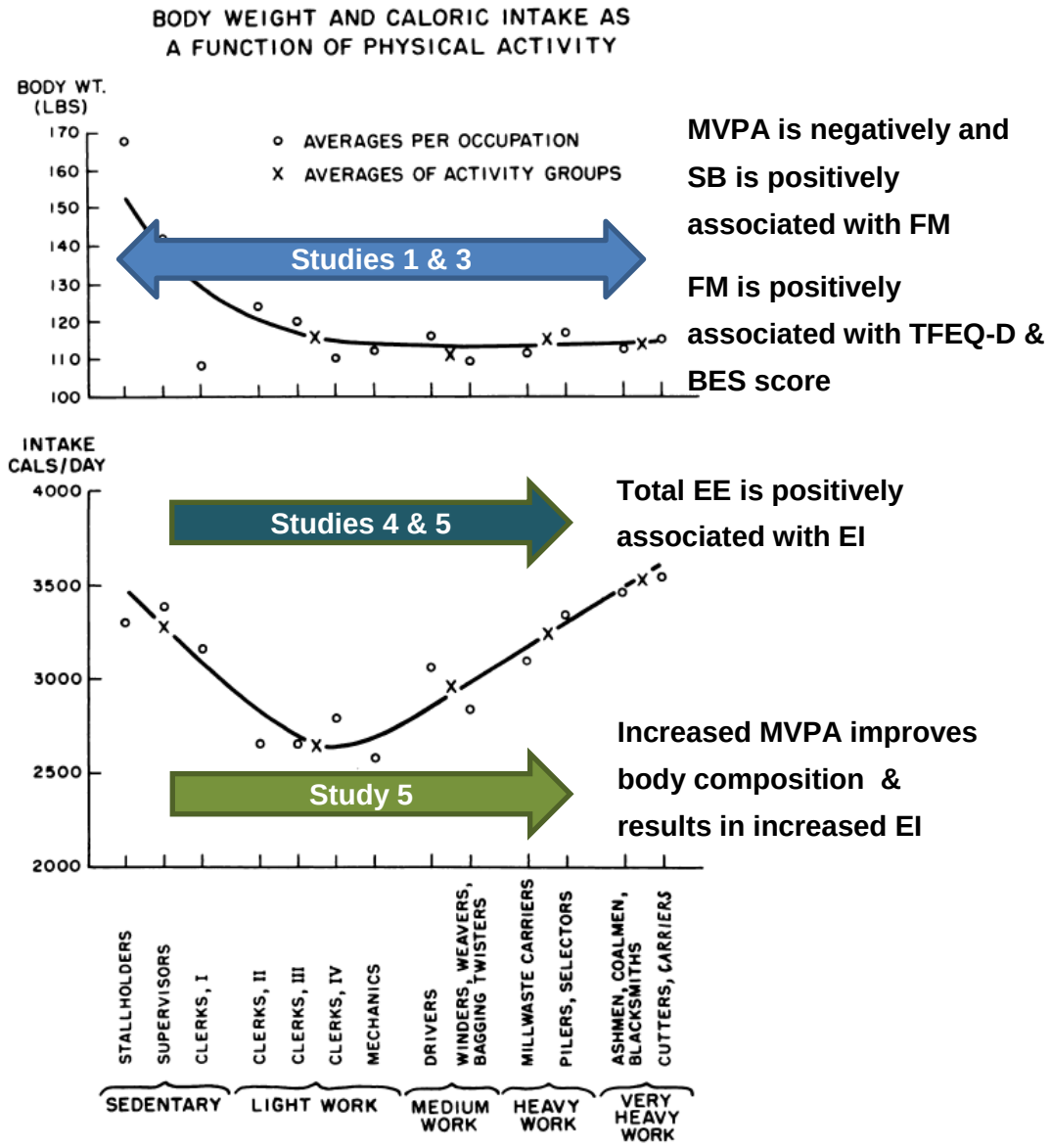


Figure 11.2 Relationship between the physical demand of occupation (EE) and EI (lower panel) and physical demand of occupation (EE) and body mass, source: (Mayer et al., 1956)

11.10 Conclusion

This thesis has examined the role of free-living PA and SB in body mass regulation and appetite control within an energy balance framework. Cross-sectional (Study 1 and 6) and experimental data (Study 5) support the role of PA (including exercise) in weight management. A major message from this work is that being more physically active is associated with lower FM, and becoming more physically active through structured exercise leads to weight loss (predominantly FM). Study 4 provided further evidence that EI is related to total EE, however, time (average per day) spent in different intensities of free-living movement behaviours was not related to eating behaviour or subjective appetite sensations. This may be a consequence of the large individual variability seen in both PA and eating behaviour. Importantly, the metabolic contributors to total EE (RMR and FFM) were associated with EI and subjective appetite sensations reflecting a drive to eat confirming previous findings. This strengthens the evidence underpinning the formulation of the major influences of appetite control within an energy balance framework. Specifically, it confirms the proposal for an excitatory orexigenic drive arising from FFM and RMR to ensure food intake meets daily basal energy requirements. Participants lost weight in response to both the exercise and diet interventions. It is important to note that although weight loss was smaller in the exercise intervention, the body composition changes were more favourable. The work of this thesis did not find evidence for compensatory change in free-living PA or SB to defend against diet or exercise-induced weight loss. Any compensation appears to be mediated through mechanisms concerning EI and adherence to dietary recommendations. It could be deduced that a combination of increased EE (through exercise) and reduced EI are likely to produce greater weight loss and more favourable changes in body composition than either exercise or diet alone.

11.11 Continuation of this line of research

The programme of work presented in this thesis has led to a number of publications in peer review journals and conference papers. Additionally, one paper is currently under review and several more papers will be submitted for publication including:

- A novel integrative procedure for identifying and integrating three-dimensions of objectively measured free-living sedentary behaviour. BMC Public Health (under review) – **Study 2**
- Disentangling the relationship between sedentariness and obesity: low activity intensity, but not posture, is associated with adiposity in overweight women – **Study 3**

- Total energy expenditure, but not individual components of physical activity, is associated with homeostatic appetite control and total energy intake – **Study 4**
- An aerobic exercise intervention in overweight women decreased fat mass but was partially compensated by increased appetite but not by increased sedentary behaviour or decreased non-exercise physical activity – **Study 5**
- Mild diet-induced weight loss does not lead to compensatory changes in physical activity or sedentary behaviour – **Study 6**

This chapter has highlighted a number of important findings arising from the experimental studies within this thesis. However, some important questions remain unanswered and warrant further investigation. These include:

- What is the role played by FM in mediating the relationships between PA and EI?
- Does coercive restriction of PA and enforced SB lead to increased FM and appetite dysregulation?
- In active individuals is time spent in different intensities of free-living activity associated with EI and subjective appetite sensations?
- How is the metabolic demand for energy arising from FFM and RMR translated in to a drive to eat and subsequent eating behaviour?

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Appendix A **Eating behaviour trait questionnaires**

A.1 Three Factor Eating Questionnaire (TFEQ)

This booklet contains a number of statements. Each statement should be answered either TRUE or FALSE. Read each statement and decide how you feel about it in PART 1.

If you agree with the statement , or if you feel that it is true about you then circle T next to the statement.

If you disagree with a statement, or if you feel that it is false as applied to you, circle the F next to the statement.

1) When I smell a sizzling steak or see a juicy piece of meat I find it very difficult to keep from eating, even if I have just finished a meal. T F
2) I usually eat too much at social occasions, like parties and picnics. T F
3) I am usually so hungry that I eat more than 3 times a day. T F
4) When I have eaten my quota of calories I am usually very good about not eating any more. T F
5) Dieting is so hard for me because I just get too hungry. T F
6) I deliberately take small helpings as a means of controlling my weight. T F
7) Sometimes things just taste so good that I keep on eating, even when I am no longer hungry. T F
8) Since I am often hungry, I sometimes wish that while I am eating an expert would tell me that I have had enough or that I can have something more to eat. T F
9) When I feel anxious I find myself eating. T F
10) Life is too short to worry about dieting. T F
11) Since my weight goes up and down, I have gone on reducing diets more than once. T F

12) I often feel so hungry I just have to eat something. T F

13) When I am with someone who is overeating I usually overeat too. T F

14) I have a pretty good idea of the number of calories in common foods. T F

15) Sometimes when I start eating, I just can't seem to stop. T F

16) It is not difficult for me to leave something on my plate. T F

17) At certain times of the day I get hungry because I have gotten used to eating then. T F

18) While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it. T F

19) Being with someone who is overeating often makes me hungry enough to eat also. T F

20) When I feel blue I often overeat. T F

21) I enjoy eating too much to spoil it by counting calories or watching my weight. T F

22) When I see a real delicacy I often get so hungry that I have to eat it right away. T F

23) I often stop eating when I am not really full as a conscious means of limiting the amount I eat. T F

24) I get so hungry my stomach feels like a bottomless pit. T F

25) My weight has hardly changed at all in the last ten years. T F

26) I am always hungry so it is hard for me to stop eating before I finish the food on my plate. T F

27) When I feel lonely, I console myself by eating. T F

28) I consciously hold back at meals in order not to gain weight. T F

29) I sometimes get very hungry late in the evening or at night. T F

30) I eat anything I want, anytime. T F

31) Without even thinking about it I take a long time to eat. T F

32) I count calories as a conscious means of controlling my weight. T F

33) I do not eat some foods because they make me fat. T F

34) I am always hungry enough to eat at anytime. T F

35) I pay a great deal of attention to changes in my figure. T F

36) While on a diet, if I eat food that is not allowed, I often then splurge and eat other high calorie foods. T F

Please answer the following questions by circling the number above the response that is appropriate to you.

37) How often are you dieting in a conscious effort to control your weight?

1	2	3	4
rarely	sometimes	usually	always

38) Would a weight fluctuation of 5lbs (2kg) affect the way you live your life?

1	2	3	4
not at all	slightly	moderately	very much

39) How often do you feel hungry?

1	2	3	4
only at meal times	sometimes between meals	often between meals	almost always

40) Do your feelings of guilt about overeating help you to control your food intake?

1	2	3	4
never	rarely	often	always

41) How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours?

1	2	3	4
easy	slightly difficult	moderately difficult	very difficult

42) How conscious are you of what you are eating?

1	2	3	4
not at all	slightly	moderately	extremely

43) How frequently do you avoid 'stocking up' on tempting foods?

1	2	3	4
Almost never	seldom	usually	almost always

44) How likely are you to shop for 'low calorie' foods?

1	2	3	4
unlikely	slightly likely	moderately likely	very likely

45) Do you eat sensibly in front of others and splurge alone?

1	2	3	4
never	rarely	often	always

46) How likely are you to consciously eat slowly in order to cut down on how much you eat?

1	2	3	4
unlikely	slightly likely	moderately likely	very likely

47) How frequently do you skip dessert because you are no longer hungry?

1	2	3	4
never	seldom	at least once a week	almost every day

48) How likely are you to consciously eat less than you want?

1	2	3	4
unlikely	slightly likely	moderately likely	very likely

49) Do you go on eating binges even though you are not hungry?

1	2	3	4
never	rarely	sometimes	at least once a week

50) On a scale of 0-5 where 0 means no restraint in eating (eat whatever you want, whenever you want it), and 5 means total restraint (constantly limiting food intake and never 'giving in'). What number would you give yourself?

0

Eat whatever you want, whenever you want it.

1

Usually eat whatever you want, whenever you want it.

2

Often eat whatever you want, whenever you want it.

3

Often limit food intake, but often 'give in'.

4

Usually limit food, rarely ‘give in’.

5

Constantly limiting food intake, never 'giving in'.

51) To what extent does this statement describe your eating behaviour?

'I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.'

1	2	3	4
not like me	little like me	pretty good description of me	describes me perfectly

A.2 Binge Eating Scale (BES)

Instructions: Below are groups of numbered statements. Read all of the statements in each group and mark on this sheet the one that describes you the best by circling the good number.

A.

1. I don't feel self-conscious about my weight or body size when I'm with others.

2. I feel concerned about how I look to others, but it normally does not make me feel disappointed with myself.

3. I do get self-conscious about my appearance and weight which makes me feel disappointed in myself.

4. I feel very self-conscious about my weight and frequently, I feel intense shame and disgust for myself. I try to avoid social contacts because of my self-consciousness.

B.

1. I don't have any difficulty eating slowly in the proper manner.

2. Although I seem to 'gobble down' foods, I don't end up feeling stuffed because of eating too much.

3. At times, I tend to eat quickly and then, I feel uncomfortably full afterwards.

4. I have the habit of bolting down my food, without really chewing it. When this happens I usually feel uncomfortably stuffed because I've eaten too much.

C.

1. I feel capable to control my eating urges when I want to.

2. I feel like I have failed to control my eating more than the average person.

3. I feel utterly helpless when it comes to feeling in control of my eating urges.

4. Because I feel so helpless about controlling my eating I have become very desperate about trying to get in control.

D.

1. I don't have the habit of eating when I'm bored.

2. I sometimes eat when I'm bored, but often I'm able to 'get busy' and get my mind off food.

3. I have a regular habit of eating when I'm bored, but occasionally, I can use some other activity to get my mind off eating.

4. I have a strong habit of eating when I'm bored. Nothing seems to help me break the habit.

E.

1. I'm usually physically hungry when I eat something.

2. Occasionally, I eat something on impulse even though I really am not hungry.

3. I have the regular habit of eating foods, that I might not really enjoy, to satisfy a hungry feeling even though physically, I don't need the food.

4. Even though I'm not physically hungry, I get a hungry feeling in my mouth that only seems to be satisfied when I eat a food, like a sandwich, that fills my mouth. Sometimes, when I eat the food to satisfy my mouth hunger, I then spit the food out so I won't gain weight.

F.

1. I don't feel any guilt or self-hate after I overeat.

2. After I overeat, occasionally I feel guilt or self-hate.

3. Almost all the time I experience strong guilt or self-hate after I overeat.

G.

1. I don't lose total control of my eating when dieting even after periods when I overeat.

2. Sometimes when I eat a 'forbidden food' on a diet, I feel like I 'blew it' and eat even more.

3. Frequently, I have the habit of saying to myself, 'I've blown it now, why not go all the way' when I overeat on a diet. When that happens I eat even more.

4. I have a regular habit of starting strict diets for myself, but I break the diets by going on an eating binge. My life seems to be either a 'feast' or 'famine'.

H.

1. I rarely eat so much food that I feel uncomfortably stuffed afterwards.

2. Usually about once a month, I eat such a quantity of food, I end up feeling very stuffed.

3. I have regular periods during the month when I eat large amounts of food, either at mealtime or at snacks.

4. I eat so much food that I regularly feel quite uncomfortable after eating and sometimes a bit nauseous.

I.

1. My level of calorie intake does not go up very high or go down very low on a regular basis.

2. Sometimes after I overeat, I will try to reduce my caloric intake to almost nothing to compensate for the excess calories I've eaten.

3. I have a regular habit of overeating during the night. It seems that my routine is not to be hungry in the morning but overeat in the evening.

4. In my adult years, I have had week-long periods where I practically starve myself. This follows periods when I overeat. It seems I live a life of either 'feast or famine'.

J.

1. I usually am able to stop eating when I want to. I know when 'enough is enough'.

2. Every so often, I experience a compulsion to eat which I can't seem to control.

3. Frequently, I experience strong urges to eat which I seem unable to control, but at other times I can control my eating urges.

4. I feel incapable of controlling urges to eat. I have a fear of not being able to stop eating voluntarily.

K.

1. I don't have any problem stopping eating when I feel full.

2. I usually can stop eating when I feel full but occasionally overeat leaving me feeling uncomfortably stuffed.

3. I have a problem stopping eating once I start and usually I feel uncomfortably stuffed after I eat a meal.

4. Because I have a problem not being able to stop eating when I want, I sometimes have to induce vomiting to relieve my stuffed feeling.

L.

1. I seem to eat just as much when I'm with others (family, social gatherings) as when I'm by myself.

2. Sometimes, when I'm with other persons, I don't eat as much as I want to eat because I'm self-conscious about my eating.

3. Frequently, I eat only a small amount of food when others are present, because I'm very embarrassed about my eating.

4. I feel so ashamed about overeating that I pick times to overeat when I know no one will see me. I feel like a 'closet eater'.

M.

1. I eat three meals a day with only an occasional between meal snack.

2. I eat 3 meals a day, but I also normally snack between meals.

3. When I am snacking heavily, I get in the habit of skipping regular meals.

4. There are regular periods when I seem to be continually eating, with no planned meals.

N.

1. I don't think much about trying to control unwanted eating urges.

2. At least some of the time, I feel my thoughts are pre-occupied with trying to control my eating urges.

3. I feel that frequently I spend much time thinking about how much I ate or about trying not to eat anymore.

4. It seems to me that most of my waking hours are pre-occupied by thoughts about eating or not eating. I feel like I'm constantly struggling not to eat.

O.

1. I don't think about food a great deal.

2. I have strong cravings for food but they last only for brief periods of time.

3. I have days when I can't seem to think about anything else but food.

4. Most of my days seem to be pre-occupied with thoughts about food. I feel like I live to eat.

P.

1. I usually know whether or not I'm physically hungry. I take the right portion of food to satisfy me.

2. Occasionally, I feel uncertain about knowing whether or not I'm physically hungry. At these times it's hard to know how much food I should take to satisfy me.

3. Even though I might know how many calories I should eat, I don't have any idea what is a 'normal' amount of food for me

A.3 Control of Eating Questionnaire (CoEQ)

Please read each question carefully and put a mark through the line at the point that best represents your experience. Answer all questions according to your experience over the last 7 days.

1. How hungry have you felt?

Not at all hungry ——— Extremely hungry

2. How full have you felt?

Not at all full ——— Extremely full

3. How strong was your desire to eat sweet foods?

Not at all strong ——— Extremely strong

4. How strong was your desire to eat savoury foods?

Not at all strong ——— Extremely strong

5. How happy have you felt?

Not at all happy ——— Extremely happy

6. How anxious have you felt?

Not at all anxious ——— Extremely anxious

7. How alert have you felt?

Not at all alert ——— Extremely alert

8. How contented have you felt?

Not at all contented ——— Extremely contented

A food craving is a strong urge to eat a particular food or drink

9. During the last 7 days how often have you had food cravings?

Not at all _____ Very often

10. How strong have any food cravings been?

Not at all _____ Extremely strong

strong

11. How difficult has it been to resist any food cravings?

Not at all _____ Extremely difficult

difficult

12. How often have you eaten in response to food cravings?

Not at all _____ After every one

How often have you had food cravings for the following types of food/drink?

13. Chocolate or chocolate flavoured foods

Not at all _____ Extremely often

14. Other sweet foods (cakes, pastries, biscuits, etc)

Not at all _____ Extremely often

15. Fruit or fruit juice

Not at all _____ Extremely often

16. Dairy foods (cheese, yoghurts, milk, etc)

Not at all _____ Extremely often

17. Starchy foods (bread, rice, pasta, etc)

Not at all _____ Extremely often

18. Savoury foods (french fries, crisps, burgers, pizza, etc)

Not at all _____ Extremely often

19. Generally, how difficult has it been to control your eating?

Not at all difficult ——————— Extremely difficult

20. Which one food makes it most difficult for you to control eating?

.....

21. How difficult has it been to resist eating this food during the last 7 days?

Not at all difficult ——————— Extremely difficult

Appendix B **Diet intervention**

B.1 Low energy dense diet

The LED diet was a commercial weight loss diet. The commercial weight loss diet is a multi-component weight management programme which promotes food optimisation (LED meals), compassion, group support, behaviour change techniques and tailored PA advice (Body Magic)[1]. Participants on the commercial weight loss diet attended weekly meeting where consultants recorded attendance and weight. The first day of the weight management programme was the day they enrolled on the commercial weight loss programme. A screening visit to the HARU was arranged within 2-4 weeks of commencing the weight loss programme.

B.2 Calorie restrictive diet

The calorie restrictive diet was the NHS Choices weight loss plan, a structured calorie restricting, self-led programme (NHS-Choices, 2016). In line with the National Institute for Health and Care Excellence (NICE) guidance on weight loss, the NHS Choices programme recommends a 600 kcal/d reduction in EI to promote gradual and sustainable weight loss (0.5-1 kg/week) (NICE, 2014). Individuals who sign up to the NHS Choices programme receive weekly advice on diet and PA such as portion control and promoting breakfast consumption and are encouraged to take part in challenges such as ‘Couch to 5k’. A diet and PA diary can be downloaded to help participants track their progress and there is also optional email support and a smart phone application to support meal preparation. The first day of the weight management programme was on the day of the screening visit .

B.3 Probe day meals

B.3.1 High and low energy dense test meals

The LED test meals were designed to be consistent with the a low energy dense commercial weight loss programme. LED meals were designed to be <0.8 kcal/g and <30% fat. The high energy dense (HED) meals were designed to provide the same number of calories, however, the energy density was >2.5 kcal/g and consisted of >50% fat. To determine meal size, participants were allocated to one of three bands based on their daily energy requirements. Daily energy requirement was calculated by

[1] Advice on increasing PA is a standard component of the commercial weight loss programme membership. However, it was not possible to obtain data on the specific information each participant received.

multiplying RMR by 1.4, the sedentary PA level (Food and Agriculture Organization, 2001). The three bands were: small ≤ 2000-2500 kcal/d, medium 2501-3000 kcal/d and large ≥ 3001 kcal/d. As participants were following a weight loss programme, the test meals were designed to allow a 20% energy deficit (80% of RMR x 1.4). The breakfast provided 20%, the lunch 30%, dinner was estimated to provide 30% and the snack box 20% (depending on how much of the *ad libitum* dinner and snack box participants consumed). All meals were prepared in the HARU kitchen following laboratory standard operating procedures with the exception of the LED chilli which was prepared by the commercial weight loss company, packaged, frozen and dispatched to the University of Leeds. All study foods were weighed before and after consumption to the nearest 0.1 g to confirm all of the meal had been consumed at breakfast and lunch and in the case of dinner and snack box, to calculate how much food had been consumed.

B.3.2 Fixed breakfast

The LED breakfast (Figure 11.3A) consisted of scrambled eggs, wholegrain toast with margarine, baked beans, tomatoes, mushrooms, mango, grapes, strawberries and clementine. The HED breakfast (Figure 11.3B) consisted of scrambled eggs mixed with double cream and butter, seeded wholegrain toast with butter, tomatoes, mushrooms and a Danish pecan pastry. Participants received 350 g of water and an optional tea or coffee (175 g of water, and optional 40 g of semi-skimmed milk). Participants were instructed to take as long as was necessary to consume all of the food and hot drink provided and to consume as much or as little of the water as they liked. Table 11.1 provides serving size, macronutrient composition and energy density of the high and low energy density breakfast for the small energy requirement band. The medium band provided 440 kcal and the large band provided 520 kcal. All three bands provided the same proportion of fat, carbohydrate and protein. The breakfast meals were matched for energy content only between the high and low energy dense days.

Table 11.1 Serving size, macronutrient composition and energy content of LED and HED breakfast based on the small energy requirement band

Meal (small)	Serving (g)	kcal	ED (kcal/g)	PRO (g)	Fat (g)	CHO (g)	Fibre (g)	PRO (%)	Fat (%)	CHO (%)
LED BF	544.5	360.0	0.7	17.6	8.5	56.7	10.3	19.6	21.4	59.1
HED BF	122.0	360.1	3.0	9.5	26.1	23.4	2.2	10.5	65.1	24.4

ED, energy density; PRO, protein; CHO, carbohydrate; BF, breakfast

A B

Figure 11.3 LED (A) and HED (B) breakfast, images show small energy requirement band

B.3.3 Fixed lunch

Lunch was provided four hours after breakfast. The LED lunch (Figure 11.4A) consisted of a jacket potato, tuna in brine mixed with mayonnaise, sweetcorn and crème fraiche, tomatoes, lettuce, yellow pepper, red onion, vinaigrette dressing and strawberry yoghurt. The HED lunch (Figure 11.4B) comprised of a jacket potato with butter, tuna in oil mixed with sunflower oil and mayonnaise, tomatoes and lettuce dressed with olive oil and a chocolate mousse. Participants received 350 g of water to drink *ad libitum*. Participants were instructed to take as long as was necessary to consume all of the food provided. Table 11.2 provides serving size, macronutrient composition and energy density of the high and low energy density lunch meals for the small energy requirement band. The medium band provided 660 kcal and the large band provided 780 kcal. All three bands provided the same proportion of fat, carbohydrate and protein. The lunch meals were matched for energy content only between the high and low energy dense days.

Table 11.2 Serving size, macronutrient composition and energy content of LED and HED lunch based on the small energy requirement band

Meal (small)	Serving (g)	kcal	ED (kcal/g)	PRO (g)	Fat (g)	CHO (g)	Fibre (g)	PRO (%)	Fat (%)	CHO (%)
LED Lunch	693.7	540	0.8	37.3	16.9	63.6	6.5	27.6	28.2	44.2
HED Lunch	217.8	540	2.5	14.0	39.7	33.8	2.1	10.3	66.2	23.4

ED, energy density; PRO, protein; CHO, carbohydrate

A **B**

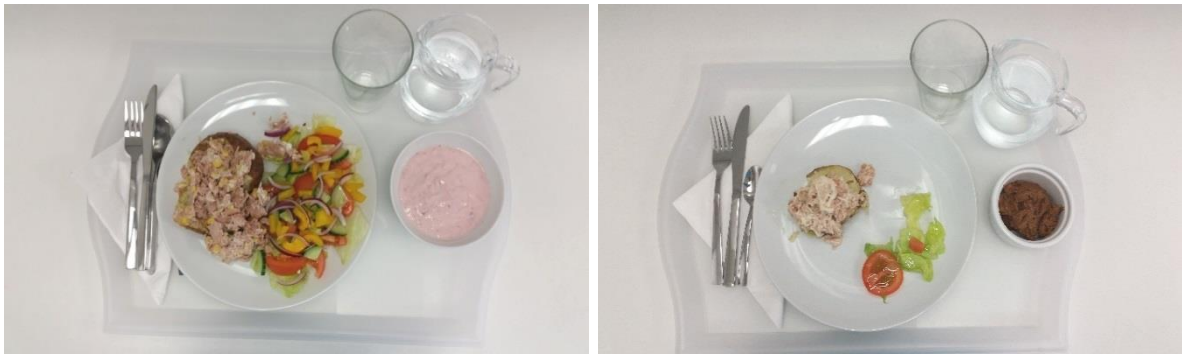

Figure 11.4 LED (A) and HED (B) lunch, images show small energy requirement band

B.3.4 *Ad libitum* dinner

Dinner was served four hours after lunch. The LED dinner (Figure 11.5) consisted of a beef-based chilli, long grain rice, cheese, tomatoes, red onion, yellow peppers, lettuce, cucumber and bananas and natural yoghurt (sweetened). The HED dinner (Figure 11.6) consisted of beef-based chilli, tortilla crisps, sour cream mixed with mayonnaise and double cream, cheese and chocolate brownies. Participants were also provided with 350 g of water. Participants were instructed to take as much time as they needed and to consume as much or as little of the food and water as they wanted but to eat until they reached a comfortable level of fullness. Participants were provided with a serving plate and were asked to eat foods they placed on the plate. An empty bowl was also provided with the LED dinner for participants to mix the banana and yoghurt if they wished. Table 11.3 provides serving size, macronutrient composition and energy density of the high and low energy density dinner meals. The *ad libitum* dinner was the same for all three energy requirement bands.

Table 11.3 Serving size, macronutrient composition and energy content of LED and HED dinner

Meal	Serving (g)	kcal	ED (kcal/g)	PRO (g)	Fat (g)	CHO (g)	Fibre (g)	PRO (%)	Fat (%)	CHO (%)
LED Din	2358.3	1788.6	0.8	127.4	26.8	276.9	23.6	28.5	13.5	58.1
HED Din	1839.0	4729.8	2.6	124.7	305.0	396.0	65.0	10.5	58.0	31.4

ED, energy density; PRO, protein; CHO, carbohydrate; Din, dinner

Figure 11.5 LED *ad libitum* dinner

Figure 11.6 HED *ad libitum* dinner

B.3.5 *Ad libitum* snack box

After the dinner, participants were provided with a snack box to take home with them that evening. The snack box contained a selection of savoury and sweet snacks packaged in clear food bags and plastic containers. The LED snack box (Figure 11.7A) contained 2 x yoghurt, grapes, pineapple, carrots, cucumber, cottage cheese, ham, Curly Wurly and crisps. The HED snack box (Figure 11.7B) contained 2 x trifle, cheese twists, Ryvita slims, houmous, mini sausages, chocolate buttons, crisps and flapjack. Participants were instructed they could eat as much or as little as they liked from the selection of foods, but they should not share or dispose of any foods, eat any foods other than those provided in the snack box and that they should avoid alcohol and caffeinated drinks. Participants were instructed to return the snack box the next day containing any packaging from foods they had eaten and any uneaten food. Table 11.4 provides serving size, macronutrient composition and energy density of the high and low energy density snack box. The *ad libitum* snack box was the same for all three energy requirement bands.

Table 11.4 Serving size, macronutrient composition and energy content of LED and HED snack foods

Meal	Serving (g)	kcal	ED (kcal/g)	PRO (g)	Fat (g)	CHO (g)	Fibre (g)	PRO (%)	Fat (%)	CHO (%)
LED SB	1073.0	909.2	0.8	48.3	19.6	143.8	9.8	21.3	19.4	59.3
HED SB	1073.0	3915.0	3.6	80.4	199.1	480.4	29.2	8.2	45.8	46.0

ED, energy density; PRO, protein; CHO, carbohydrate; SB, snack box

A **B**

Figure 11.7 LED (A) and HED (B) snack foods